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Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.01

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01NS117918
NIH Grant R21NS104394
NIH Grant R21NS119732

Title: Mir-375-3p regulates nelavls expression and promotes cell survival during neurod1-mediated astrocyte-to-neuron reprogramming

Authors: *X. CHEN[1], I. SOKIRNIY[3], X. WANG[4], M. JIANG[1], N. MSEIS-JACKSON[1], C. WILLIAMS[2], N. JIANG[1], B. PULS[4], K. MAYES[1], Q. DU[1], Y. SHI[1], H. LI[1];
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Abstract: Astrocyte-to-neuron reprogramming holds promise in regenerative medicine. To understand microRNA function during this process, we performed RNA-seq on human astrocytes with NeuroD1 overexpression. Here, we reported that NeuroD1 induced drastic upregulation of two miRNAs, miR-375-3p and miR-124-3p, as well as many neuronal genes. Further analysis revealed that miR-375-3p targeted neuronal ELAVL genes (nELAVLs), which encode a family of RNA-binding proteins and were also upregulated by NeuroD1. By overexpression and knockdown experiments, we showed that manipulating miR-375-3p level can regulate nELAVLs expression during NeuroD1-mediated reprogramming, and miR-375-3p overexpression promoted cell survival without interfering neuronal reprogramming process. Interestingly, overexpression of miR-375-3p-refractory ELAVL4 induced cell death in human astrocytes and abolished the cell survival-promoting effect of miR-375-3p during reprogramming. Therefore, we propose that miR-375-3p modulates the level of upregulated nELAVLs expression during NeuroD1-mediated neuronal reprogramming, and that miR-375-3p overexpression increases NeuroD1-mediated reprogramming efficiency by reducing cell death.

Disclosures: X. Chen: None. **I. Sokirniy:** None. **X. Wang:** None. **M. Jiang:** None. **N. Mseis-Jackson:** None. **C. Williams:** None. **N. Jiang:** None. **B. Puls:** None. **K. Mayes:** None. **Q. Du:** None. **Y. Shi:** None. **H. Li:** None.

Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH T32

Title: Rna-binding protein imp plays multiple roles in generating neural diversity

Authors: *J. MUNROE[1], C. DOE[2];
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Abstract: RNA-binding protein Imp plays multiple roles in generating neural diversity
Sensory perception and motor output can generate complex behaviors. Generating appropriate behavior requires a complex and diverse nervous system, which arises during development. Both *Drosophila* and primates have neural stem cells that generate intermediate neural progenitors (INPs) which expand neuronal diversity, called type II neuroblasts (T2NB) in fly and OSVZ progenitors in primates. In *Drosophila*, each successively born INP will generate a distinct family of neurons, but little is known about how INP/neuronal fates are diversified. One potential model is temporal patterning, where changes in gene expression in the T2NB over time lead to changes in INP/neuronal identity. One candidate temporal factor is the RNA-binding protein Imp, which forms a high-to-low protein gradient in T2NBs through development. During this Imp expression window, three different neuronal subtypes are generated consecutively as Imp levels decrease. These three subtypes all reside in the central complex, a brain structure important for celestial navigation. We hypothesize that distinct Imp levels specify the three distinct neural cell types. To test this, we used *Drosophila* genetics to knock-down Imp in T2NBs, eliminating its protein gradient, and assayed the three neuronal subtypes for altered cell numbers or axon/dendrite morphology. Surprisingly, Imp was not required in T2NBs to specify neuronal identity. Rather, loss of Imp resulted in a delay of T2NBs to exit from quiescence in early larval stages. Lastly, we found that Imp expression persists into post-mitotic neurons, and neuron-specific Imp knockdown led to abnormal neuronal morphology. We conclude that Imp has two distinct roles in brain development: first acting in T2NBs to promote exit from mitotic quiescence, and second acting in post-mitotic neurons to establish proper axon/dendrite morphology.

Disclosures: **J. Munroe:** None. **C. Doe:** None.

Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.03

Topic: A.03. Stem Cells and Reprogramming

Support: CIRM TRAN1-08525

Title: Developing Potential Therapy for Canavan Disease Using Human Induced Pluripotent Stem Cells

Authors: *N. PRAKASH[1], L. FENG[2], J. CHAO[2], Y. SHI[2];
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Abstract: Objective: To develop a potential cell-based therapy for Canavan disease (CD) using human induced pluripotent stem cells (iPSCs).
Background: Canavan disease is a fatal leukodystrophy caused by mutation of the aspartoacylase (ASPA) gene, which leads to deficiency in ASPA activity, accumulation of the substrate N-acetyl-L-aspartate (NAA), demyelination, and spongy degeneration of the brain. There is neither a cure nor a standard treatment for this disease.
Design/Methods: In this study, we established human iPSC-based cell therapeutic candidates for CD. We first established Good manufacturing process (GMP)-compatible methods for human iPSC derivation, expansion, and differentiation. We then generated iPSCs from CD patient fibroblast cells and differentiated these iPSCs into iNPCs. To reconstitute ASPA activity which is deficient in both CD patients and mouse models, we developed ASPA iNPCs by introducing a functional ASPA gene through lentiviral transduction. We then transplanted the ASPA iNPCs into Nur7 mouse brains (CD mouse model).
Results: Compared to control Nur7 mice, ASPA iNPC-transplanted Nur7 mice showed statistically significant: 1. Elevated ASPA activity, 2. Reduced NAA levels, 3. Reduced brain vacuolization, 4. Improved myelination, 5. Improved rotarod performance, 6. Improved grip strength, and 7. Improved survival. There were no signs of tumorigenicity. These effects were present at 3 months and sustained at least 6 months after transplantation.
Conclusions: iNPCs derived from CD patient iPSCs that were transduced with a functional ASPA gene are able to ameliorate disease phenotypes (biochemical, cellular, and functional) in a CD mouse model . The therapeutic effect is long-lasting, showing no diminishing effect 3 and 6 months post-transplantation.

Disclosures: **N. Prakash:** A. Employment/Salary (full or part-time):; City of Hope Medical Group. **L. Feng:** A. Employment/Salary (full or part-time):; City of Hope. **J. Chao:** A. Employment/Salary (full or part-time):; City of Hope. **Y. Shi:** A. Employment/Salary (full or part-time):; City of Hope. 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.; CIRM TRAN1-08525.

Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.04

Topic: A.03. Stem Cells and Reprogramming

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NRF-2019R1A2C2006740

Title: Maternal exposure to polystyrene nanoplastics causes brain abnormalities in progeny

Authors: *B. JEONG[1], J. BAEK[1], J. KOO[1], S. PARK[1], Y.-K. RYU[2], K.-S. KIM[2], S. ZHANG[3], C. CHUNG[3], R. DOGAN[4], H.-S. CHOI[4], D. UM[4], T.-K. KIM[4], W. LEE[5], J. JEONG[5], W.-H. SHIN[7], J.-R. LEE[6], N.-S. KIM[6], D. LEE[6];
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Abstract: As global plastic production continues to grow, microplastics released from a massive quantity of plastic wastes have become a critical environmental concern. These microplastic particles are found in a wide range of living organisms in a diverse array of ecosystems. In this study, we investigated the biological effects of polystyrene nanoplastic (PSNP) on development of the central nervous system using cultured neural stem cells (NSCs) and mice exposed to PSNP during developmental stages. Our study demonstrates that maternal administration of PSNP during gestation and lactating periods altered the functioning of NSCs, neural cell compositions, and brain histology in progeny. Similarly, PSNP-induced molecular and functional defects were also observed in cultured NSCs in vitro. Finally, we show that the abnormal brain development caused by exposure to high concentrations of PSNP results in neurophysiological and cognitive deficits in a gender-specific manner. Our data demonstrate the possibility that exposure to high amounts of PSNP may increase the risk of neurodevelopmental defects.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: A.03. Stem Cells and Reprogramming

Support: Wings for Life
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Title: High Density Neural Stem Cell Plating Biases Differentiation Along a Neuronal Lineage and Alters NOTCH/Wnt Signaling

Authors: *W. B. MCINTYRE[1,2], N. HEJRATI[2], Z. LOU[1,2], M. KARIMZADEH3[3], Y. RIAZALHOSSEINI[3], M. KHAZAEI[2], M. FEHLINGS[2,4];
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Abstract: RATIONALE: Transplantation of Neural Stem Cells (NSCs) is a promising regenerative strategy to promote neural repair following spinal cord injury (SCI) because NSCs can replace the cellular niche lost due to trauma. However, large-scale manufacturing of induced pluripotent stem cell (iPSC) lines can produce variability in cultured cells. Plating density is an amendable parameter during mass manufacturing of cell lines, which can regulate the survival, proliferation, differentiation, and fate choice of stem cells. **OBJECTIVE**: To assess transcriptomic and fate choice discrepancies of transplanted, human (h)iPSC-NSCs plated at high or low seeding densities. **METHODS**: hiPSC-NSCs were expanded *in vitro* at either a low ($5x10^4$ cells/ml) or high ($2.5x10^5$ cells/ml) plating density for 7 days. Subsequently, hiPSC-NSCs were **(1)** characterized for their ability to differentiate into neurons, astrocytes, and oligodendrocytes *in vitro*; **(2)** transplanted into an immunodeficient (RNU Nude Rat) Cervical 6/7 contusion model of SCI; **(3)** and isolated for RNA-sequencing after *in vitro* culture and *in vivo* transplantation. **RESULTS:** Compared to a lower hiPSC-NSC culturing density, a higher density promoted differentiation towards a more neuronal fate (High: 45%; Low: 23% Neurons/total cells), and less mature astrocytes (High: 30%; Low: 47% Astrocytes/total cells). Following Gene Ontology (GO) transcriptomic analyses, enriched genes involving Neurogenesis, *NOTCH*, and *Wnt* signaling were differentially expressed. Modulation of these pathways are highly associated with the regulation of pro-neuronal transcription factors, which were upregulated in response to higher-density hiPSC-NSC culturing. Enhanced *NOTCH/Wnt* signaling was maintained in transplanted hiPSC-NSCs that were cultured at a higher density, which enhanced neuron and synaptic development-related biological pathways. **CONCLUSIONS:** Culturing hiPSC-NSCs at a higher density may bias their differentiation fate towards a neuronal lineage. Moreover, this study highlights the importance of precise control of cell culture density in the development of hiPSC-NSC transplantation therapies. Ideally, consistent culturing techniques during the manufacturing stages of hiPSC-NSC culture can promote more robust cell therapeutics for SCI.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

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Program #/Poster #: 265.06

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01NS119472

Title: The role of human brain organoid age in determining transplantation outcomes in the rat motor cortex

Authors: R. BLUE[1], **M. FU**[2], P. HARARY[1], *D. JGAMADZE[1], I. RAHAMAN[1], S. SINGH[1], M. DEDHIA[1], G.-L. MING[1], H. SONG[1], H.-C. I. CHEN[1];
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Abstract: Human brain organoids have potential to be used as a substrate for brain repair following injury, however the factors affecting survival and integration of transplanted organoids are unknown. In this study, we characterize the survival and growth of organoids of different ages by volume, presence of neural progenitors, and angiogenesis. Human forebrain organoids at two ages (d20-25, d55-60) were transplanted into the motor cortex of adult rats immediately following an aspiration injury. The first cohort was survived for 1 month post transplantation (MPT, n=6); the second cohort was survived for 2 MPT (n=16). Axon projections from the organoids were seen in the adjacent host cortex at both timepoints. At the time of perfusion, transplanted organoids of both age groups contained neural progenitors that were positive for Sox2, Pax6, and nestin. Additionally, there was evidence of ingrowth of blood vessels from the host (CD31+/GFP- cells) within the organoid grafts. Survival of organoids post-transplantation was >90% overall, with 11/11 (100%) of d20-25 aged organoids surviving and 9/11 (82%) of d55-60 aged organoids surviving. Pre-transplantation organoid size at d20-25 was significantly smaller than d55-60 ($p<0.0001$). However, average organoid volume at 2 MPT was significantly increased from pre-transplant volume in only d20-25 organoids ($p=0.0156$) and not d55-60 organoids ($p=0.1518$). Younger organoids also trended toward having a larger end-volume, with the mean d20-25 volume being 3.1x larger than d55-60 at 1MPT ($p=0.3211$) and 1.74x larger at 2MPT ($p=0.5090$). Older organoids, however, had less deviation from the pial surface than did younger organoids at 2 MPT, with d55-60 having less overgrowth of the aspiration cavity than d20-25, though the difference is not statistically significant ($p=0.1288$). These data suggest that while younger organoids have a greater rate of growth than do older organoids, older organoids may have a more predictable end-volume with less deviation from the pial surface at 2MPT, and thus more potential for application in clinical use. Though further investigation is needed to clarify the influence of organoid maturity on integration, the survival rate and degree of post-transplant growth provide initial insight into this relationship.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant NS110586
The Rudi Schulte Research Institute

Title: Ventricular-subventricular zone alterations in a mouse model of post-infectious hydrocephalus

Authors: *J. HERMAN[1], S. KADIAN[1], A. MEZGER[1], M. MARTLAND[1], Y. WANG[2], F. MANDINO[3], E. LAKE[3], P. VERARDI[2], J. C. CONOVER[1];
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Abstract: Here, we explore stem cell mechanics and cytoarchitectural changes in the ventricular-subventricular zone (V-SVZ) following viral exposure in a mouse model of hydrocephalus. Congenital hydrocephalus, a common birth defect affecting 1 in 770 infants, is characterized by expansion of the cerebroventricular system. One common cause of this disorder is infection, resulting in post-infectious hydrocephalus (PIH). PIH can occur following maternal infection during pregnancy, resulting in viral and inflammatory exposure to the developing fetal and neonatal brain. Our group is interested in the effects of PIH on the V-SVZ, an active stem cell niche present throughout embryonic development and retained exclusively along the lateral wall of the lateral ventricles postnatally. V-SVZ stem cells populate the developing brain with newly generated neurons and glial cells, including multi-ciliated ependymal cells, generated through the process of ependymogenesis. In the neonate, a monolayer of ependymal cells lines the ventricles providing a protective barrier and fluid/solute exchange system. Ependymal cells also provide laminar flow of cerebrospinal fluid at the ventricle surface via their apically-oriented motile cilia.We have developed a novel mouse model of PIH that is based on intracerebroventricular injection of influenza or a non-virulent component of influenza (neuraminidase) at two specific timepoints: embryonic day 16 (prior to the development of the ependyma) and postnatal day 4 (following completion of the ependymal monolayer). Heat-inactivated influenza and saline were used as controls. Influenza was found by immunohistochemistry to target ependymal cells. For both the postnatal and embryonic timepoints, PIH was observed in approximately 60% of mice injected with either neuraminidase or influenza. This was confirmed by serial section reconstructions of the lateral ventricles or by MRI (11.7T small animal) at post-natal day 30. Based on MRI acquisitions, regions of lateral ventricle expansion showed ependymal denudation and accompanying astrogliosis. Additionally, olfactory bulb neurogenesis was assessed to determine the chronic effects on V-SVZ stem cell output. Our PIH model also provides a means to assess inflammatory response and ultimately the reparative capacity of the V-SVZ stem cell niche at different developmental time points.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

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Topic: A.03. Stem Cells and Reprogramming

Support: DST SERB Grant CRG/2021/005847

Title: Differential Hes-1 promoter activation in regulating stemness in neocortical development

Authors: ***J. JAMES**[1,2,3], P. A. RIYA[1,2], B. BASU[1,3], V. MEERA[1,2], S. SURYA[1,2];
[1]Rajiv Gandhi Ctr. For Biotech., Trivandrum, India; [2]The Univ. of Kerala, Thiruvananthapuram, India; [3]Regional Ctr. for Biotech., Faridabad, India

Abstract: We have demonstrated the existence of a new subclass of NIHes-1 expressing neural stem cells and NDHes-1 expressing neural progenitors/RGCs in the developing neocortex. The new subclass of NIHes-1 expressing neural stem cells are quite unique since they are not dependent on cell-cell interaction as observed with NDHes-1 expressing RGCs. Therefore, it is important to completely characterize this unique subpopulation of NIHes-1 expressing neural stem cells which will have profound implications in terms of maintenance/expansion of neural stem cells in the ventricular zone of developing neocortex. We asked the following question. A) What is the role of NIHes-1 and NDHes-1 expressing cells in the developing neocortex? To address these questions we have custom generated a new conditional Knockout mice model with floxed NIHes-1 promoter region (NIHes-1f/f) from Cyagen Biosciences Inc. We have generated a Nestin CreERT2: NIHes-1f/f conditional knockout mouse model for knocking out non-canonical Hes-1 promoter region (NIHes-1) in Nestin+ve neural stem cells. This will be the most appropriate tool to understand the functional significance of this newly identified subclass of NIHes-1 expressing neural stem cells in developing neocortex. We injected tamoxifen at E14 which is known as the peak of neurogenesis and collected the neocortex at E18 for all our experiments. From immunocytochemical analysis and transcriptomic analysis we found that upon knocking out NIHes-1 expression a shift from non-canonical Notch activation to canonical Notch activation occurred resulting in premature differentiation. We could also find an upregulation of early maturation signature as well as the reduction of a pool of dividing primitive neural stem cell population upon knocking out NIHes-1 expression. Thus, we can conclude that NIHes-1 promoter activation at the embryonic stages maintains the cycling neural stem cell population and in the absence of NIHes-1, early maturation of the primitive stem cell pool is taking place.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

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Title: Rejuvenation of neural stem cell function and cognition through single cell pharmacotranscriptomics with ROOT

Authors: *L. PENG[1,2,3], M. M. BAY[1,4,5], A. B. SCHROER[9], A. IBRAYEVA[1,2,6], N. ZHANG[1,2], L. SUMROW[1,2], J. TSOI[7,2], J. WU[1,2], A. HIDALGO[1,2], J. LEVI[1,2,6], W. XIANG[1,2,6], S. A. VILLEDA[10,9,11,12], M. A. BONAGUIDI[1,2,6,8];
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Abstract: Cognitive impairment increases with age and worsens in dementia. Regenerative medicine offers the unrealized potential to treat cognitive impairment. However, endogenous neural stem cell (NSC) function declines with age of unclear mechanisms. Here, we developed a single cell computational drug screening technology to reinstate NSC activity, neurogenesis, and cognition in older mice by mimicking younger NSC programs. “Revealing Origins and Ontological Targets (ROOT)” reconstructed single cell transcriptomes into cell-lineage pseudotimes and identified transcriptomic signatures (TS) that define age-related NSC activity loss. ROOT then screened 1309 clinically relevant small molecules for their ability to recapitulate the TS gene networks mediating NSC activity. Three of the top 5 TS-mimicking compounds increased NSC activation when administered to middle-aged mice. Among them, SS sustained NSC proliferation, expanded the NSC pool, increased neurogenesis, and improved cognitive function. We therefore demonstrate the utility of a new single cell pharmacotranscriptomics platform by revealing unexpected neural stem cell capacity for

regenerative medicine. The identified compound SS represents a lead target toward treatment of cognitive impairment.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

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Title: Exercise slows neural stem cell aging and AD risk with clonal selection

Authors: *W. XIANG[1], L. PENG[2], M. M. BAY[3], M. LIU[4], T. DING[4], J. WU[4], J. TSOI[4], L. SUMROW[4], J. LEVI[1], M. MARING[4], N. ZHANG[4], T. REINISTO[4], A. IBRAYEVA[6], L. EKMAN[4], K. HAMANE[4], M. A. BONAGUIDI[5];
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Abstract: Exercise acts as a countermeasure against aging and Alzheimer's disease (AD). Several lines of evidence indicate that the beneficial effects of exercise target neurogenesis and synaptic plasticity. However, how exercise shapes neural stem cell (NSC) function to maintain lifelong hippocampal plasticity remains unknown. Here, we utilize single-cell technologies to uncover exercise slows NSC aging and reduces AD risk. *In vivo* single cell lineage tracing revealed that exercise promotes clonal selection by activating quiescent NSCs for self-renewal and depleting neurogenic NSCs. Interestingly, NSC symmetric self-renewal compensated for those lost to differentiation allowing for enhanced neurogenesis without prematurely depleting the overall NSC pool. Prolonged clonal tracing further showed that the selected self-renewed NSCs are more resistant to aging-related NSC depletion. Single cell RNA-sequencing and multiple bioinformatic analyses determined that exercise slows NSC molecular aging. In particular, exercise mitigates an age-associated increase of AD risk gene expression within NSCs. Our results demonstrate a new regenerative function of exercise to promote healthier brain aging.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

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Program #/Poster #: 265.11

Topic: A.03. Stem Cells and Reprogramming

Support: CIHR

Title: Transplantation of inducible oligodendrogenic neural progenitor cells promotes neuroregeneration after cervical spinal cord injury

Authors: *K. PIECZONKA[1], K. YOKOTA[3], J. WANG[3], N. HEJRATI[3], J. HONG[3], M. KHAZAEI[3], M. G. FEHLINGS[2];
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Abstract: Spinal cord injury (SCI) results in the loss of myelinating oligodendrocytes, ultimately contributing to impaired neural communication. Neural progenitor cell (NPC) transplantation is an attractive approach to replace the neural cells that have been lost following SCI and to promote beneficial trophic effects. However, the injury microenvironment predominantly directs NPCs to differentiate into scar-forming astrocytes, at the expense of oligodendrocytes and neurons. In order to promote oligodendrocyte differentiation, we aimed to generate inducible oligodendrogenic NPCs (ioNPCs) and to characterise the neuroregenerative role of the cells in cervical SCI. Human ioNPCs were prepared by engineering iPSC-NPCs to express Olig2 under the control of the conditional doxycycline-inducible Tet-ON promoter. The cells were then treated with doxycycline in order to identify the optimal doxycycline treatment timeline. To characterise the cells in vitro, RT-qPCR and immunocytochemistry were used. For in vivo assessments, a cervical SCI was induced in RNU immunodeficient rats followed by transplantation with either ioNPCs or vehicle. Behavioural recovery was monitored weekly for fifteen weeks post SCI. Following sacrifice, histological methods were used to determine ioNPC differentiation, remyelination and tissue preservation. Additionally, bulk RNA sequencing was performed in order to assess the transcriptomic hallmarks of ioNPC transplantation. We found that several genes involved in oligodendroglial lineage determination were upregulated in the ioNPCs compared to the unbiased NPCs that were not treated with doxycycline in vitro. These genes included OLIG1, OLIG2, PDGFRA and NKX6.1. Immunostaining showed that the ioNPCs gave rise to an increased proportion of O1+ oligodendrocytes than the unbiased NPCs in vitro. Enhanced oligodendroglial differentiation was also observed in vivo. Additionally, immunohistochemical analyses demonstrated that ioNPC transplantation contributed to MBP+

remyelination and that tissue integrity was improved in the ioNPC group. During the transcriptomic analyses, it was found that several pathways were differentially expressed in the ioNPC transplantation group. Importantly, ioNPC transplantation correlated with significantly better grip strength compared to vehicle ($p < 0.05$). Overall, this work suggests that ioNPCs can promote remyelination and several other neuroregenerative effects which correlate with functional recovery post SCI.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.12

Topic: A.03. Stem Cells and Reprogramming

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Title: Subventricular zone neural stem/precursor cell-microglia cross-communication after stroke

Authors: *S. NATH[1], J. CHOI[3], P. CONFORTI[2], R. SANKOWSKI[5], L. AMANN[2], Y.-H. CHU[2], C. GALANIS[2], S. DESHPANDE[2], A. VLACHOS[6], M. PRINZ[2], J. K. LEE[4], C. SCHACHTRUP[2];
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Abstract: Adult neural stem/precursor cells (NSPCs) of the subventricular zone (SVZ) serve as a reservoir for brain plasticity and repair, and microglia, key innate immune cells, are integral components of the SVZ stem cell niche. To identify microglial states governing NSPC behavior, we performed single-cell RNA sequencing and cross-communication analysis of NSPCs and microglia after cortical photothrombotic ischemia (PT) in mice. Here, we show a stroke-induced cell-cycle arrest, followed by cell death of NSPCs during their neuronal lineage progression within the SVZ. We identified a discrete stroke-associated microglial state that phagocytoses apoptotic NSPCs within the stem cell niche. Furthermore, pharmacologic depletion of microglia increased NSPC survival and formation of doublecortin-expressing neuroblasts within the SVZ. Finally, we developed a repository of interacting ligand-receptor pairs potentially driving novel

facets of NSPCs and microglia cross-communication after stroke. Thus, we propose that SVZ niche stroke-associated microglia prevent the SVZ neurogenic response after stroke.

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Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.13

Topic: A.03. Stem Cells and Reprogramming

Title: Neural crest stem cells are the source of directly reprogrammed neurons in the murine skin

Authors: *J. J. BELAIR-HICKEY, A. FAHMY, R. SAJIID, B. TAKABE, D. VAN DER KOOY;
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Abstract: Direct reprogramming involves converting one differentiated cell type immediately into another through overexpression of transcription factors that specify cell fate. Unlike other types of reprogramming, where mature post-mitotic cells are brought back to a proliferative stem cell state, direct reprogramming is thought to occur between two terminally differentiated cell types. In many examples of direct reprogramming questions regarding the heterogeneity and biases of the starting cell population remain largely unexplored. This heterogeneity may arise both at the level of embryonic developmental lineage as well as the maturity of cells. Our study investigates heterogeneity in the often-used paradigm of directly reprogramming neurons from cells in the murine skin. The data shown here indicate lineage and cell type heterogeneity in the starting skin cell population and favor an elite model of direct reprogramming. In this model a subset of cells in the skin cultures have an intrinsic and enhanced ability to turn into neurons when being forced to express the neuron fate specifying transcription factors Brn2, Ascl1, and Myt1l. Krt15-Cre; TdTomato mice mark epidermal lineage cells. When skin from these mice was neuronally reprogrammed none of the resulting neurons were of an epidermal lineage. When skin cells are neuronally reprogrammed from a Wnt1-Cre; TdTomato mouse that marks the neural crest (NC) lineage all the resulting neurons are derived from the NC. Furthermore, when NC and non-NC cells of the skin are purified using FACS, approximately 95% of the reprogramming occurs in the NC purified population. These data indicate a cell-autonomous bias for NC cells in the skin to be directly reprogrammed to neurons. Within the NC compartment in the skin there are differing levels of cellular maturity including a population of NC stem and progenitor cells marked by expression of Sox2. When these NC stem cells are killed off *in vitro* or *in vivo* using a Sox2-Cre; ROSA-DTA mouse, approximately 70% and 80% of neuron reprogramming was eliminated, respectively. Overall, these results support a model where

directed differentiation of a neural crest stem cell population explains most of what was previously thought to be direct neuronal reprogramming from a non-neural cell type in the skin.

Disclosures: J.J. Belair-Hickey: None. **A. Fahmy:** None. **R. Sajiid:** None. **B. Takabe:** None. **D. van der Kooy:** None.

Poster

265. Neural Stem Cells: *In Vivo* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 265.14

Topic: A.03. Stem Cells and Reprogramming

Support: NIH NS100514

Title: Sequential direct reprogramming of human NG2 glia to neurons using a novel switch expression system

Authors: *A. BARAL, E. REISENBIGLER, M. MARCINIAK, R. A. MARR, D. A. PETERSON;
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Abstract: Reprogramming cell fate has vast potential to provide a deeper understanding of brain development and to be harnessed for therapeutic use. As neurons are not added to the adult brain, apart from some spatially-restricted regions, reprogramming glia into neurons provides a novel option to replenish a population of neurons lost to disease or injury. However, current approaches to cellular reprogramming using continuous forced single or multiple instructive genes do not allow control of the orchestrated sequence of instructive signals seen in normal development. This can result in low efficiency of cellular reprogramming with a substantial loss of cells following initial induction. To address this limitation, we have engineered a regulatable gene expression system to control temporal expression of sequential genes instructive for survival and subtype specification of neurons induced from NG2 glia. This novel switch expression system for reprogramming is an innovative approach that is able to more closely mimic in vivo lineage specification by permitting control of the timing of lineage specification. We targeted in-vitro a mitotically active population of human-derived NG2 glia also known as oligodendrocyte progenitor cells (OPCs) to reprogram into neurons using retroviral gene delivery. To achieve neuronal induction, we express the developmental pioneering factor neurogenin-2 (Ngn2) with a fluorescent reporter. The Ngn2-GFP construct is expressed in the first position of a dual construct tamoxifen inducible CRE recombinase system with Bcl2-DsRed in the second. Addition of tamoxifen leads to an excision of the first construct and expression of the second gene set. Here, we tested the capacity of human-derived OPCs to reprogram and the use of the switch design to induce a second cell survival gene set. Forced expression of Ngn2 successfully induced a neuronal phenotype. Different durations of expression were evaluated. Limiting the duration of inductive signals to 3 days post infection appeared optimal for neuronal

reprogramming. The switch also enabled the expression of the second gene set expressing Bcl2 as an anti-apoptotic factor. Ongoing quantitative analysis indicates that this switch from pro-neuronal geneset to a pro-survival gene set may lead to an increase in reprogrammed cell survival. Ability to direct cell fate offers a powerful tool to enable regulation of sequential gene expression that could be applied to many applications including increased cell survival and neuronal subtype specification.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

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Program #/Poster #: 266.01

Topic: A.03. Stem Cells and Reprogramming

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Title: Constitutive activation of canonical Wnt signaling disrupts choroid plexus epithelial fate

Authors: *A. PARICHHA[1], V. SURESH[1], M. CHATTERJEE[2], A. KSHIRSAGAR[3], L. REUVEN[3], T. OLENDER[3], S. TRNSKI[4], M. RASONJA[4], V. RADOSEVIC[4], M. TAKETO[5], M. HOLTZMAN[6], N. MILOSEVIC[4], O. REINER[3], S. TOLE[1];
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Abstract: The choroid plexus secretes cerebrospinal fluid and is critical for the development and function of the brain. In the telencephalon, the choroid plexus epithelium arises from the Wnt-expressing cortical hem. Canonical Wnt signaling pathway molecule such as nuclear β-CATENIN is expressed in the mouse and human embryonic choroid plexus epithelium indicating that this pathway is active. Point mutations in human β-CATENIN are known to result in the constitutive activation of canonical Wnt signaling. In a mouse model that recapitulates this perturbation, we report a loss of choroid plexus epithelial identity and an apparent transformation

of this tissue to a neuronal identity. Aspects of this phenomenon are recapitulated in human embryonic stem cell derived organoids. The choroid plexus is also disrupted when β-Catenin is conditionally inactivated. Together, our results indicate that canonical Wnt signaling is required in a precise and regulated manner for normal choroid plexus development in the mammalian brain.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

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Program #/Poster #: 266.02

Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01NS117918
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NIH Grant R21NS119732

Title: Two-photon imaging analysis of in vivo neuronal reprogramming in the mouse cortex

Authors: *K. MAYES, J. SWORD, S. KIROV, H. LI;
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Abstract: Injury to the Central Nervous System (CNS) can lead to reactive astrocytes forming a glial scar around the injury site. In vivo reprogramming is an emerging field of regenerative medicine for neural repair where, through the overexpression of neuronal transcription factors such as NeuroD1 (ND1), endogenous glial cells can reprogram into functioning neurons. To achieve this reprograming, we injected ND1 plasmids with a CAG promotor packaged into a retrovirus into the cortex of wild-type adult mice. In order to observe the reprogramming process in vivo, we performed craniotomies and placed windows through which two-photon microscopy could be utilized to image the infected cells labeled with GFP. This imaging was used to assess morphological differences between ND1 injected mice and those injected with a GFP control vector. Cell morphology and characteristics related to the amount of primary branching, overall cell shape, symmetry, layout, and number of processes were assessed based on the two-photon imaging to characterize infected cells as neuron-like, such as those found post injection in the ND1 group, or whether they maintained glial-like cell morphology and characteristics, which we saw in the GFP control group. ND1-injected mice also showed less proliferating cell pairs compared to the GFP control, which supports the fact that the ND1-infected cells have reprogrammed into mature neurons, and the GFP control cells remain glial cells. These imaging techniques can help us to further characterize these morphological changes in vivo, as well as

answer questions of cell migration and functional integration during the neuronal reprograming process.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

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Topic: A.03. Stem Cells and Reprogramming

Support: NIH Grant R01NS117918
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Title: Manipulating NeuroD1 expression by microRNA-124 to optimize neuronal conversion for spinal cord injury repair

Authors: *N. MSEIS-JACKSON[1], X. CHEN[1], M. JIANG[1], C. WILLIAMS[2], H. LI[1];
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Abstract: The mammalian spinal cord has a remarkable and complex function, yet a limited capacity for regeneration after injury. A damaged spinal cord is incapable of full regeneration, leaving patients with few suitable options for restoring lost neurons which leads to the deterioration of sensory and motor functions. *In vivo* reprogramming technology offers a potential solution for replenishing lost neurons to treat spinal cord injuries. Previous work in our lab has shown a 90% efficiency in converting astrocytes to neurons by using a transcription factor, NeuroD1 (ND1). However, ND1-converted neurons have been shown to be mainly glutamatergic neuronal subtypes. We believe that a high expression level of ND1 drives astrocytes to one specific neuronal subtype. This has led us to design a new construct (ND1-124b) that integrates ND1 expression with a microRNA-124 (miR-124) binding site in our *in vivo* reprogramming system. miR-124, a neuron-specific microRNA, is not expressed in astrocytes but is upregulated during neuronal reprogramming. Presumably, this construct still achieves a high ND1 expression level in astrocytes to initiate proper neuronal reprogramming. The inhibition of ND1 by endogenous miR-124 at a certain point during the reprogramming process will lead to a lower level of ND1 and is expected to generate diversified neuronal subtypes. Our data in human astrocyte cultures using imminoflouracence (IF) and western blot suggest that combining ND1 and a miR-124 binding site reduces ND1 expression levels while still converting astrocytes to neurons with an 80% efficiency. QRT-PCR analyses have verified the specificity of miR-124 expression upon ND1 induction among HeLa, astrocytes vs. converted-astrocytes to neuron cells. Neuronal subtypes generated by the ND1-124b construct are investigated in human astrocyte cultures. In addition, we are determining the efficiency of the

ND1-124b construct in converting reactive astrocytes to neurons and their neuronal subtypes in the mouse injured spinal cord. Our goal is to characterize this new construct in detail in its reprogramming capability and develop it into a treatment for spinal cord injury with optimized outcomes.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

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Program #/Poster #: 266.04

Topic: A.03. Stem Cells and Reprogramming

Support: Wellcome Trust 206410/Z/17/Z
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Title: Gradual loss of glial specific expression of AAV transgenes in vivo: implications for glia-to-neuron reprogramming.

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Abstract: Recent publications have reported efficient astrocyte-to-neuron lineage conversion in the adult mammalian brain using AAVs with specificity of glial expression conferred by using glial fibrillary acidic protein (Gfap) regulatory elements (Mattugini et al., 2019; Qian et al., 2020; Wu et al., 2020). Induced neurons in these studies display remarkable fidelity to mature endogenous neurons. However, the interpretation of these data is tightly bound to assumptions about the specificity of genetic tools used to restrict AAV transgene expression to astrocytes (e.g. Cre-Lox system in mGfap-Cre transgenic mice).
Given the scientific and potential translational importance of these works, we tested these assumptions by injecting two Cre-dependent AAVs (AAV5-CAG-FLEx-tdTomato, AAV5-CAG-FLEx-Bcl2-T2A-GFP) into the cortex of adult mGfap-Cre transgenic mice. We find that over time, these AAVs label an increasing proportion of cortical neurons. At 2 weeks post-injection (wpi), fewer than 20% of fluorescent cells are neurons. However, this rises steeply between 4 and 8wpi and by 12wpi more than half of cells expressing the fluorescent reporter transgenes were neurons. Driving AAV transgene expression from an hGfap truncated promoter within the viral genome (AAV5-hGfap-dTomato::dlox-GFP(rev)-dlox) also shows a substantial proportion of neurons labelled, which increases over the two months after viral injection into mouse cortex (from less than 10% to 30% neurons).

We conclude that AAVs utilising these common genetic strategies to confer astrocyte specificity are highly prone to labelling endogenous neurons. Particularly troublingly for their use in glia-to-neuron reprogramming experiments, the proportion of neurons expressing viral reporter transgenes tends to increase over time in the absence of any reprogramming factors. Without implementation of robust methods to distinguish between reprogrammed and endogenous neurons (Mattugini et al., 2019; Wang et al., 2021), this would directly confound interpretation of glia-to-neuron reprogramming experiments. Furthermore, since Cre-loxP system and AAVs are commonplace molecular tools outside of lineage reprogramming field, better understanding these dynamics is crucial for studying glial biology in vivo.

Mattugini, et al, 2019. Neuron 103, 1086-1095 e1085.Qian, et al., 2020. Nature 582, 550-556.Wang et al, 2021. Cell.Wu et al., 2020. Nat Commun 11, 1105.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

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Topic: A.03. Stem Cells and Reprogramming

Support: FETPROACT-2018-2020 HEREMES [grant number 824164]
Fondazione Telethon–Italy (Grant Number GSP20004_PAsMCT8006)

Title: Generation of hippocampal organoids: a developmental study

Authors: *F. CIARPELLA[1], R. ZAMFIR[1], A. CAMPANELLI[1], E. REN[2], G. PEDROTTI[1], E. BOTTANI[1], A. BORIOLI[3], D. CARON[4], M. DI CHIO[1], S. DOLCI[1], A. AHTIAINEN[5], N. PIAZZA[1], G. MALPELI[1], G. MALERBA[1], R. BARDONI[2], G. F. FUMAGALLI[1], J. A. HYTTINEN[5], F. BIFARI[6], G. PALAZZOLO[4], G. PANUCCIO[4], I. DECIMO[1];
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Abstract: Brain organoids are *in vitro* three-dimensional (3D) self-organized neural structures, which have been used for several applications, including the study and mimicking of brain development, the modeling of neuronal disorders and their employment in preclinical drug screening tests. Beside the use of human induced pluripotent stem cells (hiPSCs), mouse embryonic stem cells can be used to generate reproducible and standardized brain organoids in an efficient time window. We adopted multipotent neural stem cells (NSCs) isolated from embryonic subgranular zone (SGZ) to generated mature murine brain organoid in 5 weeks. By optimizing a three-phase protocol, consisting of an expansion (day 1-4), induction (day 5-14) and differentiation (day 14-36) step, we were able to mimic the stages of *in vivo* neuronal development. Strictly, by adding low concentration of the morphogen Wnt3a during the early

phases of the protocol, we specifically induced the CA3 hippocampal phenotype in mouse brain organoids. We investigated the specific hippocampal phenotype both via RT-PCR and immunofluorescence. RT-PCR analyses showed an high expression of hippocampal-related genes (e.g. Neurod1, Fzd9, Nectin3, Alk, Grik4, Sct) confirming the Wnt3a effectiveness in inducing the hippocampal patterning. In the mature organoids we found the expression of the pan-hippocampal (ZBTB20) and CA3 (Ka1) markers, hippocampal neurons ($ZBTB20^+/Map2^+$ and $Ka1^+/Map2^+$ cells) and a slight expression of the hippocampal CA1 (OCT6) and CA2 (FDZ9) markers. No expression of the dentate gyrus (DG) marker (PROX1) was found. Additionally, functional properties measured by calcium imaging (Fluo-4 AM dye analysis) revealed that organoids increased their spontaneous cellular activity during maturation, together with a progressive differentiation trough early, intermediate, and mature stages - defined by the expression of specific stemness (SOX2, Vimentin), neural progenitors (DCX) and mature neuronal (Tubb3, MAP2) markers. Overall, our results showed the establishment of murine 3D *in vitro* hippocampal model that may be a useful tool for high-throughput drug screening and disease modelling. The possibility to generate hippocampal organoids could be further exploited as an innovative tool for transplantation procedures and regenerative purposes.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

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Program #/Poster #: 266.06

Topic: A.03. Stem Cells and Reprogramming

Support: NIH grant 1R01DA053372-01A1

Title: Buprenorphine shows milder inhibitory effect on neuronal activity than methadone in human cortical organoid

Authors: *H. YAO[1], W. WU[1], J. WANG[1], G. HADDAD[1,2,3];
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Abstract: Medication assisted treatment (MAT) for opioid use disorder is an effective way to alleviate withdrawal in opioid users during pregnancy. However, medications used for the MAT, such as methadone (MET), have been implicated in the disrupted fetal brain development which can lead to neonatal abstinence syndrome in newborns and neurological deficits later in life. Since buprenorphine (BUP) has been shown to cause less severe neonatal abstinence syndrome than MET, we hypothesized that BUP has less injurious effect on neuronal activities in early

brain development. In this work, using cortical organoids generated from human induced pluripotent stem cells, we examined how MET and BUP affect the neuronal activities on a multielectrode array (MEA) system. Organoids cultured for 8 weeks were seeded in MEA plate and treated with MET or BUP for a week starting from the 11th week, an organoid age that simulates early to mid-gestation stages of human fetal brain development. Spontaneous activities were recorded before treatment (on the 11th week) and after one week treatment (on the 12th week) on a Maestro MEA machine (Axion Biosystems) and action potential firing rate (FR) was calculated and normalized to the same sample recorded prior each treatment. Our results show that, in the control group, the FR recorded on the 12th week increased by about 30% from baseline (129.8±15.3% of baseline, n=9). In the MET treated group, however, the FR decreased by about 93% from baseline (6.6±1.3% of baseline, n=9), which is significantly different from the control group ($p<0.001$). In the BUP treated group, the FR decreased by about 64% from baseline (35.4±6.2%, n=9), which is significantly different from control group ($p<0.05$). Further comparison between the two treatment groups revealed that the FR in the BUP treated group is significantly less suppressed than in the MET group (35.4±6.2% vs. 6.6±1.3%, $p<0.05$). In another experiment, we used antibodies to SYN1 and PSD-95, to examine if the synapse formation was reduced in the MAT medication-treated organoids. Indeed, we observed much less PSD-95 immuno reactivity in MET treated organoids than control and BUP treated organoids in the cryosections. In summary, both MET and BUP inhibit neuronal activities in human cortical organoids but BUP imposed less severe suppression on the neuronal activity, which may be due to the less severely disrupted synaptogenesis in organoids.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

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Program #/Poster #: 266.07

Topic: A.03. Stem Cells and Reprogramming

Support: FAPESP 2017/052423

Title: The effect of cannabidiol on the viability of neural progenitor cells

Authors: *S. ROMARIZ, M. L. QUINTELLA, K. R. SILVA, D. CANDIDO, B. G. DE MELO, M. PORCIONATTO, B. LONGO;
Univ. Federal de Sao Paulo, Univ. Federal de São Paulo, São Paulo, Brazil

Abstract: Cannabidiol (CBD) is the most abundant non-psychotropic cannabinoid found in *Cannabis Sativa*. Recent studies have demonstrated therapeutic effects in several diseases of the central nervous system, such as severe epilepsies in children. Despite the increasing use of CBD-based therapies in children, whose brains are still developing, few studies have evaluated the toxic effect of CBD on the developing brain. Neural progenitor cells (NPC) can be isolated and

cultured in two- and three-dimensional (2D and 3D), making both useful tools for studying the effects of CBD. Our goal was to investigate the toxic effect of CBD on NPC in 2D and 3D *in vitro*. The telencephalon region of rat embryos (E14.5) was dissected to obtain NPC. NPC were seeded in 48-well plates pre-coated with laminin and poly-L-lysine at 100.000 cells/ml (2D culture). For 3D culture, 1 x 10^3 of NPC were mixed with fibrinogen solution, 5% gelatin, 2.5% GelMA. The bioink was transferred to a syringe, which in turn was connected to the 3D bioprinter. The bioprint design consisted of a 6 x 6 x 4 mm grid, and the bioprinted scaffolds were transferred to a 24-well plate. Cell viability analysis was measured using the following technique: MTT, which directly reflects mitochondrial activity, Flow Cytometryto estimate the degree of apoptosis, and Live/Dead-Viability/Cytotoxicity in 3Dculture. The colony formation assay was performed to assess the proliferation of NPC in the presence of CBD. Cells were exposed to CBD at concentrations of 1µm, 5µm, and 10µm for 24h. All experiments were done in triplicate. According to the results obtained, there is a significant decrease in viable cells and a significant increase of cells in late apoptosis in the 5µm and 10µm CBD groups when compared to the CTRL and 1µM CBD groups (p&It0.01). MTT data showed that the presence of CBD at 5µM and 10µM concentrations produces a significant increase in cellular toxicity when compared to the CTRL and 1µM CBD groups (p&It0.5).The presence of CBD at 5µM and 10µM concentrations significantly reduced the proliferative capacity of NPC, observed by the reduction of the size and formation of new neurospheres when compared to the CTRL and 1µM groups (p&It0.01).The 1µm dose did not present significant differences compared to the control (p&gt0.5). Our data showed that CBD at higher concentrations, such as 5µM and 10µM, induces cell death by apoptosis and decreases cell viability and proliferative capacity of NPC. Therefore, it is possible that any exposure of high doses of CBD during stages in which cell division and proliferation are essential for a correct neurodevelopmental processes, might cause adverse effects for the developing central nervous system.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.08

Topic: A.03. Stem Cells and Reprogramming

Support: Korea National Research Foundation Grant 2017M3C7A1029610
Korea National Research Foundation Grant 2019R1A2C1087192
Korea Ministry of Health & Welfare Grant HI20C0253
Korea Ministry of Health & Welfare Grant HU21C0113

Title: Impaired migration of autologous induced neural stem cells from patients with schizophrenia and implications for genetic risk for psychosis

Authors: J. LEE[1], S. SONG[2], J. LEE[2], J. KANG[2], E. CHOE[3], T. LEE[1], M.-W. CHON[4], M. KIM[1], S. KIM[5], M.-S. CHUN[6], J. KWON[1], ***M.-S. CHANG**[2];
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Abstract: Stem cell technologies have presented explicit evidence of the neurodevelopmental hypothesis of schizophrenia. However, few studies investigated relevance of the schizophrenia genetic liability and the use of genetic reprogramming on pluripotent stem cells to the impaired neurodevelopment shown by stem cells. Therefore, this study sought to investigate the cellular phenotypes of induced neural stem cells (iNSCs) derived without genetic modification from patients with schizophrenia and from genetic high risk (GHR) individuals. Three patients with a diagnosis of schizophrenia, 3 GHR individuals who had two or more relatives with schizophrenia, and 3 healthy volunteers participated. iNSCs were derived using a small molecule-based lineage switch method, and their gene expression levels and migration capabilities were examined. Demographic characteristics were not different among the groups (age, $\chi^2 = 5.637$, $P = .060$; education, $\chi^2 = 2.111$, $P = .348$). All participants stayed well during the follow-up except one GHR individual who developed psychosis 1.5 years later. Migration capacity was impaired in iNSCs from patients with schizophrenia (SZ-iNSCs) compared to iNSCs from GHR individuals or controls ($P < .001$). iNSCs from a GHR individual who later developed schizophrenia showed migratory impairment that was similar to SZ-iNSCs. Gene expression levels of *Sox2* in SZ-iNSCs were significantly lower than those in controls ($P = .028$). Defective migration in genetically unmodified SZ-iNSCs is the first direct demonstration of neurodevelopmental abnormalities in schizophrenia. Additionally, alterations in gene expression in SZ-iNSCs suggest mechanisms by which genetic liability leads to aberrant neurodevelopment.

Disclosures: J. Lee: None. **S. Song:** None. **J. Lee:** None. **J. Kang:** None. **E. Choe:** None. **T. Lee:** None. **M. Chon:** None. **M. Kim:** None. **S. Kim:** None. **M. Chun:** None. **J. Kwon:** None. **M. Chang:** None.

Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.09

Topic: A.03. Stem Cells and Reprogramming

Support: KBRI basic research program through the Korea Brain Research Institute funded by the Ministry of Science and ICT (22-BR-01-03)
NRF (2022R1A2C1009376)

Title: The role of FASN-dependent lipogenesis related mitochondria using human derived-iPSCs and cerebral organoids during neurodevelopment

Authors: *S. NOH[1,2], J. MUN[1];
[1]Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of; [2]Daegu Gyeongbuk Inst. of Sci. and Techonology (DGIST), Daegu, Korea, Republic of

Abstract: Lipid synthesis produces various products such as fatty acids, phospholipids, glycolipids, sterols, and sphingolipids, which are important metabolic processes that constitute membranes and tissues during the development of the nervous system. Among the various pathways for lipid synthesis, de novo fatty acid synthesis (FAS) is a pathway that produces long-chain fatty acids, using the fatty acid synthase enzyme (FASN). FASN is involved in the regulation of neural stem cell activity. In a recent study, human FASN gene point mutation R1819W has been reported with intellectual disability and developmental epileptic encephalopathy. It indicated an association of FASN-dependent lipogenesis metabolism in human brain development and disease. Mitochondria are important to regulate cell proliferation, differentiation, and maturation precisely and sequentially through mitochondrial dynamics and metabolic shifts during embryonic and neurogenesis in stem cell biology. In a recent study, it is confirmed that mitochondrial dysfunction is induced by FASN-dependent lipogenesis inhibition in iPSCs. This indicates that mitochondria associated lipogenesis is essential for maintaining pluripotency and cellular activity of stem cells. Based on discovery that mitochondrial and lipid metabolism suggests the importance of stem cell function and developmental potential. However, it remains unclear how lipid and mitochondrial metabolism are related to neuronal and brain development. To link between FASN-dependent lipogenesis and mitochondria, we mainly focused on between mitochondria and lipid changes on neuronal and brain development in FASN-deficient conditions. Here, we proposed that will aim to examine: (1) how deficient FASN-dependent lipogenesis affects mitochondria and lipids in human neural stem cells and (2) how deficient-FASN affects brain development using cerebral organoids. First, we aim to study whether FASN-dependent lipid metabolism is related to mitochondria in NSCs. We used iPSC-derived NSCs to examine how FASN affects mitochondria and lipids. Next, we will examine how FASN affects the brain development and maturation process, we used human induced-cerebral organoids to examine cell proliferation and structural integrity according to developmental stage. Furthermore, it will help understand energy metabolism related neuronal disorders during embryonic brain development.

Disclosures: **S. Noh:** None. **J. Mun:** None.

Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.10

Topic: A.03. Stem Cells and Reprogramming

Support: R01DA044859

Title: Human stem-cell derived brain models from postmortem subjects

Authors: *E. F. MENDEZ, L. STERTZ, S. V. MOVVA, K. MORIEL, T. D. MEYER, G. R. FRIES, C. WALSS-BASS;
Louis A. Faillace, MD, Dept. of Psychiatry and Behavioral Sci., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Modeling brain disorders at the molecular level continues to be a challenge in the field. Human-derived induced pluripotent stem cell models of brain promise to address limitations of animal models, but how well these models accurately reflect normal brain cell function as well as deterioration in disease remains under question. In particular, certain neurological and psychiatric diseases as well as neurodevelopment itself are known to affect brain cellular ageing which can be measured by DNA methylation biomarkers known as the epigenetic clocks. Understanding the process of epigenetic ageing in human-derived cell models would greatly advance the field. We have engineered a novel induced pluripotent stem cell-derived model of neural progenitor cells and neurons from cultured postmortem human skin fibroblasts, which we directly compare to brain tissue from the donor source. These cell lines (n = 2 human subjects, 2 clones each), were validated for stem cell, neural progenitor cell, and neuronal markers. We assessed the maturity of these models across differentiation from stem cells to neurons using epigenetic clocks trained on adult (Horvath) and fetal (Steg) human tissue. Within each human subject postmortem frontal cortex epigenetic age parallels that of cultured skin fibroblasts; stem cell induction from fibroblasts cells effectively sets the epigenetic clock to an embryonic age; and differentiation of stem cells to neural progenitor cells and then to neurons progressively ages the cells. This study introduced an induced pluripotent stem cell model from postmortem human fibroblasts that can be directly compared to corresponding brain tissue. Differentiation of the stem cells into neurons effectively aged the cells via epigenetic readouts which can be used as a molecular biomarker for neurodevelopment and diseases of brain ageing.

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Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.11

Topic: A.03. Stem Cells and Reprogramming

Title: A 3D human model for preclinical drug screening using a myelinated nerve-on-a-chip micro-physiological system

Authors: *M. TERRAL[1], C. ROUNTREE[1], E. SCHMIDT[1], M. J. MOORE[1,2,3], J. L. CURLEY[1];

[1]AxoSim, Inc., New Orleans, LA; [2]Dept. of Biomed. Engin., [3]Brain Inst., Tulane Univ., New Orleans, LA

Abstract: Preclinical animal models are historically expensive and low-throughput and have largely failed to deliver results that translate to success in the human system. Peripheral nerves, in particular, lack predictive human-relevant *in vitro* drug screening models, with less than 7% of neurological drug candidates reaching the marketplace. Micro-physiological systems (MPS), including organs-on-chips, which utilize human induced pluripotent stem cell (iPSC)-derived cells to emulate specific organ systems, have emerged as promising screening platforms to bridge the gap between preclinical and clinical success. However, engineering 3D tissues relevant to the nervous system, especially peripheral nerves (PN), is challenging because of the complex ultrastructure and necessity of functional outputs, including Schwann cell myelination and electrophysiological measurements. AxoSim has developed an all-human NerveSim® MPS platform, using human iPSC-derived sensory neurons and primary human Schwann cells. This platform has been shown to exhibit crucial aspects of PN physiology and function, displaying robust neurite outgrowth, with 5% axonal myelination and measurable electrical activity, acting as a promising screening platform for improving pre-clinical success. The NerveSim® MPS is fabricated using a digital projection photolithography technique to produce a dual-hydrogel scaffold with an outer cell-restrictive polymer mold and a cell-permissive inner gel. Coculture spheroids were produced from human sensory neurons and Schwann cells in round-bottom multiwell plates, then plated in one end of the inner cell-permissive gel. Spheroids were cultured for 4 weeks in a series of culture media that promote neurite outgrowth through the gel, Schwann cell migration and alignment with axons, and ultimately, Schwann cell myelination. Robust neurite outgrowth of ~5mm was consistently observed, with Schwann cell migration and alignment along neuronal axons confirmed by S100 immunostaining. Schwann cell myelination (~5%) was confirmed with myelin basic protein (MBP) immunostaining, as well as by concentric lamination seen in ultrastructure electron microscopy (EM) imaging. We have successfully engineered a 3D human model of the myelinated peripheral nerve, which can provide clinically relevant and functional readouts. Current efforts are focused first on increasing the number of myelinated nerve fibers in the NerveSim® sample, and second, on quantifying the effects of well-known demyelinating compounds, such as Cuprizone, to further establish the efficacy of NerveSim® as a drug screening platform.

Disclosures: M. Terral: None. **C. Rountree:** None. **E. Schmidt:** None. **M.J. Moore:** None. **J.L. Curley:** None.

Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.12

Topic: A.03. Stem Cells and Reprogramming

Title: Understanding the role of glucose metabolism in cell fate specification events during cortical development

Authors: *J. MIL;
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Abstract: Neural stem cells (NSCs) give rise to fully differentiated mature neurons during the development of the human cortex, the outermost layer of the brain. During this process, there is a metabolic transition in NSCs from glycolysis to mitochondrial oxidative phosphorylation (OXPHOS) to accommodate for the changing metabolic demands that are essential for NSCs to activate and differentiate to a neuronal lineage. Although this metabolic feature is a key step during neurogenesis, the molecular mechanisms and factors influencing the metabolic transition remains unclear. Cortical organoids have revolutionized the field of brain development as they act as a novel system to study and recapitulate the development of the human cortex. Moreover, cortical organoids provide us with the opportunity to understand the molecular mechanisms essential for NSCs cell-fate decisions. Here, we establish the metabolic profile of cortical organoids over the course of 15 weeks across three cell lines. We introduce glycolytic pharmacological inhibitors at two developing timepoints and characterize the impact manipulating metabolism during the transition of a NSC to a differentiated neuron. Our preliminary results indicate that the treatment of glycolytic inhibitors on cortical organoids is promising in recapitulating the metabolic hallmark of decreased glycolysis and increased OXPHOS. Using organoids as our model system also allows us to study the influence of metabolic reprogramming on neuronal maturation and morphology. By identifying the influential metabolic pathways during neurogenesis, we can provide new insights as to how dysregulation of cell fate and maturation may impact brain development and identify the etiology of neurodevelopmental disorders driven by metabolic mutations.

Disclosures: J. Mil: None.

Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.13

Topic: A.03. Stem Cells and Reprogramming

Support: CDC

Title: A functional link between Notch signaling and COVID-19 through the analysis of variants discovered from sewage and clinical samples

Authors: *V. VO, C. CHANG, A. HARRINGTON, H. BAKER, E. OH;
Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: Notch signaling is a master regulator of neurological function in both development and disease and has been linked to the mediation of viral infections. Recent studies have demonstrated that SARS-CoV-2 infection in macaques results in an increase in Notch signaling and that protein coding regions of the viral genome can interact with Notch signaling proteins. Here we show that the expression of the SARS-CoV-2 protein-coding genome can modulate Notch signaling at several levels, including receptor-binding and transcriptional pathways. In addition, we express SARS-CoV-2 mutations identified through wastewater surveillance to understand how ultra-rare mutations can impact Notch signaling using luciferase and proteomic assays. Our findings demonstrate a unique complex between SARS-CoV-2 proteins and the Notch signaling pathway. Taken together, the findings highlight a link between the Notch pathway and COVID-19-associated neurological events.

Disclosures: V. Vo: None. **C. Chang:** None. **A. Harrington:** None. **H. Baker:** None. **E. Oh:** None.

Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.14

Topic: A.03. Stem Cells and Reprogramming

Support: NIH R01 NS096238
NIH T32 GM07628
NIH F31 NS120608

Title: Mapping the Time Course of mTORC1 Signaling and Quiescence in the Developing Brain

Authors: *L. C. GEBEN[1,2], G. V. RUSHING[2,3], A. A. BROCKMAN[2], Z. M. LANAGHAN[1], S. R. SWEET[3], K. C. ESS[2,3,5], J. M. IRISH[2,4], R. A. IHRIE[2,6];
[1]Pharmacol., [2]Cell & Developmental Biol., [3]Neurosci., [4]Pathology, Microbiology & Immunol., Vanderbilt Univ., Nashville, TN; [5]Neurol., [6]Neurolog. Surgery, Vanderbilt Univ. Med. Ctr., Nashville, TN

Abstract: The plasticity of the mammalian brain is determined by the number and proliferation capacity of neural stem cells (NSCs). NSCs exist in heterogeneous populations that are organized in space and across time. Different regional NSC populations have varying levels of activation of the mammalian target of rapamycin (mTOR), the master regulator of cell size and growth, while different temporal populations of NSCs are variably proliferative or quiescent. Together, these positionings in space and time are essential in coordinating neurogenesis.
The pool of NSCs that populates the adult brain is established during prenatal development from a subset of embryonic NSCs that temporarily quiesce. This quiescent phenotype persists until postnatal reactivation and is characterized by limited entry into the mitotic phase of the cell cycle and decreased cellular proliferation. Published studies in rodents have shown that ablation of the

quiescent pool of embryonic NSCs significantly decreases the number of adult NSCs and results in impaired cognitive performance. Despite understanding the importance of quiescence in forming the adult brain, the mechanisms driving regulation of and entry into quiescence are not known.
Using per-cell measurements of protein levels in murine NSC cultures, we found that mTOR activity transiently decreases with induction of quiescence and increases with return to cycle, suggesting this kinase as a regulator of quiescence entry and exit. Surprisingly, the multiple downstream phosphorylation targets of mTOR respond differentially to the induction of quiescence, suggesting activation of each target by mTOR may elicit distinct cellular functions. These phosphorylation targets are expressed independently, not coordinately, in embryonic NSCs which may further indicate their diverging contributions to cellular proliferation. This work also revealed that embryonic and postnatal NSCs have differing responses to the induction of quiescence, including differences observed across diverse spatial populations. Complementary immunostaining of intact embryonic mouse brain confirmed these patterns. While ongoing experiments strive to determine the necessity and sufficiency of decreased mTOR activity in inducing NSC quiescence, these existing data suggest mTOR may uniquely integrate several temporal and spatial variables to regulate the proliferative capacity of NSCs.
Results from this work may inform treatments for diseases where NSC numbers and activity are known to be altered during development, including brain cancers and neurodegenerative disease, by highlighting mTOR as a drug target to stimulate or inhibit the expansion of NSCs.

Disclosures: L.C. Geben: None. **G.V. Rushing:** None. **A.A. Brockman:** None. **Z.M. Lanaghan:** None. **S.R. Sweet:** None. **K.C. Ess:** None. **J.M. Irish:** None. **R.A. Ihrie:** None.

Poster

266. Neural Stem Cells: *In Vitro* Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 266.15

Topic: A.03. Stem Cells and Reprogramming

Title: Emergence and properties of human brain wave-like activity in human iPSC-3D cortical circuits

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

Abstract: Human brain wave activity prominently exhibits oscillatory network events (0.5—100 Hz) generated from neuronal assemblies within the human brain parenchyma. During fetal human brain development, oscillatory activity emerges, reflects functional consequences of brain ontogeny, and influences the development of the immature brain. Although rodent models have largely expanded our knowledge about how neuronal cells contribute to oscillatory activity, species-specific differences might limit the translatability of animal models. Human induced

pluripotent stem cell-derived (iPSC) neural *in vitro* models are widely used to study cellular aspects of human brain ontogeny. However, utilizing human iPSC neural in vitro models to assess the emergence and properties of oscillatory activity in human neuronal circuits in vitro is in its infancy. We utilized an *in vitro* human iPSC-derived 3D cortical aggregate model and applied microelectrode array (MEA) technology to assess properties of emerging oscillatory activity and its relation to synchronous neuronal network activity in cortical circuits. We demonstrate that our human iPSC-derived 3D cortical aggregate *in vitro* model permits assessing the emergence and properties of oscillatory activity generated from human iPSC-derived neuronal circuits within few weeks. In detail, we reveal that oscillatory slow-wave activity (< 1 Hz), phase-locked to synchronous network bursting, emerges already within 14 days *in vitro* followed by consecutive stages of emerging delta (1—4 Hz), theta (4—11 Hz), beta (11—30 Hz), and gamma (30—55 Hz) oscillatory activity, accompanied by stage-specific changes in neuronal network burst pattern characteristics. We provide a classification of neuronal mesoscale functional ontogeny stages of developing human iPSC-cortical circuits, where each stage is defined by specific oscillatory activity and characteristic synchronous neuronal bursting patterns. See also poster "*Pharmacological manipulation of human brain wave-like activity in human iPSC-3D cortical circuits*".

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

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.01

Topic: A.05. Axon and Dendrite Development

Support: BBSRC Grant: BB/G007632/1
NASA/INSGC grant

Title: Nell2 acts as an inhibitory guidance molecule that mediates stereotyped pruning of a long hippocampal mossy fiber branch during murine postnatal development

Authors: C. M. NAKAMOTO, M. DUNSON, C. MEYERS, ***M. NAKAMOTO**;
Biol., Valparaiso Univ., Valparaiso, IN

Abstract: Correct functioning of the nervous system is critically dependent on the proper formation of the neural network. Whereas the initial broad pattern of neuronal connectivity is set up through progressive events, including axon outgrowth and synaptogenesis, the more precise and mature circuity is established by regressive events, such as axon pruning and synapse elimination. Axon pruning events facilitate removal of exuberant or misguided axon branches, while the neuronal cell bodies remain intact. In early postnatal development (~Postnatal day (P) 10) of the mouse hippocampus, granule cells of the dentate gyrus extend two bundles of axons (mossy fibers) to CA3: the main (suprapyramidal) bundle, which is directed to apical dendrites

of CA3 pyramidal neurons; and the infrapyramidal bundle (IPB), which makes transient synaptic connections with basal dendrites of CA3 pyramidal neurons. Later in development, distal IPB synapses are eliminated and IPB axons are pruned, whereas the main bundle is maintained. However, the molecular mechanisms of IPB axon pruning are not well understood. Nell2 (also known as Nel) is a multi-modular extracellular glycoprotein that is predominantly expressed in the nervous system. Nell2 exerts diverse functions in neural development, including neuronal proliferation, differentiation and survival. We have previously shown that Nell2 acts as an inhibitory guidance cue for retinal axons that is essential for establishment of the eye-specific retinogeniculate projection. In this study, we investigated the function of Nell2 in stereotyped IPB pruning in the mouse hippocampus. By using RNA in situ hybridization, we found that Nell2 is strongly expressed in the hippocampus at the time of IPB pruning (P15-30). In vitro, Nell2 inhibited axon outgrowth and induced growth cone collapse in hippocampal neurons. In addition, treatment with Nell2 decreased the numbers of synapses in cultured hippocampal neurons. Furthermore, pruning of the IPB was defective in Nell2 null mice. Taken together, these results suggest that Nell2 acts as an inhibitory guidance molecule for hippocampal axons and is an essential for stereotypical pruning of IPB axons.

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Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.02

Topic: A.05. Axon and Dendrite Development

Support: JSPS Grant KM101-2587054400
JSPS Grant KM100-2633200
JSPS Grant KM101-18K1483900

Title: Thalamocortical axons control the cytoarchitecture of neocortical layers by area-specific supply of VGF

Authors: *H. SATO[1], J. HATAKEYAMA[1], T. IWASATO[2], K. ARAKI[1], N. YAMAMOTO[3], K. SHIMAMURA[1];
[1]Kumamoto Univ., Kumamoto Univ., Kumamoto City / Kumamoto, Japan; [2]Natl. Inst. of Genet., Natl. Inst. of Genet., Shizuoka, Japan; [3]Osaka Univ, Grad Sch. Frontier Biosci, Osaka Univ, Grad Sch. Frontier Biosci, Suita, Osaka, Japan

Abstract: Neuronal abundance and thickness of each cortical layer are specific to each area, but how this fundamental feature arises during development remains poorly understood. While some of area-specific features are controlled by intrinsic cues such as morphogens and transcription factors, the exact influence and mechanisms of action by cues extrinsic to the cortex, in

particular the thalamic axons, have not been fully established. Here, we identify a thalamus-derived factor, VGF, which is indispensable for thalamocortical axons to maintain the proper amount of layer 4 neurons in the mouse sensory cortices. This process is prerequisite for further maturation of the primary somatosensory area, such as barrel field formation instructed by a neuronal activity-dependent mechanism. Our results provide an actual case in which highly site-specific axon projection confers further regional complexity upon the target field through locally secreting signaling molecules from axon terminals.
Reference:
Sato H, Hatakeyama J, Iwasato T, Araki K, Yamamoto N, Shimamura K (2022) eLife 11:e67549.

Disclosures: H. Sato: None. **J. Hatakeyama:** None. **T. Iwasato:** None. **K. Araki:** None. **N. Yamamoto:** None. **K. Shimamura:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.03

Topic: A.05. Axon and Dendrite Development

Support: CIHR

Title: Programmed cell death 1 ligand 2 plays a role in retinal axon guidance during development

Authors: *X. CHEN[1,2], H. HARADA[2], P. MONNIER[1,2];
[1]Physiol., Univ. of Toronto, TORONTO, ON, Canada; [2]Univ. Hlth. Network, Toronto, ON, Canada

Abstract: During development, axons and dendrites extend from cell bodies in response to intrinsic and extrinsic cues. This axon guidance process is critical for proper synaptic activities in adulthood. Understanding the mechanisms that regulate axon guidance and the development of neural circuit formation may help to find a cure for neural disorders and regenerate the nervous system. A previous study from our lab investigated Repulsive Guidance Molecule b (RGMb), a guidance molecule which inhibits axon outgrowth in retinal ganglion cells (RGC). RGMb negatively regulated the Wnt pathway by depredating the Wnt receptor of the Low-density lipoprotein receptor-related protein 5 (LRP5) therefore inhibiting axon outgrowth. However, we could not confirm the direct interaction between RGMb and LRP5. Evidence shows the expression of Programmed cell death-1 ligand 2 (PD-L2) in the developing central nervous system (CNS) such as the retina, and it is responsible for maintaining proper RGC numbers during development as a programmed cell death ligand. Yet, no studies show the role of PD-L2 in axon guidance. Since PD-L2 is also known to be the ligand of RGMb, we hypothesize that PD-L2 is the mediator between RGMb and LRP5. In this study, we investigated the new function

of PD-L2 in the developing visual system by using chick and mouse models. Our data shows that PD-L2 overexpression increased axon length in the chick embryo RGC primary culture. The axon length was suppressed by PD-L2 overexpression in RGMb/Wnt knockdown conditions. We also studied the PD-L1 and PD-L2 double knockout animals and the data reflected aberrant retinal axon projection to the superior colliculus. These findings imply a new function of PD-L2 and the potential interaction of PD-L2 with RGMb and Wnt signaling in the developing CNS.

Disclosures: X. Chen: None. **H. Harada:** None. **P. Monnier:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.04

Topic: A.05. Axon and Dendrite Development

Support: NRF-2022R1A2C3003589
NRF-2018R1A5A1024261
KAIST-N11210255

Title: Visuomotor anomalies in achiasmatic Vax1^{aa} mice

Authors: *J. KIM[1], K. MIN[2];
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Abstract: In binocular animals that exhibit stereoscopic visual responses, the axons of retinal ganglion cells (RGCs) connect to brain areas bilaterally by forming a commissure called the optic chiasm (OC). Ventral anterior homeobox 1 (Vax1) contributes to formation of the OC, acting endogenously in optic pathway cells and exogenously in growing RGC axons. Here, we generated *Vax1$^{AA/AA}$* mice expressing the Vax1^{AA} mutant, which is incapable of intercellular transfer. We found that RGC axons cannot take up Vax1^{AA} protein from *Vax1$^{AA/AA}$* mouse optic stalk (OS) cells, of which maturation is delayed, and fail to access the midline. Consequently, RGC axons of *Vax1$^{AA/AA}$* mice connect exclusively to ipsilateral brain areas, resulting in the reduced visual acuity and the abnormal oculomotor responses. Together, our study provides physiological evidences for the necessity of intercellular transfer of Vax1 and the importance of the bilateral RGC axon projection in visuomotor responses.

Disclosures: J. Kim: None. **K. Min:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.05

Topic: A.05. Axon and Dendrite Development

Support: NSF #1822517
NSF #2112862
NIH #MH117488
California NanoSystems Institute

Title: An experimental toolbox for the analysis of single serotonergic axons in the mouse brain

Authors: K. C. MAYS, J. H. HAIMAN, *S. JANUSONIS;
Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: The self-organization of the serotonergic matrix in the brain is a key unsolved problem in neuroscience. This matrix is composed of extremely long axons (fibers) that originate in the brainstem, invade nearly all brain regions, and accumulate in remarkably high densities in many of them. Serotonergic fibers possess a number of intriguing properties, including the ability to robustly regenerate in the adult brain, the strongly stochastic trajectories, and the poorly understood but consistent association with neural plasticity. We developed several experimental methods that can be used to capture the individual trajectories of serotonergic fibers in the mouse brain, including regions with high fiber densities. These data are essential for stochastic modeling efforts that currently utilize two different frameworks (a step-wise random walk based on the von Mises-Fisher directional distribution and the superdiffusive fractional Brownian motion). In one approach, we show that serotonergic fibers can be experimentally isolated by using transgenic mice with the inducible Cre (under the Tph2-promoter), crossed with a Cre reporter line. While the overall labeling intensity falls below that of the best constitutive model in the field (Migliarini et al., 2013), the inducible Cre allows for control over how many fibers are labeled in high-density regions, thus facilitating their semi-automated tracing. A particularly powerful approach is based on the Brainbow toolbox (Cai et al., 2013) which can be used to randomly “color-code” individual axons. We have developed the first implementation of Brainbow-tagging in the serotonergic system (based on intracranial AAV-injections) and demonstrate its potential in downstream stochastic analyses. In particular, we show that some apparent branching points are different fibers crossing at distances below the limit of optical resolution (even in high-power confocal imaging). Finally, we demonstrate the feasibility of imaging single serotonergic fibers with CUBIC-based tissue clearing and high-resolution light-sheet microscopy (with a 20X objective). This experimental toolbox, integrated with stochastic modeling, can advance the current understanding of the dynamics, robustness, and plasticity of the brain serotonergic system.

Disclosures: K.C. Mays: None. **J.H. Haiman:** None. **S. Janusonis:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.06

Topic: A.05. Axon and Dendrite Development

Support: R01 NS104055
R01 NS045523
T32 AG000222
F32 AG067661
Max & Anne Wien Professor of Life Sciences fund
DEARS Foundation

Title: Development of ultra-low-input nanoRibo-seq enables quantification of translational control, revealing broad uORF translation by subtype-specific neurons

Authors: *J. FROBERG[1], D. TILLMAN[2], O. DURAK[2], B. BUDNIK[3], J. D. MACKLIS[2];
[2]Harvard Univ., [1]Harvard Univ., Cambridge, MA; [3]Wyss Inst., Boston, MA

Abstract: While increasingly powerful approaches enable investigation of transcription using small samples of RNA, approaches to investigate translational regulation in small populations of specific cell types, and/or (sub)-cellular contexts are lacking. Comprehensive investigation of mRNAs actively translated into proteins from ultra-low input material would provide important insight into molecular machinery and mechanisms underlying many cellular, developmental, and disease processes in vivo. Such investigations are limited by the large input required for state-of-the-art Ribo-seq. We present an optimized, ultra-low input "nanoRibo-seq" approach using 10^2 - 10^3-fold less input material than standard approaches, demonstrated here in subtype-specific neurons. nanoRibo-seq requires as few as 2.5K neurons, and exhibits rigorous quality control features: 1) strong enrichment for CDS versus UTRs and non-CDS; 2) narrow, distinct length distributions over CDS; 3) ribosome P-sites predominantly in-frame to annotated CDS; and 4) sufficient ribosome-protected fragment (RPF) coverage across thousands of mRNAs. We calculate translation efficiencies from paired Ribo-seq and alkaline fragmented control libraries from "callosal projection neurons" (CPN), revealing divergence between mRNA abundance and RPF abundance for hundreds of genes. Intriguingly, we identify substantial translation of upstream ORFs in the 5' UTRs of genes involved in axon guidance and synapse assembly. We combined nanoRibo-seq with ultra-low input proteomics to compare translational output and protein abundance between CPN and subcerebral projection neurons (SCPN). Both translation efficiency, and protein abundance are tightly correlated between CPN and SCPN, with dozens of significantly different mRNAs/proteins. nanoRibo-seq enables previously inaccessible investigation of translational regulation by small, specific cell populations in normal or perturbed contexts.

Disclosures: J. Froberg: None. **D. Tillman:** None. **O. Durak:** None. **B. Budnik:** None. **J.D. Macklis:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.07

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R15NS124008

Title: Axon initial segment morphology is regulated by early postnatal GABA signaling

Authors: D. J. HINES[1], R. ALI RODRIGUEZ[1], V. VOLK[2], A. CONTRERAS[3], C. FITZPATRICK[2], **R. M. HINES**[1];
[1]Interdisciplinary Neuroscience, Psychological and Brain Sci., Univ. of Nevada Las Vegas, Las Vegas, NV; [2]Mechanical and Biomed. Engin., Boise State, Boise, ID; [3]Interdisciplinary Neuroscience, Psychological and Brain Sci., Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: The axon initial segment (AIS) is a highly specialized compartment of neuronal cells that controls cell firing. In the mature cortex, GABAergic chandelier cells pattern neural activity by contacting large groups of principal neurons at the AIS forming axo-axonic synapses that exert shunting inhibition. Axo-axonic synapses are enriched with GABAARs containing the α2 subunit and specific interacting partners whose genes have been implicated in disorders of neurodevelopment. α2 subunit expression is also notably high in early postnatal cortex before the formation of axo-axonic synapses. We have found that in parallel with strong downregulation of α2 subunit expression there is a shift in cortical pyramidal cell AIS morphology from tortuous to linear. We generated a mouse model with a substitution mutation in the GABAAR α2 subunit (*Gabra2*-1) and found that this prevents developmental downregulation of α2 expression and prevents the linearization of the AIS. Mature *Gabra2*-1 cortical cells are characterized by tortuous AISs, which have reduced numbers of axo-axonic synapses. *Gabra2*-1 mice also display spontaneous seizures beginning around day 9 of postnatal development, along with a number of features of syndromic intellectual disability. Our studies suggest that the α2 subunit of $GABA_A$Rs plays an early role in organizing the AIS, and that this organization is essential for axo-axonic synapse formation and the control of cortical activity.

Disclosures: D.J. Hines: None. **R. Ali Rodriguez:** None. **V. Volk:** None. **A. Contreras:** None. **C. Fitzpatrick:** None. **R.M. Hines:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.08

Topic: A.05. Axon and Dendrite Development

Support: National Institutes of Health Grants R35 NS122073
Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Biotinylation by Neurofascin Antibody Recognition Maps the Extracellular Axon Initial Segment Proteome

Authors: *Y. OGAWA[1], B. C. LIM[1], J. A. OSES-PRIETO[2], B. VIJAYARAGAV[3], Y. ESHED-EISENBACH[3], E. PELES[3], A. L. BURLINGAME[2], M. N. RASBAND[4];
[1]Baylor Col. of Med., Houston, TX; [2]Univ. of California San Francisco, San Francisco, CA; [3]Weizmann Inst. Sci., Rehovot, Israel; [4]Neurosci., Baylor Col. of Med. Dept. of Neurosci., Houston, TX

Abstract: Axon initial segment (AIS) membrane proteins mediate key biological processes in neurons. For example, AIS Na^+ and K^+ channels initiate action potentials, while other AIS membrane proteins assemble AIS-specific perineuronal nets and inhibitory synapses. However, relatively few AIS membrane proteins have been reported. Here, using biotinylation by antibody recognition, we report the extracellular Neurofascin proximity proteome across five developmental timepoints. We identified all previously reported AIS cell adhesion molecules and extracellular membrane-associated proteins. We used CRISPR/Cas9 and homology independent genome editing to determine the distributions of membrane proteins in close proximity to Neurofascin. Among these, we found that Contactin-1 is enriched at the AIS and is required to recruit TenascinR to the AIS-extracellular matrix. In addition, we mapped other membrane proteins to axonal, AIS, and dendritic domains. This strategy enables flexible and temporally resolved proteomic profiling as a discovery tool to elucidate the proximity proteomes of surface membrane proteins.

Disclosures: Y. Ogawa: None. **B.C. Lim:** None. **J.A. Oses-Prieto:** None. **B. Vijayaragav:** None. **Y. Eshed-Eisenbach:** None. **E. Peles:** None. **A.L. Burlingame:** None. **M.N. Rasband:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.09

Topic: A.05. Axon and Dendrite Development

Support: NIH Pioneer Award DP1 NS106665
Allen Frontiers Group
Foundation Jean-Jacques et Felicia Lopez-Loreta pour excellence academique
NSF Graduate Research Fellowship
NSF-Simons Center Quantitative Biology Initiative at Harvard

Title: Growth cone molecular machinery locally implements the development of subtype-specific neocortical circuitry

Authors: *P. VEERARAGHAVAN[1], A. K. ENGMANN[1], J. J. HATCH[1], Y. ITOH[1], T. ADDISON[1], M. LIU[1], A. POULOPOULOS[2], J. D. MACKLIS[1];
[1]Dept of Stem Cell and Regenerative Biol. and Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [2]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: During development, growth cones (GCs) of diverse cortical or other projection neuron (PN) subtypes navigate complex extracellular environments to reach distant, subtype-specific targets. These axon-terminal structures respond to guidance signals in a subtype- and stage/context-specific fashion to construct specific functional circuitry. Local protein synthesis of axonally-trafficked transcripts is required for directional responses to at least some guidance cues, and previous work from our lab and others have shown that GC transcriptomes are distinct from their parent somata. However, the composition of subtype-specific GC-localized molecular machinery, the mechanisms to establish subtype-specific spatial segregation of transcriptomes, and dynamic regulation of these processes are essentially unknown.
The work presented here investigates subcellular transcriptomes of two subtypes of cortical projection neurons with highly distinct connectivity and, subsequently, function: callosal projection neurons (CPN), which form interhemispheric connections and corticothalamic projection neurons (CThPN), which send projections corticofugally- away from the cortex, ultimately targeting specific nuclei of the thalamus. We find that the local, GC-enriched transcriptomes are at least partially subtype-specific, with a pronounced portion of nonoverlapping transcripts, and that many of the CPN- and CThPN-specific GC-localized transcripts are associated with neurodevelopmental diseases, strongly suggesting an important function of subtype-specific subcellular machinery in the generation of functional circuitry. Further, we identify that the subtype-specific GC-localized transcriptomes are likely established by both, transcriptional as well as RNA trafficking mechanisms and, lastly, that the composition of subcellular transcriptomes is dynamically regulated across developmental time point, likely reflecting local enrichment of context-specific molecular mechanisms to enable correct axon guidance and circuit formation. Newly accessible subcellular investigation of distinct subtypes of neurons critically enables a deepened mechanistic understanding of the development of diverse and functional connectivity in the brain and will provide new insight into potential etiology of neurodevelopmental and neuropsychiatric disorders.

Disclosures: P. Veeraraghavan: None. **A.K. Engmann:** None. **J.J. Hatch:** None. **Y. Itoh:** None. **T. Addison:** None. **M. Liu:** None. **A. Poulopoulos:** None. **J.D. Macklis:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.10

Topic: A.05. Axon and Dendrite Development

Title: Identification of the translating circular RNA molecule in retinal ganglion cells using viral TRAP

Authors: *B. BASU[1,2], S. LALITHA[1,3], A. PAL[1], P. A. RIYA[1,3], A. C. PANDA[4], J. JAMES[1,2,3]; [1]Regenerative Biol., Rajiv Gandhi Ctr. for Biotech., Thiruvananthapuram, India; [2]Regional Ctr. for Biotech., Faridabad, India; [3]The Univ. of Kerala, Thiruvananthapuram, India; [4]Inst. of Life Sci., Bhubaneswar, India

Abstract: Retinal Ganglion Cell (RGC) genesis, fate specification and its axon guidance are concomitant processes that start during embryonic stages. This humongous task is achieved by coordination between the environmental cues and fine-tuning of intracellular regulatory molecules. There are few reports available about microRNA mediated control of RGC genesis and axon path finding but till now no finding has been reported regarding the regulatory role of circular RNAs (circRNAs) in embryonic retina. To understand the global expression of circular RNA in embryonic retina we have performed rRNA-depleted bulk RNA-Sequencing of E18 mouse retina and two different circular RNA finding tools i.e., CIRIquant and CIRCexplorer3 were employed for identifying their genomic and chromosomal locus. We found that around 1898 circular RNAs were unanimously identified by both the algorithm and majority of them were originated from exonic region. Experimentally we validated few circRNAs using combination of strategies i.e., RNaseR mediated linear transcript digestion, divergent primers across the back-splice junction (BSJ) specific for circRNAs, and sanger sequencing of the PCR products. We functionally classified the host genes of the circRNAs by gene ontology enrichment analysis. We found that functional groups related to microtubule organization and axon guidance were overrepresented amongst the enriched gene ontology terms. At this crucial developmental timepoint when axon guidance of young neurons is very important, biogenesis of circRNAs provoked us to find its regulatory role specifically in RGCs. We wanted to know whether circular RNA is associated with the translating ribosome. We used viral Translating Ribosome Affinity Purification (vTRAP) method by genetically defining the retinal ganglion cells using Pou4f-1 promoter. The vTRAP sample was subjected to rRNA-depleted bulk RNA-Seq analysis for identifying translating circRNAs. Further analysis with CIRCexplorer3 reported only 35 circular RNA to be associated with translating polyribosome. Comparing our data with riboCIRC database we found two candidate circRNAs i.e., circTcf20 and circSmad1 were reported to be translating in other tissues and cell lines. Further analysis with IRESfinder.py and Coding Potential Assessment Tools (CPAT) revealed that circTcf20 had the highest coding probability score of 1. Further exploration of their peptides and their characterization might provide us a clue of their regulatory role.

Disclosures: B. Basu: None. **S. Lalitha:** None. **A. Pal:** None. **P.A. Riya:** None. **A.C. Panda:** None. **J. James:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.11

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant DP1 NS10665-04
NIH Grant R01 NS045523-16

Title: Subcellular growth cone protein machinery regulating subtype-specific cortical projection neuron circuit development identified via ultra-low-input proteomics

Authors: *D. E. TILLMAN[1], O. DURAK[1], P. VEERARGHAVAN[1], J. E. FROBERG[1], B. BUDNIK[2], J. D. MACKLIS[1];
[1]Stem Cell and Regenerative Biology, Ctr. for Brain Sci., [2]Wyss Inst., Harvard Univ., Cambridge, MA

Abstract: Cerebral cortex projection neurons (PN) have critical roles in sensory, motor, cognitive, and behavioral circuits. During development, PN build intricate circuitry by extending axons through diverse extracellular environments, then innervating specific targets located at great distances (10^3 - 10^5 cell body diameters) from their nucleus-containing cell bodies (somata). This precise navigation is regulated by growth cones (GCs): semi-autonomous, subcellular compartments at tips of growing axons that rapidly integrate extracellular signals to control axon pathfinding. Proper GC function enables PN to form subtype-specific connectivity with synaptic targets. For example, callosal PN (CPN) innervate contralateral cortex, subcerebral PN (SCPN) innervate locations in brainstem and spinal cord, and corticothalamic PN (CThPN) innervate specific thalamic nuclei. Previous work has identified molecular controls over subtype-specific PN development via regulation of transcription in somata. Additionally, protein synthesis and degradation in GCs is required for directional responses to some guidance cues. However, molecular mechanisms linking nuclear transcription to GC protein abundances have not been elucidated, functions of distinct GC proteins in axon pathfinding are poorly understood, and even less is known about roles of GC proteins in specific PN subtypes. Recently, our lab developed experimental and analytical approaches to purify GCs and their parent somata from specific PN subtypes in developing mouse cortex, then quantitatively "map" RNA and protein species between these subcellular compartments. We integrated these approaches with advances in ultra-low-input mass spectrometry to generate a proteome of subtype-specific GCs 40% deeper than previous work, using only 50% of the input material. We then developed an analytical workflow to quantify the purity of isolated GCs, and increased GC purity 4-fold by incorporating additional biochemical purification steps. Using these optimized procedures, we purified CPN GCs before and after midline crossing, then performed ultra-low-input mass spectrometry to identify differentially abundant proteins. We selected candidates for functional investigation in CPN midline crossing. These investigations promise to reveal molecular mechanisms controlling construction, function, and maintenance of cortical circuitry, and will enable future investigations of how proteomic regulation in distinct subtypes, stages, and/or subcellular compartments contributes to normal circuit development and disorders of nervous system circuitry.

Disclosures: D.E. Tillman: None. **O. Durak:** None. **P. Veerarghavan:** None. **J.E. Froberg:** None. **B. Budnik:** None. **J.D. Macklis:** None.

Poster

267. Axon Dynamics: New Mechanisms and Techniques

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 267.12

Topic: A.01. Neurogenesis and Gliogenesis

Support: NSERC Discovery Grant RGPIN-2021-03866
Howard Hughes Medical Institute

Title: A novel chemigenetic approach for birthdate color-coding of emerging neurons in developing zebrafish

Authors: *M. KOYAMA[1,2], L. ZHANG[3,2], F. DU[1], D. MARK[1];
[1]Cell and Systems Biol., Univ. of Toronto, Toronto, ON, Canada; [2]Janelia Res. Campus, Ashburn, VA; [3]Natl. Inst. of Hlth., Besethda, MD

Abstract: The temporal pattern of neuron specification contributes to the generation of neuronal diversity in the brain. Recently, such accretion of neuron types and their maturation late in development have been indicated to play a critical role in the sophistication of brain functions and behavior during development. However, it has not been possible to directly visualize anatomical and functional development of a series of emerging neuronal cell-types in maturing animals. To address this, we developed a novel chemigenetic birthdating approach that maps neurons to a range of colors based on their birthdates in zebrafish. This technique is based on the coupling of transgenic lines expressing the HaloTag protein in differentiating neurons with a chromatic variety of Janelia Flour HaloTag binding dyes. First, we characterized how well these dyes permeate the nervous system of developing zebrafish by bath application and how long the dyes persist in the developing zebrafish after transfer to a dye-free medium. Using a transgenic line expressing nuclear-localized HaloTag pan-neuronally, we determined that dyes permeate easily and stay bound to HaloTag until at least 6 dpf. Second, we tested which of these dyes can be used in combination to label multiple age groups in the same animal. We found that Janelia Flour (JF) 635 , 585 and 525 nm dyes can be loaded in three successive days to label distinct groups of neurons. Third, we compared the spatial distribution of these distinct groups of neurons to the age groups identified by EdU staining. Here, we used JF 585 to block the binding to neurons that were older than the neuronal population of interest and JF635 to label the desired population. We performed this procedure at five time points and found that our HaloTag-based birthdating approach revealed a pattern consistent with EdU staining. We also tested if it is possible to visualize specific cellular processes of each age group. Using a pan-neuronal line that localizes HaloTag to axon terminals, we revealed that axon terminals are spatially clustered based on birthdate, consistent with previous observations. We are currently pursuing the addition of calcium imaging to our method to add the ability to functionally characterize neurons based on birthdate. Taken together, this work provides a novel and highly flexible approach to visualize the anatomical and functional development of emerging neurons.

Disclosures: **M. Koyama:** None. **L. Zhang:** None. **F. Du:** None. **D. Mark:** None.

Poster

268. Autism: Environment and Pathology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 268.01

Topic: A.07. Developmental Disorders

Support: NIEHS P30ES030283
American Epilepsy Society

Title: Cerebellar Neuroinflammation disrupts the Blood Brain Barrier Integrity and may impair Cerebellum-regulated Cognitive Function in Two Mouse Models of Autism.

Authors: R. JAGADAPILLAI[1], J. CAI[1], G. N. BARNES[2], *E. GOZAL[1];
[2]Neurol., [1]Dept of Pediatrics PRI / Univ. of Louisville, Louisville, KY

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition with both genetic and environmental etiology. Recent genetic and neuropathological studies have suggested that incongruous interactions between cerebral vascular endothelial cells, microglia, and neurons may underlie impaired neurodevelopment in ASD subjects. GABA signaling in cerebral endothelial cells controls the migration and differentiation of interneurons in cerebral cortex, and when altered in mice endothelial cell, results in autism-like behaviors. Neuroinflammation and oxidative stress can induce endothelial injury and potential breach of the BBB, which may also stem from ASD-associated genetic abnormalities. Thus, altered neurovascular interactions resulting in changes of network functional connectivity, may promote autistic behavior in humans. Evidence from human and pre-clinical models of autism suggest that the cerebellum may play a pivotal role in the expression of autistic behavior. The cerebellum and in particular Purkinje cells through alpha/beta range rhythms may modulate gamma oscillations (>30 Hz) which are critical for communication between neurons in distinct brain regions to facilitate gamma-rhythmic synchronization between communicating neuronal groups. Here we investigated whether neuroinflammation and oxidative stress may disrupt BBB integrity within the cerebellum in two murine models of autism. Both ROS and albumin leakage staining are increased in the cerebellar cortex of GABAergic-specific semaphorin 3 F (Sema 3F) KO and BTBR models of ASD, compared to their wild types. Interestingly, western blotting and immunofluorescent staining show that ZO1, one of the BBB tight junction proteins, significantly decreased in the cerebellar cortex of BTBR and Sema 3F KO mice, with occasional cells containing increased cytoplasmic Z01. Immunoprecipitation experiments suggest that Z01 can bind to the subcellular location/trafficking protein 14-3-3, which levels have been shown to be altered in brain and in blood of ASD patients, suggesting improper protein trafficking. In summary, the neuroinflammatory response in these preclinical murine models of autism is associated with cerebellar BBB leakage and possible abnormal trafficking of BBB tight junction proteins, thereby further promoting dysfunction of the BBB in ASD subjects. These data support the hypothesis that abnormal neurovascular signaling in the cerebellum may impair its ability to coordinate communication between neuronal groups in social, cognitive, and corticostriatal networks, thus promoting the appearance of autistic behaviors in children with ASD.

Disclosures: **R. Jagadapillai:** None. **J. Cai:** None. **G.N. Barnes:** None. **E. Gozal:** None.

Poster

268. Autism: Environment and Pathology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 268.02

Topic: A.07. Developmental Disorders

Title: An Angiotensin Converting Enzyme (ACE) inhibitor leads to reduced microglial activation in a mouse model of ASD induced by in utero exposure to maternal anti-Caspr2 antibodies

Authors: *B. SPIELMAN, C. BAGNALL-MOREAU, C. CRUZ, L. BRIMBERG;
Feinstein Inst. for Med. Res., Manhasset, NY

Abstract: The presence of circulating maternal anti-brain antibodies (IgG) in the in utero environment has been associated with an increased risk of having a child with Autism spectrum disorder (ASD). Caspr2 protein (Contactin-associated protein-like 2, encoded by the ASD risk gene, CNTNAP2) is a common target of these maternal antibodies. We have developed a mouse model in which dams are immunized with human Caspr2 protein, leading them to produce endogenous anti-Caspr2 IgG. We have shown that male, but not female offspring born to dams harboring anti-Caspr2 IgG throughout gestation are more likely to exhibit neurodevelopmental phenotypes including increased repetitive behaviors (grooming and marble burying) and impaired social behavior. Here, we examine the role of microglia in mediating the effect of in utero exposure to anti-Caspr2 IgG ("anti-Caspr2") or to control IgG ("control"). Microglia in the hippocampus of anti-Caspr2 male, but not female mice, appear to be activated at embryonic day E18.5. This microglial activation persists into adulthood. We also observe decreased dendritic arborization and reduced dendritic spines on pyramidal cells in the CA1 region of the hippocampus in the anti-Caspr2 males. We hypothesized that early suppression of the microglia using captopril, an FDA approved drug for hypertension and heart failure that has previously been shown to suppress microglia in Alzheimer's disease and neuropsychiatric lupus, will prevent the decreased dendritic arborization and reduced dendritic spines in the anti-Caspr2 male mice. We show that captopril suppresses microglial activation in anti-Caspr2 male mice compared to vehicle-treated anti-Caspr2 male mice. Furthermore, we demonstrate that in anti-Caspr2 males, captopril treatment preserves dendritic arborization and dendritic spine density of pyramidal cells in the CA1 region of the hippocampus. Investigation into whether the microglia in the captopril-treated anti-Caspr2 male mice are engulfing less synaptic material than the microglia in the vehicle-treated anti-Caspr2 male mice is currently ongoing. Additionally, assessment of the effect of captopril on social novelty behavior in the anti-Caspr2 males is currently being analyzed. Altogether, our model can be utilized to examine microglial activation in ASD and to assess microglia as targets for potential therapeutic strategies.

Disclosures: **B. Spielman:** None. **C. Bagnall-Moreau:** None. **C. Cruz:** None. **L. Brimberg:** None.

Poster

268. Autism: Environment and Pathology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 268.03

Topic: A.07. Developmental Disorders

Support: a Canadian Institutes of Health Research Operating Grant

Title: Association between prenatal antibiotic exposure and autism spectrum disorder: a population-based cohort study from British Columbia, Canada

Authors: *A. S. NITSCHKE, H. ABREU DO VALLE, B. A. VALLANCE, C. BICKFORD, A. IP, N. LANPHEAR, B. LANPHEAR, W. WEIKUM, T. F. OBERLANDER, G. E. HANLEY; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Antibiotics are among the most used medications during pregnancy, and while their short-term benefits are clear, their potential long-term effects are underexplored. Prenatal antibiotic exposure induces changes in the maternal microbiome, which could in turn influence development of the infant's microbiome-gut-brain axis. We assessed whether prenatal antibiotic exposure increases the risk of autism spectrum disorder (ASD) in offspring. This retrospective cohort study included everyone who delivered a live singleton term infant in the British Columbia, Canada between April 1st 2000 and December 31st 2014 (n = 569,953 children born to 366, 152 mothers). The outcome was an ASD diagnosis made through the British Columbia Autism Assessment Network, with follow-up to December 31st 2016. Cox proportional hazards models were used to estimate unadjusted and adjusted hazard ratios (HR). The analysis was stratified by pregnancy trimester of exposure to antibiotics, cumulative duration of exposure, class of antibiotic, sex and mode of delivery. To assess confounding by indication, we stratified by different sites of infection (i.e., genital, urinary, respiratory tract) and infectious agents (i.e., viral, bacterial). Further, to account for unmeasured confounding, we ran a conditional logistic regression of discordant sibling pairs. Prenatal antibiotic use was associated with a statistically significant increase in risk of ASD (aHR 1.08 [95% CI 1.02-1.13]). Significant associations were observed for exposure during the second trimester (aHR 1.08 [95% CI 1.02-1.15]), as well as exposure to penicillins and other beta lactams (aHRs 1.06 [95% CI 1.00-1.12] and 1.10 [95% CI 1.00-1.20], respectively). Analysis based on cumulative duration of antibiotic exposure showed a dose response relationship, with the highest risk observed when exposure lasted for 15 days or longer (aHR 1.10 [95% CI 1.00-1.20]). In the sub-cohort of pregnancies treated for urinary tract infections, the aHR was 1.10 (95% CI 0.98-1.25). The relationship was no longer significant in the conditional logistic regression (aHR 1.05 [95% CI 0.93-1.18]). In conclusion, prenatal antibiotic exposure was associated with a small increase in risk of ASD in offspring. These findings may suggest prenatal origins of neurodevelopmental risk, possibly via metabolic

programming by the maternal microbiome and a disrupted early developing microbiome-gut-brain axis. However, given the possibility of residual confounding, these results should be confirmed in future studies.

Disclosures: **A.S. Nitschke:** None. **H. Abreu do Valle:** None. **B.A. Vallance:** None. **C. Bickford:** None. **A. Ip:** None. **N. Lanphear:** None. **B. Lanphear:** None. **W. Weikum:** A. Employment/Salary (full or part-time):; author disclosed that the BC Children's Hospital Foundation funds a portion of her salary. **T.F. Oberlander:** None. **G.E. Hanley:** None.

Poster

268. Autism: Environment and Pathology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 268.04

Topic: A.07. Developmental Disorders

Support: Brain & Behavior Research Foundation NARSAD Young Investigator Grant 28298 (S.A.B.)
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NIH NIA 1T32 AG067952-01 (L.M.M.)
John Stanton Endowed Scholarship (C.M.D.G.)

Title: Diet-induced negative selection of SCFA-producing taxa among maternal gut microbiota impairs descendant social behavior

Authors: C. M. DI GESÙ[1], L. M. MATZ[2], I. J. BOLDING[2], R. FULTZ[2], K. L. HOFFMAN[3], A. MARINO GAMMAZZA[4], J. F. PETROSINO[3], *S. A. BUFFINGTON[2];
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Abstract: Neurodevelopmental disorders, including autism spectrum disorder (ASD), are highly heritable; however, consensus is growing behind a two-hit model in which environmental exposures can promote disease in genetically predisposed individuals. Among environmental factors, those that impact the maternal gut microbiome during pregnancy are emerging as critical modulators of neurodevelopment and long-term behavioral outcomes in offspring. Specifically, disruption, or 'dysbiosis,' of the maternal gut microbiome during pregnancy is associated with adverse neurodevelopmental outcomes. Previously, we showed that maternal high-fat diet (MHFD) in mice induces gut dysbiosis, social dysfunction, and underlying synaptic plasticity deficits in male offspring (F_1). Here, we reasoned that MHFD would likewise induce dysbiosis in *female* offspring (F_1), thereby recapitulating the adverse *in utero* environment experienced by the

F_1 males and resulting social dysfunction in the F_2 generation, even in the absence of a dietary challenge. Metataxonomic sequencing revealed a significant reduction in microbial richness among female F_1 MHFD offspring, with a specific decrease in the abundance of short-chain fatty acid (SCFA)-producing taxa. Despite recovery of richness in the F_2 generation, F_2 social behavior remained impaired, implicating dysbiosis of the *maternal* gut microbiome in offspring social deficits. Post-weaning supplementation with *Limosilactobacillus* (*L.*) *reuteri* was sufficient to rescue F_2 generation social deficits. Unexpectedly, *L. reuteri* exerted a differential impact on the microbiome of control *versus* MHFD-descendant F_2 mice, revealing a relative instability of the MHFD lineage microbiome. We also observed a near-tripling of discriminant taxa between *L. reuteri*-treated F_2 females *versus* males. This previously unreported heightened responsiveness of the female gut microbiome to probiotic modulation presented an opportunity for intervention: probiotic targeting of the maternal gut microbiome during pregnancy to relieve offspring social dysfunction. Hence, we developed a 7-strain probiotic cocktail consisting of immunomodulatory taxa and administered it to control and HFD-fed dams during pregnancy and lactation. Antenatal targeting of the maternal gut microbiome was sufficient to restore neurotypical social behavior in the MHFD-descendant F_1 generation. Our results link maternal lineage HFD to instability of descendant microbial communities and maladaptive social behavior. Moreover, they highlight the potential for therapeutic targeting of the maternal gut microbiome to improve neurobehavioral outcomes in descendants.

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Poster

268. Autism: Environment and Pathology

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Topic: A.07. Developmental Disorders

Support: R00HD087523
R01MH122447
R01ES029511
P30ES023515
U2CES026561
R35ES030435

Title: Identifying critical windows of increased inflammation in early life associated with autism spectrum disorder using a novel protocol for extracting temporal biomarkers from deciduous teeth

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder affecting various cognitive functions such as learning and communication, and affects 1 in 44 children in the United States (CDC). Inflammation, particularly in utero, has been identified in prior research as playing a role in the pathogenesis of ASD. However, further investigation has been hampered by the lack of a temporal biomarker capable of providing insight into the prenatal inflammatory events associated with the development of ASD.
Baby teeth begin forming in utero and grow in an incremental pattern (akin to growth rings in a tree), incorporating environmental exposure and biological response data at discrete points in time, from approximately the second trimester until tooth shedding in childhood. We previously showed initial results from the development of an immunohistochemistry (IHC) protocol conducted on decalcified tooth sections that measured the inflammatory markers, C-reactive protein (CRP) and cortisol, as well as a structural protein, dentin matrix protein-1 (DMP). Here, we present new results from method optimization, validation and application in a study of children with ASD. Overall, this novel technology provides daily profiles of inflammation during fetal development and early childhood.
Optimal decalcification occurred after 5 weeks soaking in an EDTA solution, depending on tooth mass. We validated tooth-CRP as a marker of inflammation by comparing medical records with temporal CRP profiles extracted from teeth donated by two children and a non-human primate. We then applied this technology to a national study in Sweden. Because ASD has heritability of 50% to 80%, we applied this technology to a cohort of monozygotic and dizygotic twins to control for underlying genetics (n=66; 14 ASD cases). Distributed lag models (rDLM), a time series statistical analysis method, was used to measure the time-varying effect of inflammation against ASD diagnosis, while adjusting for all past exposure values in the time series. We found statistically significant third trimester inflammation in children eventually diagnosed with ASD relative to controls. We then replicated our findings in a cohort of children from the US (n=47; 23 ASD cases). Not only do these findings reinforce the importance of in utero inflammation in the pathogenesis of ASD, but they also serve to validate a novel technology for the investigation of other biomarkers.

Disclosures: R.J.J. Coenen: None. **D. Dumitriu:** None. **E. Baldwin:** None. **L. Hammond:** None. **D.S. Peterka:** None. **R. Palmer:** None. **S. Bölte:** None. **C. Austin:** None. **P. Curtin:** None. **M. Arora:** None.

Poster

268. Autism: Environment and Pathology

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Program #/Poster #: 268.06

Topic: H.12. Aging and Development

Support: NIGMS- GM113109 NIH
Start up funds from K-State to BP

Title: Enlarged anterior cingulate cortices are implicated in cognitive deficits in adolescent female ASD-modeled rats

Authors: C. KING[1], I. MALI[2], M. PAYNE[2], S. BOSSMANN[2], T. R. MAZE[2], T. DAVISON[2], *B. PLAKKE[2];
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Abstract: In order to develop better treatments for autism spectrum disorder (ASD) it is critical to understand the developmental trajectory of the disorder and the accompanying brain changes. This study used the valproic acid (VPA) model to induce ASD-like symptoms in rodents. Prior studies have demonstrated that VPA animals are impaired on executive function tasks, paralleling results in humans with ASD. Pregnant dams were injected with 600 mg/kg VPA or saline on gestational age 12. To control for the litter effect, one male and one female pup per litter were assigned to an experimental condition. There were 32 rats that completed the set-shifting task (9 male controls, 7 VPA males, 9 control females, 7 VPA females). Data analysis compared male and female data separately based on past findings (McKinnell et al.,2020). Researchers that conducted behavior and volumetric segmentation for brain regions were blind-to-condition. The VPA adolescent female rats were impaired on the set-shifting task and had enlarged frontal cortices compared to control females. In addition, adolescent VPA females with enlarged frontal cortices performed the worst of all groups across the entire task. This deficit observed in the VPA female rats mirrors those observed in females with ASD, suggesting that ASD-like symptom progression in the VPA model is similar to that observed in humans. These novel findings highlight the importance of studying the brain at different developmental stages and implicate overgrowth of the anterior cingulate cortex is impacting executive functioning deficits found in ASD.

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Poster

268. Autism: Environment and Pathology

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Program #/Poster #: 268.07

Topic: A.07. Developmental Disorders

Support: Gerber Foundation Grant (CME & MBB)
NIH HD097327 (MBB)
NIH EY027881 (PAR)

Title: Personalized brain nutrition in very preterm infants: a pilot study of nutritional biomarkers using exosomal RNAseq and blood micronutrient ICP-MS

Authors: *C. M. ELITT[1,2], M. ROSS[1], J. WANG[1], L. BALAJ[3,2], M. B. BELFORT[4,2], P. A. ROSENBERG[1,2];
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Abstract: Very preterm infants are at high risk for brain injury and altered development in the neonatal period, leading to later cognitive, motor, and behavioral challenges including autism. The pathogenesis of brain injury and abnormal development remains unclear. Nutritional factors may be neuroprotective, but progress has been limited due in part to a lack of biomarkers that are feasible to assess in neonates. Exosomes, small membrane-bound extracellular vesicles (EVs) that can be isolated from peripheral human blood, provide a window into intracellular processes including gene expression in the brain. We piloted sequencing RNA from plasma extracellular vesicles (EVs). As a first approach, we compared EV gene expression in a very preterm cohort with an established adult cohort. We hypothesized that the gene expression profile in preterm infants would differ from adults. We also hypothesized that there would be variability in blood micronutrient concentrations between infants and within individual infants over time. From 8 very preterm infants (Cohort 1, gestational age, 23-30 weeks), we collected 0.5-1 mL of whole blood at term equivalent age. We isolated EV RNA from plasma using the Qiagen exoRNeasy kit and sequenced/analyzed RNA using the QIAseq Ultraplex RNA secondary analysis package. In a second group of very preterm infants (Cohort 2, n=5, gestational age, 23-30 weeks), we measured zinc, copper, and iron concentrations using inductively coupled plasma mass spectroscopy (ICP-MS) at birth, 2 weeks, 4 weeks, and discharge. In Cohort 1, mean infant plasma EV RNA concentration was 326 pg/uL (range, 79-1175). The mean number of unique genes identifiable in preterm infant samples was 6217 (range, 559-14165). The number of identifiable genes highly correlated with RNA concentration (r=0.91). Comparing gene expression between infant and adult cohorts, 562 genes were significantly different (adjusted $p<0.05$). In Cohort 2, mean zinc concentration per patient was 2320 ug/L (range, 1414-4305), mean copper concentration per patient was 870 ug/L (range, 717-1063) and mean iron concentration per patient was 335427 ug/L (range, 287730-447230). In conclusion, RNAseq can be performed on plasma EV RNA isolated from small volumes of blood from preterm infants. Significant differences in gene expression between preterm infants and adults supports the validity of this approach to study brain development and injury in preterm infants. Variability in micronutrient concentrations may reflect differences in intake, absorption, or clearance. Future work will consider how micronutrients may drive changes in gene expression relevant to brain development.

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Poster

268. Autism: Environment and Pathology

Location: SDCC Halls B-H

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Program #/Poster #: 268.08

Topic: A.07. Developmental Disorders

Support: Health Research Council of New Zealand (17/052)

Title: Efficacy of dietary zinc supplementation on reversing behavioral and synaptic deficits found in mouse models of autism spectrum disorder

Authors: *K. LEE[1], Y. JUNG[1], Y. VYAS[2], Z. MILLS[1], I. SKELTON[1], C. ABRAHAM[3], E. KIM[4], Y.-P. HSUEH[5], J. M. MONTGOMERY[1];
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Abstract: Autism Spectrum Disorders (ASDs) are neurodevelopmental disorders characterized by impaired social interaction and communication, and repetitive behaviours. Of numerous genetic mutations discovered in people affected by ASD, many converge on the pathways involved in synaptic transmission in the brain. This suggests that synaptic dysfunction, in part, contributes to the pathogenesis of ASD. Interestingly, low zinc levels are associated with ASD in humans, and zinc plays a critical role in modulating synaptic transmission. We have recently shown that increasing dietary zinc can reverse ASD-associated behaviours by restoring synaptic deficits in *Shank3*$^{-/-}$ mice - a mouse model of ASD with a mutation in the highly zinc-responsive synaptic protein SHANK3. Here, we have examined the efficacy and breadth of dietary zinc supplementation as an effective treatment strategy using other ASD mice: *Shank2*$^{-/-}$ and *Tbr1*$^{+/-}$. After weaning (21 days old), *Shank2*$^{-/-}$ and *Tbr1*$^{+/-}$ mice of both sexes were fed with either a normal zinc diet (30 parts per million; ppm) or a high zinc diet (150 ppm) for 6-8 weeks. Then we performed behavioural tests, electrophysiology and immunohistochemistry to validate the synaptic mechanisms underpinning the high zinc diet-induced behavioural rescue in these mice. In the three-chamber social behaviour test, both *Shank2*$^{-/-}$ (n = 9) and *Tbr1*$^{+/-}$ (n = 11) mice fed with a normal zinc diet displayed significantly reduced interaction time with a stranger mouse than an empty cup when compared to wild-type mice, and this social interaction deficit was corrected by high dietary zinc. For *Tbr1*$^{+/-}$ mice, high dietary zinc reversed the impairment in auditory fear memory in these mice through the restoration of *N*-methyl-D-aspartate receptor (NMDAR) function at thalamic-lateral amygdalar synapses. This was, in part, attributed to the high zinc diet-induced increase in the synaptic density of the GluN1 NMDAR subunit. In contrast, although *Shank2*$^{-/-}$ mice demonstrated a significantly reduced NMDAR-mediated synaptic response at hippocampal Schaffer collateral-CA1 synapses, the high zinc diet did not rescue NMDAR hypofunction nor induce changes in the synaptic GluN1 density. Together, our data demonstrate that the therapeutic outcome delivered by dietary zinc supplementation at the behavioural level differentially rescues synapse dysfunction, depending on the ASD-associated genetic mutation.

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Poster

268. Autism: Environment and Pathology

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Program #/Poster #: 268.09

Topic: H.12. Aging and Development

Support: P20GM103418- DRPP to Dr. Bethany Plakke
NIGMS- GM113109 NIH
Start up funds from K-State to Dr. Bethany Plakke

Title: Exercise improves cognitive performance in a rodent model of autism

Authors: *A. E. PAHUA, C. KING, T. DAVISON, I. MALI, M. PAYNE, B. PLAKKE; Psychological Sciences, Behavioral Neurosci., Kansas State Univ., Manhattan, KS

Abstract: Autism Spectrum Disorder (ASD) is classified as a neurodevelopmental disorder with core symptoms including, social deficits, impaired communication, and restricted, repetitive and stereotyped behaviors. Previous studies have found that exercise interventions improve spatial memory, cognitive ability and enhance behavioral outcomes. The current study examined the effects of aerobic exercise on attentional set-shifting in the valproic acid (VPA) rat model of autism. On gestational day 12, pregnant Long-Evans rats received a single dose intraperitoneal injection of either saline or VPA (600 mg/kg valproic acid). All behavioral data collection and brain segmentation was conducted by blind-to-condition researchers. Using a rodent treadmill, exercise was started on PND 40 and animals ran on a 0% slope for 30 minutes/day, 5 days/week for 4 weeks. Attentional set shifting was conducted in the fourth week of running. Data was separated by sex based on past findings. N=88 (Male: Exercise control 9, Sedentary Controls 9, VPA Exercise 12, VPA Sedentary 13; Female: Exercise control 10, Sedentary Control 9, VPA Exercise 13, VPA Sedentary13). A two-way ANOVA (condition: control vs VPA, treatment: exercise, sedentary) found main effects of treatment for both male and female rats. This indicated that exercise groups were significantly better at making extra-dimensional shifts of attention compared to sedentary groups. This behavioral data indicates that exercise can improve some forms of executive function even in a model of ASD. Past research demonstrated that overgrowth of lobule VI impaired cognition in VPA ras. Here, preliminary volume data indicates that exercise is modulating this overgrowth, where exercised rats have decreased lobule VI volumes compared to sedentary rats. This data supports the hypothesis that exercise can improve brain volume regulation mechanisms which may improve cognition within ASD.

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Poster

268. Autism: Environment and Pathology

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Program #/Poster #: 268.10

Topic: A.07. Developmental Disorders

Support: IBS R015-D1
NRF-2019M3E5D2A01060299
NRF-2019R1A2C1085566

Title: Idiosyncrasy of latent neural state dynamics in ASD during movie watching

Authors: *E. JI[1], J.-E. LEE[2,5], S.-J. HONG[3,5], W. SHIM[3,5,4];
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Abstract: Individuals with autism spectrum disorder (ASD) have difficulties in understanding other people's mental states and social narratives. Naturalistic paradigms such as a movie-watching fMRI provide a novel opportunity to examine dynamically changing brain states in typical and atypical conditions of children while they process narrative information. By leveraging a large sample data of ASD (n=87) and typically developing (TD; n=55) brains from Healthy Brain Network, here we examined the latent neural state dynamics of their large-scale functional networks during movie-watching fMRI. By fitting a hidden Markov model separately for TD and ASD subjects and finding corresponding states between the groups based on a matching algorithm, we inferred four low-dimensional latent states: the default mode network (DMN), dorsal attention network (DAN), somatosensory motor (SM), and base states. We found that neural state dynamics were more desynchronized across ASD subjects compared to the TD group. On the one hand, in the TD brains, DMN and DAN states were mainly alternated, each of which reflects internal and external information processing, respectively, in understanding the narrative structure of the movie (Song, Park, Park, & Shim, 2021). On the other hand, in ASD, in addition to DMN and DAN states, the SM state occurred at comparable frequencies and exhibited longer dwell times compared to those in TD, which may be associated with inattention or disengagement of ASD subjects during movie watching. These results suggest that the idiosyncrasy of neural state dynamics during processing naturalistic stimuli could link to the pathological neural mechanisms underlying ASD.

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Poster

268. Autism: Environment and Pathology

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Program #/Poster #: 268.11

Topic: A.07. Developmental Disorders

Support: NINDS NS088776

Title: Complement system signaling in the hippocampus is altered by neonatal status epilepticus

Authors: *D. S. COELHO, D. A. NARVAIZ, S. CHILUKURI, J. N. LUGO, Jr.;
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Abstract: The developing brain is uniquely susceptible to seizures. Seizures during the early postnatal period can have deleterious consequences to brain development leading to behavioral outcomes such as deficits in communication, cognition and increased anxiety. However, the mechanisms by which neonatal seizures disrupt neurodevelopment are unclear. Microglia are resident brain immune cells that respond to insults and exhibit important roles in development such as synapse pruning, maturation and function. Synaptic pruning is an important developmental process that has shown to be altered in developmental disorders such as autism spectrum disorder and schizophrenia. The pruning of synapses occurs during development in synapses that are tagged by the complement proteins C1q and C3. These complement proteins are increased in response to status epilepticus (SE) in adult rodents. Thus, we hypothesize that neonatal SE leads to the upregulation of the complement system. This increase in complement signaling can potentially alter synaptic pruning and lead to behavioral deficits later in life. To test this hypothesis mice were treated with 2 mg/kg kainic acid intraperitoneally at postnatal day 10 to induce SE. Following SE, hippocampal microglial activation was assessed at 1, 2, 3, 4, and 5 days, 2 weeks and 1 month after SE using flow cytometry to quantify MHCII+ myeloid cells. Expression of the complement system proteins C1q, C3 and C4 were measured at 1 day, 2 days and 2 weeks after SE by RT-qPCR. Our data indicate that microglial activation was significantly increased by SE ($p < 0.001$) with the highest activation occurring at one- and two-days post SE ($p < 0.001$). Complement system proteins expression was also altered in a time dependent manner. C4 expression was decreased one day after SE, and C3 and C1q expression was increased 2 weeks after SE ($p < 0.05$). Together, our findings suggest that microglial activation and complement system expression were altered after SE in a time-dependent manner. Full characterization of these cellular responses will enable us to identify a critical window where the use of therapeutic interventions might prevent the deleterious consequences that SE during early life can have on neurodevelopment.

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Poster

268. Autism: Environment and Pathology

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Program #/Poster #: 268.12

Topic: A.07. Developmental Disorders

Support: FAPESP
CNPq
INCT-Regenera
CAPES

Title: Zika virus infection impairs neurogenesis and increases the prevalence of neurodevelopmental disorders in children born with the congenital syndrome

Authors: C. BENAZZATO[1], F. LOJUDICE[2], F. RUSSO[1,4], A. MANUCCI[3], V. VAN DER LINDEN[5], P. JUNGMANN[6], M. C. SOGAYAR[2,7], A. B. CARDOSO[3], ***P. BELTRÃO-BRAGA**[1,4];
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Abstract: The Zika virus (ZIKV) infection has been associated with mild manifestations in humans,namely, fever, rash, arthralgia, and conjunctivitis. In 2015, the first cases of microcephaly weredescribed in the Northeastern region of Brazil associated with a vertical infection caused by ZIKV,later known as the Congenital Zika Syndrome (CZS). *In vitro* studies using cells infected with ZIKVshowed that it is neurotropic virus, leading to cell death. Astrocytes were reported as a reservoirof ZIKV in the fetal brain and mild apoptosis combined with inefficient antiviral response cancontribute to the establishment of a chronic cerebral infection associated with abnormalities ofneurodevelopment. However, the mechanisms underlying Zika infection duringneurodevelopment are still poorly known. Neural modeling of cells derived from children bornwith CZS could help to understand the impact of Zika infection during neurodevelopment. Here,we investigated the effects of ZIKV vertical infection using neurons and astrocytes derived frominduced pluripotent stem cells (iPSC) from babies born with CZS. Patients were followed sincebirth, being currently under 5-6 years of age. Patients did not present any neurological disorder,but some were diagnosed with Autism Spectrum Disorder (ASD). The neuroprogenitor cells(NPCs) produced from iPSCs developed into neurons and astrocytes, which did not showmorphological differences when compared with cells from the control group. However, neuronalproduction revealed a differentiation unbalance in neurons and astrocytes among CZS patients,since the ones diagnosed with ASD (named as CZS-B subgroup) presented fewer astrocytes duringneuronal differentiation. Investigation of the number of synapses in the iPSC-derived neuronsfrom both CZS-A and CZS-B groups showed lower levels of pre-and postsynaptic proteins and oftheir co-localization, indicating that these patients have a significant reduction in synapsescompared to the control group. In addition, CZS-B patients presented a significant reduction inpostsynaptic protein and its colocalization compared with the CZS-A subgroup. Moreover, lessglutamate production and uptake were observed in neurons and astrocytes derived from bothsubgroups of patients with CZS, but with similar patterns between patient subgroups. Thesepreliminary data reveal that ZIKV vertical infection probably causes permanent disturbance in thebrain during fetogenesis, even in the absence of the virus after birth.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Program #/Poster #: 269.01

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: I01 BX003759

Title: Neurotensin receptor 1 biased ligand attenuates neurotensin-mediated excitation of ventral tegmental area dopamine neurons and dopamine release in the nucleus accumbens

Authors: *S. M. SINGHAL[1], V. ZELL[1], A. B. PINKERTON[2,3], T. S. HNASKO[1,4];
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Abstract: Strong expression of the G-protein coupled receptor (GPCR) neurotensin receptor 1 (NTR1) in ventral tegmental area (VTA) dopamine neurons and terminals makes it an attractive target to modulate dopamine neuron function and release, and related behaviors or pathologies. Indeed, recent studies have identified a novel class of a biased ligand that acts on NTR1, and which has shown promising effects in preclinical models of addiction, without side effects associated with unbiased NTR1 agonism. In cells, SBI-0654553 (SBI-553) acts as a positive allosteric modulator of β-arrestin signaling downstream of NTR1 activation but antagonizes NTR1 G-protein signaling. Using cell-attached recordings from VTA dopamine neurons in acute brain slices from mice, we discovered that SBI-553 did not independently affect spontaneous firing, but blocked neurotensin (NT) mediated increase in firing. Moreover, SBI-553 also antagonized the effects of NT on dopamine D2 auto-receptor (D2R) signaling, potentially through its inhibitory effects on the G-protein coupled pathway. Using fast-scan cyclic voltammetry, we measured evoked dopamine release in the nucleus accumbens shell and also observed antagonistic effects of SBI-553 on NT-induced increase in dopamine release. Overall, these results suggest that SBI-553 has an inhibitory effect on mesolimbic dopamine activity and release, which could contribute to its efficacy in animal models of psychostimulant abuse.

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Poster

269. Neuropeptides and Other Signaling Molecules

Location: SDCC Halls B-H

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Program #/Poster #: 269.02

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH R01NS094597
NIH R21DA044515
M NIH T32GM067550

Title: Diversity of peptide neurotransmitter production modeled by neuropeptidome proteolysis of secretory vesicles at intravesicular and extracellular pH

Authors: *V. PHAN, S. PODVIN, Z. JIANG, M. C. YOON, V. HOOK, A. J. O'DONOGHUE; Skaggs Sch. of Pharm. and Pharmaceut. Sci., UCSD, La Jolla, CA

Abstract: Neuropeptides mediate cell-cell signaling as neurotransmitters in the nervous system. The neuropeptidome is the entire spectrum of peptides generated from precursors by proteolysis within dense core secretory vesicles (DCSV). DCSV neuropeptides and contents are released to the extracellular environment where further processing for neuropeptide formation may occur. To assess the entire DCSV proteolytic capacity for production of neuropeptidomes at intravesicular pH 5.5 and extracellular pH 7.2 conditions, neuropeptidomics, proteomics, and proteolysis analyzed by neuropeptide cleavage profiling were conducted using dense core secretory vesicles (DCSV) purified from adrenal medulla (known as chromaffingranules) as a model for neuropeptide biosynthesis. This secretory vesicle neuropeptidome consisted of 1239 unique peptides derived from 15 proneuropeptides that were colocalized with 64 proteases. Distinct neuropeptidomes were generated at the internal DCSV pH of 5.5 compared to the extracellular pH of 7.2. Class-specific protease inhibitors differentially regulated neuropeptidome production resulting from specific cleavages, involving aspartic, cysteine, serine, and metallo proteases. The cleavage properties of DCSV were further assessed by cleavage profiling assays using a synthetic peptide library containing diverse cleavage sites for endopeptidases and exopeptidases. Parallel inhibitor-sensitive cleavages for neuropeptidome production and peptide library proteolysis led to elucidation of six DCSV proteases possessing endogenous cleavage properties for neuropeptidome production, represented by cathepsins A, B, C, D, and L, as well as carboxypeptidase E (CPE). The cleavage profiles of these six enzymes represented the majority of DCSV proteolytic cleavages utilized for neuropeptidome production. These proteases have been reported to participate in the production of neuropeptides. These novel findings provide new insight into the DCSV proteolytic system for production of distinct neuropeptidomes at the internal CG pH of 5.5 and at the extracellular pH of 7.2.

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Poster

269. Neuropeptides and Other Signaling Molecules

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 269.03

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

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Title: Neuropeptidergic modulation of fear memories in the neocortex

Authors: *S. MELZER[1], E. NEWMARK[2], G. O. MIZUNO[3], B. RIGHETTI[4], L. TIAN[5], B. L. SABATINI[2];
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Abstract: Inhibitory neurons throughout the mammalian cortex are powerful regulators of circuit excitability and plasticity, thus controlling cortical functions such as learning, memory and perception. All major inhibitory cortical cell types are marked by differential expression of neuropeptide receptors, suggesting diverse peptidergic modulation of cortical processing. We found that the neuropeptide gastrin-releasing peptide (GRP) serves as an important regulator of cortical memory formation through selective targeting of one specific type of inhibitory neurons that is marked by vasoactive intestinal peptide (VIP) expression. Using in vivo imaging, CRISPR/Cas9-mediated knockout and a combination of molecular and electrophysiological techniques, we deciphered underlying signaling mechanisms and behavioral functions. Our data establish peptidergic regulation of cortical disinhibitory microcircuits as a mechanism to regulate auditory fear memories.

Disclosures: S. Melzer: None. **E. Newmark:** None. **G.O. Mizuno:** Other; Co-founder Seven Biosciences. **B. Righetti:** None. **L. Tian:** Other; Co-founder Seven Biosciences. **B.L. Sabatini:** None.

Poster

269. Neuropeptides and Other Signaling Molecules

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 269.04

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NSERC Grant 46292

Title: Investigating postsynaptic modulation of muscle contraction by neuropeptides in Drosophila

Authors: J. H. JUNG, Z. E. GAGNON, L. J. WASILEWICZ, J. MERCIER;
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Abstract: Body wall muscles of third instar Drosophila larvae are innervated by motorneurons that release glutamate and the neuropeptide, proctolin (RYLPT), as a co-transmitter. Glutamate depolarizes the muscles and elicits contraction, and proctolin enlarges contractions without altering the EJP. Another neuropeptide, DPKQDFMRFamide, is released as a neurohormone and increases contractions by increasing transmitter release and by direct action on muscle cells. We are examining postsynaptic effect of these peptides by examining their ability to alter contractions elicited by bath application of glutamate. Both peptides increase the peak amplitude of glutamate-evoked contraction, and both increase the peak amplitude of contractions elicited by caffeine, which releases calcium from the sarcoplasmic reticulum. By itself proctolin induces very small contractions, and its ability to enlarge glutamate-evoked contractions is much larger. The combined effect of proctolin and glutamate on contraction is much larger than the sum of the contractions elicited by each, indicating synergy. Glutamate increases calcium-induced fluorescence in larvae expressing GCaMP6 only in the plasma membrane of muscle cells. These calcium signals include an initial peak at about 500 ms followed by rapid decay. Proctolin increases the calcium signal after the initial peak, prolonging the increase in cytoplasmic calcium. Calcium release is normally limited by holo-calmodulin at high calcium levels, which inhibits ryanodine receptors. We examined the possibility that proctolin inhibits calmodulin and prevents ryanodine receptor inhibition, increasing cytoplasmic calcium. W7, a calmodulin inhibitor, increased glutamate-evoked contraction and induced contractions on its own. Unlike proctolin-induced contractions, however, W7-induced contractions were much larger, and they occurred in calcium-free saline. Thus, inhibition of ryanodine receptors by calmodulin does not precisely mimick proctolin's effect. Inhibiting calmodulin did not alter proctolin's ability to enhance glutamate-evoked contraction. We are currently using Shibire larvae to determine whether both peptides can modulate glutamate-evoked contractions when transmitter release is blocked at elevated temperature.

Disclosures: J.H. Jung: None. **Z.E. Gagnon:** None. **L.J. Wasilewicz:** None. **J. Mercier:** None.

Poster

269. Neuropeptides and Other Signaling Molecules

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 269.05

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NSERC

Title: Cholecystokinin and leptin act synergistically to modulate glutamate signaling in the rat dorsomedial hypothalamus

Authors: *K. CROSBY, K. MACKINNON, C. DESJARDINS;
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Abstract: Obesity is largely caused by an imbalance in food intake and energy expenditure. In the brain, the dorsomedial hypothalamus (DMH) is an important player in the regulation of appetite and metabolism. Cholecystokinin (CCK), a key satiety hormone, acts in the DMH to suppress food intake, but the underlying mechanisms are not well understood. There is evidence that CCK increases GABA release onto DMH neurons in rats, but despite reports of extensive glutamatergic projections to DMH neurons, it remains unknown whether CCK affects glutamate signaling in this region. We hypothesized that CCK decreases glutamate release onto putative orexigenic neurons in the DMH, an effect that would be consistent with the appetite-suppressing effects of CCK. To test this, we used young male Sprague Dawley rats and performed patch clamp electrophysiology to record glutamatergic currents in DMH neurons. We report that CCK had no effect on glutamate transmission onto DMH neurons. Because CCK has been reported to trigger long lasting changes in the strength of GABA synapses in the DMH following high frequency stimulation (HFS), we examined whether glutamate synapses exhibit synaptic plasticity. We failed, however, to observe any long-lasting changes in synaptic strength in the presence of CCK. CCK and leptin have been reported to act synergistically to regulate appetite, so we tested the effect of co-application of CCK and leptin onto DMH neurons and observed a decrease in glutamate signaling. We also observed a long-term depression following HFS in the presence of both CCK and leptin. Leptin alone had no effect on glutamate signaling. These results suggest that CCK and leptin act synergistically in the DMH to dampen glutamate signaling onto DMH neurons, an effect that could mediate the satiety effects of these hormones. Overall, this research provides important information on how CCK and leptin act in the brain to potentially modulate appetite.

Disclosures: K. Crosby: None. **K. MacKinnon:** None. **C. Desjardins:** None.

Poster

269. Neuropeptides and Other Signaling Molecules

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Program #/Poster #: 269.06

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Neuropeptide Y receptors modulate dopamine synaptic transmission in the nucleus accumbens core

Authors: *T. W. MURRAY[1], B. A. GRUETER[2], N. K. SMITH[3], T. R. HUNT[4], C. A. GRUETER[1];
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Abstract: Neuropeptide Y (NPY) is an abundant neuropeptide that is expressed in multiple brain regions, including the nucleus accumbens. Studies have shown that NPY signaling is involved in anxiolytic and anti-depressive behavioral effects. NPY has also been linked to stress regulation and adaption through its antagonistic effects on the behavioral consequences of stress. The effects of NPY in the brain are mediated through $G_{i/o}$-coupled receptors, Y1, Y2, and Y5. NPY, along with the Y1 receptor subtype, has one of its highest levels of expression in the NAc, which is involved in a range of adaptive stress responses. Exogenous infusion of NPY in the NAc induces a robust increase in extracellular dopamine as well as conditioned place preference in a dopamine receptor-dependent manner. However, the specific mechanism of NPY's effect on dopamine and the effects of stress on NPY-mediated DA signaling remains unknown. In this study, we investigated the effects of NPY and [Leu31, Pro34]-NPY (NPY Y1 receptor agonists) on the extracellular release of dopamine in acute NAc core-containing mouse brain slices using Fast Scan Cyclic Voltammetry (FSCV). NPY significantly increased dopamine release from baseline in both male and female mice. The Y1 receptor agonist caused a significant increase in dopamine release in the female mice but had mixed effects in the male mice. The effects of the Y1 receptor agonists in both the males and females was blocked by the addition of BIBO 3304 trifluoroacetate, a Y1 receptor antagonist. Ongoing studies address NPY-DA interaction following stress. This data highlights the NPY system's involvement in the regulation of mesolimbic dopaminergic neurotransmission, which could be a possible mechanism for NPY's involvement in stress adaption.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIMH Grant 5T K00 MH123667 04
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Burroughs Wellcome Fund PDEP

Title: Photopharmacological control of oxytocin signaling in the central nervous system and periphery

Authors: *I. AHMED[1], J. LIU[1], C. BAIR-MARSHALL[1], K. A. GIENIEC[2], B. HETZLER[3], A. B. ADEWAKUN[1], C. J. ARP[3], L. KHATRI[1], G. C. VANWALLEGHEM[4], A. SEIDENBERG[1], P. COWIN[1], D. TRAUNER[3], M. CHAO[1], F. M. DAVIS[4,2], R. TSIEN[1], R. C. FROEMKE[1]; [1]New York Univ. Grossman Sch. of Med., New York, NY; [2]Univ. of New South Wales Sch. of Med. Sci., Sydney, Australia; [3]New York Univ., New York, NY; [4]Aarhus Univ., Aarhus, Denmark

Abstract: Oxytocin is a nine amino acid neurohormone that is critical for lactation and other aspects of maternal physiology (Burbach et al. 2006; Valtcheva & Froemke 2019), as well as social behavior (Insel and Young 2001; Marlin et al. 2015), and is thought to be dysregulated in several neuropsychiatric disorders (Cochran et al. 2013). Despite the importance of this molecule for these biological and neurocognitive functions, methods are lacking to deliver and activate oxytocin with spatiotemporal specificity to the brain and periphery. For precise control of oxytocin signaling in mammalian tissues, we developed caged analogs of oxytocin which are functionally inert until cage release is triggered by ultraviolet light. We validated the caged compounds in vitro using a fluorescent calcium flux assay and calcium imaging in cell culture. We further demonstrated its utility in brain tissue by performing whole-cell electrophysiological recordings in acute brain slices of mouse auditory cortex. We demonstrated that photorelease of the caged-oxytocin cause significant membrane depolarization of OXTR+ neurons (n= 9, p=0.03) but not OXTR- neurons (n=5). The effect of photolyzed caged-oxytocin was similar to the pharmacological application of oxytocin peptide, which also depolarized OXTR+ neurons (n= 13, p=0.001) but not OXTR- neurons (n= 9). We also examined UV uncaging in mouse hippocampal CA2 neurons and observed a similar depolarization in OXTR+ neurons (n= 10). In absence of photorelease, caged-oxytocin was relatively inert in brain slices. In vivo, oxytocin uncaging accelerated the onset of mouse maternal behavior in virgin females co-housed with an experienced dam and litter (N=9, p = 0.03), comparable to the action of exogenous oxytocin or optogenetic stimulation of the oxytocin system (Marlin et al. Nature 2015; Carcea et al. Nature 2021). Finally, we tested our caged compound in peripheral tissue. We examined how focal versus global oxytocin application affected Ca^{2+} wave propagation in mammary glands. Together, these results demonstrate that photopharmacological control of caged peptides is a robust tool for modulating neuropeptide signaling throughout the brain and body.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Program #/Poster #: 269.08

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Title: Brain disturbed levels of testosterone in rats treated with oleoylethanolamide and palmitoylethanolamide

Authors: *G. VIANA[1], C. VALENCIA[2], R. HERNÁNDEZ[2], G. ARANKOWSKY-SANDOVAL[3], D. ALDANA[4], S. MACHADO[5], C. IMPERATORI[6], E. MURILLO-RODRÍGUEZ[1];
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Yucatán., [1]Lab. de Neurociencias Moleculares e Integrativas. Escuela de Medicina División Ciencias de la Salud. Univ. Anáhuac Mayab, Mérida, Mexico; [3]Ctr. De Investigaciones Regionales “Dr. Hideyo Noguchi” Univ. Autónoma de Yucatán, Mérida, Mexico; [4]Unidad Mérida, Lab. de Biología y Cultivo de Moluscos Ctr. de Investigación y de Estudios Avanzados del Inst. Politécnico, Mérida, Mexico; [5]Dept. of Sports Methods and Techniques, Federal Univ. of Santa Maria, Santa María, Brazil; [6]Dept. of Human Sci., European Univ. of Rome, Rome, Italy

Abstract: Our group has published that the peroxisome proliferator-activated receptor alpha (PPARα), a nuclear receptor, is linked to the regulation of the sleep-wake cycle. For instance, the activation of PPARα by endogenous ligands such as oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) promotes wake-inducing effects and blocks the sleep rebound period after total sleep deprivation. In addition, we have demonstrated that systemic injections of either OEA or PEA enhanced the extracellular contents of neurotransmitters related to modulation of wakefulness, such as acetylcholine and 5-hydroxytryptamine by engaging PPARα activation. Interestingly, emerging data have shown that PPARs are also regulated by hormones, such as testosterone. Here, we have investigated the effects of treatments of OEA or PEA on the contents of testosterone by using *in vivo* microdialysis techniques as well as HPLC means. For this purpose, OEA or PEA were systemically injected (5, 10 or 30 mg/kg; i.p.), and testosterone was addressed by the analysis of dialysates collected from a microdialsis probe placed at the basal forebrain, a wake-related brain area, in rats. Results showed that pharmacological challenges significantly decreased the levels of testosterone. Preliminary data suggest that OEA or PEA affects brain levels of testosterone in rats and such effect may have neurobiological significance in the sleep-wake cycle modulation by PPARα.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Program #/Poster #: 269.09

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: JSPS KAKENHI Grant 20H04048

Title: In vivo imaging of exercise-induced BDNF expression in the brain using Bdnf-Luc Tg mice

Authors: *H. MAEJIMA[1], R. IKEGAMI[2], T. INOUE[2], Y. TAKAMATSU[1], M. FUKUCHI[3], S. HAGA[4], M. OZAKI[4];
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Abstract: Exercise increases the expression of BDNF in the brain, and beneficially contributes to cognitive and sensory-motor function. So far, findings regarding exercise-induced BDNF expression in the brain are based on biochemical or histochemical analyses using brain tissues from euthanized animals. Thus, it has been impossible to detect time-course changes of BDNF expression after exercise in an individual animal. In this study, we measured time-course changes of exercise-induced BDNF expression in the brain of Bdnf-Luc Tg mice using in vivo bioluminescence imaging (BLI).Ten adult male Bdnf-Luc Tg mice were divided into two groups: a 0-4 h post-exercise group (n=5), in which BLI was performed between 0 and 4 h after the end of exercise, and a 4-8 h post-exercise group (n=5), in which BLI was performed between 4 and 8 h after exercise. Exercise intervention was carried out 5 days a week for 4 weeks. BLI was performed at the timing of pre-exercise, after a single exercise, after 2 weeks exercise, and after 4 weeks exercise. We performed BLI at 0, 15, 30, 45, 60, 120, and 240 minutes after a single administration of Akalumine-HCl (TokeOni) , a luciferase substrate. We calculated numerical integration using the trapezoidal approximation from a plot showing the luminescence intensity over 240 min after TokeOni administration, in addition to the measurement of luminescence level at each time point in both groups.Although there was no significant effect of exercise in the 0-4 h post-exercise group, BLI in a 4-8 h post-exercise group showed that a single exercise acutely increased the integrated luminescence intensity and repetitive exercise for 2 weeks also increased the intensity, indicating that BDNF expression was enhanced in the brain 4-8 h after exercise. Meanwhile, such a significant increase was not detected after four weeks of exercise. In addition, BLI showed that repetitive exercise for two weeks accelerated the timing of enhanced luminescence after exercise, i.e, a single exercise significantly increased the intensity 6h after exercise, whereas two week exercise significantly increased the intensity within 4h after exercise, suggesting that repetitive exercise enhance the sensitivity to BDNF expression triggered by acute exercise. This study firstly reports that exercise enhances BDNF expression in the brain in vivo based on BLI. This study showed that BDNF expression was enhanced by a single exercise intervention, and BDNF expression was accelerated after 2 weeks of exercise, but weakened after 4 weeks of exercise. These results indicate that BLI is a useful method to measure time-dependent modification of exercise-induced BDNF expression in the brain.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIMH Grant MH067842

Title: Viral mapping of MET receptor tyrosine kinase-expressing neuronal projection patterns from medial prefrontal cortex

Authors: *A. LANJEWAR[1], Z. M. KHAN[2], K. L. EAGLESON[1], P. LEVITT[1];
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Abstract: MET receptor tyrosine kinase (MET) is a synaptic regulator that peaks temporally in the cerebral cortex during the active period of synaptogenesis. In medial prefrontal cortex (mPFC), a cortical region involved in higher order cognitive functions, MET is expressed during development in a subset of subcerebral projection neurons (SCPNs), while largely absent from intralencephalic PNs. The role of the neuron subtype-specific expression of MET in mPFC remains unknown. To determine 1) the specific projection targets of MET^+ mPFC neurons and 2) whether there are projection target specificities of MET^+ mPFC SCPNs compared to overall mPFC SCPNs, connectomics analyses was performed. At postnatal day (P) 12 in a transgenic mouse line that expresses high levels of green fluorescent protein (GFP) in MET^+ neurons (*Met*^{GFP} mice), the mPFC was stereotaxically injected with Cre Dependent on GFP viruses (CRE-DOG) and flex-tdTomato to permanently label the mPFC MET-GFP expressing neurons and their axons with tdTomato. A separate cohort of *Met*^{GFP} mice were injected in mPFC at P12 with AAV2/1-hSyn-mTurqoise2 virus to permanently label all transfected neurons at the injection site and their axons with mTurqoise2, independent of MET-GFP. Brains were then collected at various ages between P19 and P60 and processed for immunofluorescence and confocal microscopy. We find that MET^+ mPFC projections are abundant in many of the expected mPFC subcerebral targets, including modest axonal labeling in midline dorsal thalamic nuclei and dense labeling in preoptic areas of the hypothalamus and zona incerta. Surprisingly, very few labeled axons were present in the basolateral amygdala, a target that receives dense innervation from mPFC neurons, independent of MET. These results suggest that MET^+ mPFC SCPNs target specific subcortical areas, providing an opportunity to perform future studies on the involvement of MET-specific circuits in specific functions.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: VA Grant BX 11049
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NIH Grant R41MH113398

Title: Antidepressant properties of ketamine and psychedelics: A common cellular pathway?

Authors: A. KOUTSOURIS[1], A. GUNAY[1], J. SCHAPPI[3], ***M. M. RASENICK**[2,4,5]; [2]Univ. of Illinois at Chicago Col. of Med., [1]U. Illinois Col. of Med., Chicago, IL; [3]Jesse Brown VAMC, chicago, IL; [4]Jesse Brown VAMC, Chicago, IL; [5]Pax Neurosci., Glenview, IL

Abstract: Previous data suggest that the heterotrimeric G protein, Gsalpha (Gsα) is ensconced predominantly inlipid rafts in subjects with major depressive disorder (MDD), resulting in impaired stimulation ofadenylyl cyclase. Both diminished Gsα-adenylyl cyclase coupling and an increase in the proportion ofGsα in lipid rafts have been observed in MDD. Antidepressants accumulate slowly in lipid rafts andevoke translocation of Gsα out of lipid rafts toward a more productive association with adenylyl cyclase,resulting in sustained cAMP elevation and sequelae such as increased brain-derived neurotrophicfactor. We hypothesize that heightened accumulation of Gsα in lipid rafts is a biomarker for depression,and that the translocation of Gsα from those rafts is a biomarker for clinical response to antidepressants."Rapid-acting" antidepressant compounds, such as ketamine, have the same effect, but on an acceleratedtimescale. Some clinical data suggest that psychedelics, at hallucinogenic doses, evoke a long-lastingantidepressant response after a single dose. This study sought to compare the effects of these variouscompounds on cellular features of antidepressant response using cultured cells (C6 glioma, SK N SHneuroblastoma or astrocytes induced from fibroblasts derived from MDD or control subjects) exposed tofluoxetine, desipramine or ketamine (all 10 µM) or LSD (10 nM) or psilocin (psilocybin metabolite-50nM). cAMP was determined by Alpha Screen or fluorescent reporters.There were distinct differences in the response profile for the compounds. Fluoxetine and desipramineregquired three-day exposure to translocate Gsα from rafts and increase Gsα-activated adenylyl cyclase.The effects of ketamine, while the most rapid in onset, declined over a 24-hour period, returning to pre-drug values after that time. However, both LSD and psilocin (1 hr exposure followed by washout) gave aprolonged antidepressant biosignature. All experiments were repeated 4-6 times in triplicate.Antidepressants, psychedelics and ketamine all show an antidepressant biosignature, revealing amobilization of Gsα from lipid rafts and an increased signaling of GPCRs working through Gsα.Previous studies in postmortem brain and in platelets showed that this biomarker was enriched in lipidrafts from depressed subjects and reverted toward control values after effective treatment (as indicated byHAM-D). The similarities and differences in the actions of these drugs at the cellular level may lead to abetter molecular understanding of the distinct kinetic features of their actions.

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Poster

269. Neuropeptides and Other Signaling Molecules

Location: SDCC Halls B-H

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH Grant: 5R01NS107523

Title: Regulating Effector T Cell Differentiation Through Slc7a5 Amino Acid Transporter in Experimental Models of MS

Authors: *M. COZART, J. HU, M. BAYDYUK, J. HUANG;
Georgetown Univ., Washington, DC

Abstract: Multiple Sclerosis (MS) is an autoimmune disease mediated by the infiltration of autoreactive T cells into the central nervous system (CNS). T cells contribute to both acute and chronic inflammation, causing loss of the myelin sheath, a protective layer surrounding axons and made by oligodendrocytes in a process called demyelination. Excessive demyelination leads to axonal damage and progressive neurodegeneration. Interestingly, upon demyelinating injury, myelin producing oligodendrocyte precursor cells (OPCs) are recruited to the site of damage, where they differentiate into mature oligodendrocytes and restore myelin in an endogenous process known as remyelination. Unfortunately, with disease progression, remyelination fails to occur for reasons not fully understood. One possibility for remyelination failure is unresolved inflammation in the CNS caused by effector Th1 and Th17 cells. Here we hypothesize that a large neutral amino acid transporter, known as Slc7a5, promotes T-cell expansion following demyelination. We found that Slc7a5 is upregulated in CNS lesions after lysolecithin-induced demyelination in mice. To examine the effect of Slc7a5 loss of function in CD4+ T-cells on remyelination, lysolecithin-induced demyelination was performed on Slc7a5f/f CD4-Cre mice. We found that the Slc7a5f/f CD4-Cre mice displayed significant increase in oligodendrocytes and remyelination in lesions compared to control mice. Moreover, analysis of T-cell population from splenocytes of Slc7a5f/f CD4-Cre mice revealed a significant decrease in Th1 and Th17 cells and no change in regulatory T cells, or Tregs, that are known to reduce inflammation and promote remyelination. Our results suggest that Slc7a5 inhibition in T-cells reduces inflammation, leading to endogenous remyelination, and that Slc7a5 may be a potential novel immunomodulatory target for improving remyelination in MS.

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Poster

269. Neuropeptides and Other Signaling Molecules

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HHMI

Title: Synaptotagmin 9 modulates spontaneous neurotransmitter release in striatal neurons by regulating substance P secretion

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Abstract: Synaptotagmin 9 (SYT9) is a tandem C2-domain Ca^{2+} sensor for exocytosis in neuroendocrine cells; its function in neurons remains unclear. In this work, we characterized the role of SYT9 in neuronal cell biology and synaptic physiology using cultured neurons from WT and *Syt9 KO* mice. We show, via whole-cell voltage-clamp experiments, that SYT9 does not trigger rapid synaptic vesicle exocytosis in cultured cortical, hippocampal, or striatal neurons; rather, synaptotagmin 1 (SYT1) fulfills this function. We also demonstrate, via quantitative immunoblotting, that SYT9 is expressed in cultured cortical, hippocampal, and striatal neurons at levels that are ~25x less than SYT1. Only upon significant over-expression, can SYT9 rescue some degree of fast, synchronous neurotransmitter release in *Syt1* KO cortical neurons, but this is an artifact resulting from mislocalization of the over-expressed protein. We went on to observe that miniature inhibitory postsynaptic current (mIPSC) frequency was decreased in *Syt9* KO striatal neurons, with no differences observed in cortical or hippocampal neurons. To begin to elucidate the role of SYT9 in regulating mIPSCs in striatal neurons, we found, via immunocytochemistry, that endogenous SYT9 was colocalized with substance P (SP), a neuropeptide that is packaged into dense core vesicles (DCV). The neuropeptide is enriched in striatal tissue and is an agonist to the neurokinin 1 receptor (NK1R). To address whether SYT9 regulates SP secretion, we expressed a SP-pHluorin fusion protein in cultured striatal neurons and observed reductions in release in *Syt9* KOs. To determine whether SP secretion contributes to mIPSC frequency, we measured mIPSC frequency in *Syt9* KO striatal neurons in the presence of exogenous SP in the bath solution; SP supplementation fully rescued the *Syt9* KO mIPSC phenotype. Additionally, when WT striatal neurons were treated with SR140333, an NK1R antagonist, we observed a decrease in mIPSC frequency that phenocopied the *Syt9* KO phenotype. These results suggest that SYT9 serves as a Ca^{2+} sensor for SP secretion from striatal neurons, and that this secretion regulates spontaneous synaptic transmission in striatal neurons. These findings reassign the function of SYT9 in striatal neurons and open the way for future study of the function of SYT9 in other neuron types and brain regions.

Disclosures: M. Seibert: None. **C. Evans:** None. **K. Stanley:** None. **Z. Wu:** None. **E. Chapman:** None.

Poster

269. Neuropeptides and Other Signaling Molecules

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Program #/Poster #: 269.14

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH MH094527 to RDB
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Title: Serotonin transporter (SERT) Ala276 mouse: novel model to assess the biochemical, physiological, and behavioral impact of SERT Thr276 phosphorylation in vivo

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Abstract: Serotonin (5-hydroxytyramine, 5-HT) is as an essential neuromodulator of several fundamental processes, including mood, cognition, and social behavior. The presynaptic 5-HT transporter (SERT) mediates the clearance of extracellular 5-HT, tightly regulating the availability of 5-HT for synaptic and extra synaptic signaling. Consequently, alterations in SERT function have been proposed to contribute to the etiology of 5-HT associated disorders such as depression and anxiety for which the 5-HT selective reuptake inhibitors (SSRI), which antagonize SERT, are widely prescribed. Ample evidence has demonstrated that SERT function is amenable to regulation via kinase-mediated phosphorylation. SERT phosphorylation has been detected following PKC, PKG, GSK3 and p38 MAPK activation and leads to changes in catalytic activity or surface expression. *In vitro* studies revealed that SERT Ala276 is a key site targeted by PKG and that phosphorylation at this residue biases SERT conformation, leading to changes in activity and drug responses. Using SERT Ala276 knock-in (KI) mice, we are investigating the requirement for SERT Thr276 phosphorylation for 5-HT neurotransmission as well as basal and drug modulated behaviors. Preliminary data from surface biotinylation studies in dorsal striatum brain slices of adult male mice revealed an increase in SERT surface expression after treatment with the PKG activator 8-Br-cGMP, consistent with prior cell culture studies, whereas Ala276 mice show elevated basal surface SERT and a decrease in SERT surface levels after 8-Br-cGMP treatment. Recently, we reported that SERT Ala276 homozygotes displayed sex-dependent alterations in repetitive and social behavior. SERT Ala276 females exhibit a decrease in marble burying whereas males exhibit a decrease in social dominance in the tube test. Interestingly, conducting the three-chamber social preference test revealed that females, but not males, display a decrease in social preference compared to WT controls. We hypothesize that males may require SERT Thr276 phosphorylation to sustain social interactions whereas females require SERT Thr276 phosphorylation to motivate social engagement. Ongoing efforts seek to examine basal and regulated surface expression in acute brain slices from SERT WT and Ala276, and the consequences and reversal of these alterations *in vivo* with circuit-specific amperometry and fiber photometry. The results of our studies will allow us to understand how SERT regulation contributes to synaptic 5-HT homeostasis and behavior in normal and pathological states and thereby provide insights into mechanisms underlying neuropsychiatric disorders.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NICHD intramural program

Title: ProNRG2 accumulates at neuronal ER-PM contacts via VAP binding and independently of Kv2.1

Authors: *D. VULLHORST, M. S. BLOOM, N. AKELLA, A. BUONANNO;
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Abstract: Junctions between the endoplasmic reticulum (ER) and the plasma membrane (PM) are involved in important processes including calcium homeostasis, lipid transfer and regulation of intrinsic neuron excitability. We recently reported that the transmembrane proform of Neuregulin 2 (proNRG2), an important ligand of the ErbB4 receptor that regulates synaptic function and plasticity, selectively accumulates at ER-PM contacts in cortical and hippocampal GABAergic interneurons. ProNRG2 puncta are closely associated with Kv2.1, a voltage-gated potassium channel that interacts with VAP (VAMP-associated protein), an ER-transmembrane protein mediating membrane contacts with the PM and various intracellular organelles. ProNRG2 is regulated by glutamatergic signaling via NMDA receptors, like Kv2.1, and its accumulation at membrane contacts requires two conserved sequence elements located in its intracellular domain known as C- and D-boxes. However, the protein(s) targeting proNRG2 to ER-PM contacts are not known. As Kv2.1 clusters have been suggested to serve as microdomains involved in trafficking of other plasma membrane proteins, we began by exploring its possible involvement using shRNA-mediated knockdown and found that Kv2.1 is dispensable for proNRG2 targeting to ER-PM contacts. Furthermore, immunocytochemistry and immunogold electron microscopy of AAV-transduced neurons indicated that proNRG2 overexpression promotes the formation of ER-PM contacts. Because of the highly similar subcellular localization of Kv2.1 and proNRG2, we then tested whether VAP independently interacts with both Kv2.1 and proNRG2 and indeed detected VAP but not Kv2.1 in proNRG2 immunoprecipitates from cultured neurons, brain tissue and heterologous 293 cells. Unexpectedly, although neither the C- nor the D-box conform to a canonical FFAT motif, proNRG2 variants lacking either one failed to co-immunoprecipitate VAP, indicating that high-affinity proNRG2-VAP interactions involve cooperative VAP binding to two separate and noncanonical FFAT motifs in the proNRG2 ICD. Evidence from point mutation and GST pulldown experiments furthermore indicated that the C-box harbors a cryptic and phosphorylation-dependent VAP binding site (similar to the Kv2.1 PRC motif). In summary, our findings reveal proNRG2 as an independent organizer of neuronal ER-PM contacts and suggest that autocrine proNRG2/ErbB4 signaling and Kv2.1 channels function synergistically to

homeostatically regulate intrinsic excitability in GABAergic interneurons in response to NMDA receptor activation.

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Poster

269. Neuropeptides and Other Signaling Molecules

Location: SDCC Halls B-H

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Program #/Poster #: 269.16

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: 610-5210100-60057013-01

Title: Bone Morphogenic Protein Antagonism is Regulated by Neuronal Activity in the Neurogenic Niche

Authors: *S. DUNLOP[1], C.-Y. PENG[2], J. A. KESSLER[2];
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Abstract: Background: The birth of newborn neurons or neurogenesis generates new granule cell neurons that mature and incorporate into the hippocampal dentate gyrus circuit throughout the lifetime. Changes in the rate of neurogenesis are associated with changes in cognitive performance, memory, and affective behavior. Previously, our group has shown that bone morphogenic protein (BMP) signaling is a powerful negative regulator of hippocampal neurogenesis, but it is currently unknown how BMP signaling is regulated within the neurogenic niche. Here we aimed to investigate the interrelationship between BMP signaling and dentate granule neuron activity using both in vivo and in vitro models. Methods: Hippocampal neuronal progenitor cellss were cultured as neurospheres from P0-3 B6C57 wildtype mice in the presence of noggin 250ng/ml and EGF 10ng/ml. Neurospheres were dissociated and cells were plated on poly-D-lysine laminin coated adherent plates and differentiated for 28-32 days to maturity with DMEM F-12 containing NT-3 and BDNF. Prior to collection, cultures were treated with KCl to induce neuronal depolarization or vehicle. Cells were subsequently collected for protein analysis by Western blot and RNA for RT-qPCR. Additionally, we stereotaxically injected 8-12 week old wildtype B6C57 mice with an GFP expressing AAV 2/9 Camkii virus expressing either an activating (Dq) or inhibiting (Di) DREADD. DREADDs were activated by the inert clonazapine derivative CNO. Brains of these animals were then collected for both immunohistochemistry and RT-qPCR and Western analyses. Results: We observed an activity mediated increase in the expression of the BMP antagonist, noggin, with KCl-induced depolarization in cultured dentate granule neurons. Depolarization increased both noggin transcript and protein expression *in vitro* and *in vivo*. Animals expressing neuronal activating DREADD receptors (hM3Dq) showed similar activity related increase in noggin *in vivo* in response to receptor activation, indicating that hippocampal BMP antagonism is activity dependent. The effects of silencing the activity of mature granule neurons on noggin expression, both *in vitro* and *in vivo*, are in progress.

Conclusion: We localized the BMP inhibitor, noggin, to dentate gyrus granule cells. *In vitro* analyses demonstrate that noggin expression is regulated by neuronal activity. Activation of DREADDs expressed in mature granule neurons *in vivo* similarly leads to changes in BMP signaling. This work suggests that the level of BMP signaling in the adult neurogenic niche is modulated by neuronal activity through the regulation of the expression of the BMP antagonist noggin.

Disclosures: S. Dunlop: None. **C. Peng:** None. **J.A. Kessler:** None.

Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: 21H02342
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22H02494

Title: Analyses of the effect of adrenomedullin on pulsatile luteinizing hormone secretion

Authors: *Y. OSHIMO[1], M. SATO[1], F. MAGATA[1], S. OHKURA[2], F. MATSUDA[1];
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Abstract: [Background] The pulse mode of gonadotropin-releasing hormone (GnRH) release from the hypothalamus and following luteinizing hormone (LH) pulses from the pituitary induce follicular development in female animals. Kisspeptin neurons distributed in the hypothalamic arcuate nucleus (ARC) are suggested to play key roles to control GnRH/LH pulses. However, the central mechanisms regulating GnRH/LH pulses remain to be elucidated. Adrenomedullin (AM), a secretory peptide expressed in various tissues and cells including the hypothalamic paraventricular nucleus and supraoptic nucleus, is known as a biomarker of various diseases. In this study, we aimed to reveal the effects of AM on pulsatile LH secretion and investigate the pathway to induce the effects. [Method] Eight-week-old Wistar-Imamichi female rats that were ovariectomized and treated with 17β-estradiol (OVX+E_2 rats) received brain surgery so that the guide cannula for microinjection was implanted into the lateral ventricle (LV). Six days later, serial blood sampling was performed every 6 minutes for 3 hours. During the first 10 minutes of blood sampling, AM dissolved in 10 μL of saline (0 nM, 0.1 nM, or 0.5 nM) was microinjected into the LV. After the blood sampling, rats were perfused with 4% paraformaldehyde and brains were collected. LH concentrations in plasma were measured by radioimmunoassay. Brain sections including the ARC were subjected to *in situ* hybridization of the kisspeptin gene (*Kiss1*) and the number of *Kiss1*-expressing cells was counted. Brain sections from OVX+E_2 rats without any treatment were subjected to double immunohistochemistry (IHC) of either GnRH or kisspeptin with each of the AM receptor components (CALCRL or RAMP2). [Results] The

group injected with 0.1 nM AM showed lower mean basal LH levels compared to the control group, suggesting the negative effect of AM on LH secretion. The number of *Kiss1*-expressing cells did not differ between AM-injected and control groups, suggesting that AM did not influence the *Kiss1* expression. Double IHC of GnRH and CALCRL or RAMP2 and kisspeptin and CALCRL or RAMP2 did not detect colocalization of AM receptors on GnRH neurons nor ARC kisspeptin neurons, suggesting that AM does not directly affect those neurons. [Conclusion] The present results indicate that AM in the brain has a suppressive effect on LH secretion. In addition, the effect is unlikely to be caused by the direct action of AM on ARC kisspeptin neurons nor GnRH neurons.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: The Council of Higher Education (CoHE) of Turkey funded Esra Candar under "100/2000 CoHE Ph.D. Scholarship Program" in the field of "Translational Medicine."

Title: Chemoarchitecture of human dorsal, lumbar precerebellar and sacral precerebellar nuclei

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Abstract: The dorsal nucleus (D) is located in the medial part of lamina IV in T1(2)-L2(3), lumbar precerebellar nucleus (LPrCb) in lamina VII of L1-L5, and sacral precerebellar nucleus (SPrCb) in lamina VII of S1-Co1 in the human spinal cord. Thus far, studies using retrograde neuronal tracers have shown that D, LPrCb, and SPrCb neurons project to the cerebellum. Neuronal loss in the precerebellar nuclei leads to multiple system atrophy. In this study, we aimed to show the detailed chemoarchitecture of these nuclei in the human spinal cord. T1-T12, L1-L5, S1-S5, and Co1 human spinal cord segments fixed with 10% formalin were transversely cut at a thickness of 30 µm and stained with calbindin, calretinin, parvalbumin, glutamate decarboxylase 65/67 (GAD 65/67), cocaine- and amphetamine-regulated transcript peptide (CART), neuronal nuclear antigen (NeuN), choline acetyltransferase (ChAT), substance P (SP), serotonin, and enkephalin immunohistochemistry. Under light microscopy, we observed that all these neuronal markers are found in neurons of these precerebellar nuclei. Our data demonstrate a marked heterogeneity in the anatomical distribution of neurotransmitter markers in the

precerebellar nuclei. This anatomical and functional diversity may lead to diverse influences these precerebellar nuclei may exert upon the cerebellum.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: the National Institute of Mental Health Intramural Research Program, Project 1ZIAMH002386 to L.E.E.

Title: Characteristics of transcription and protein expression of RapGEF2, a guanine nucleotide exchange protein that links cAMP elevation to ERK activation in neurons and neuroendocrine cells

Authors: *D. BAKALAR, W. XU, M. SUNG, L. EIDEN;
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Abstract: The neuritogenic cAMP sensor NCS-RapGEF2 is a neuroendocrine-specific molecule connecting cAMP elevation to the activation of ERK (Emery et. al., 2013, Sci. Sig). Rapgef2 mRNA comprises two families of transcript variants that represent two distinct promoter sites in the Rapgef2 gene. One set of transcripts (NCS-Rapgef2) is expressed at high levels in adult rodent brain, with the other (NN-Rapgef2) expressed in all tissues (Jiang et. al., 2017, eNeuro). RapGEF2 protein expression correlates with that of the NCS-Rapgef2 transcript. Expression of NCS-Rapgef2 in cells lacking RapGEF2 protein confers RapGEF2 protein expression and cAMP-dependent ERK activation, while in RapGEF2-producing cells, deleting or silencing the transcript abrogates both. The role of the peripherally expressed NN transcript is unknown. We hypothesize that the NCS transcript is obligatory for protein production, and that cells with no protein will express only the NN transcript. We investigated this hypothesis in mouse brain, where IHC reveals RapGEF2 immunoreactivity in NeuN+ neurons but not in GFAP+ glia. ISH reveals that while neurons express both NCS and NN transcripts, Sox9+ astrocytes and Olig2+ oligodendrocytes do not express Rapgef2 transcripts of either variety. This raises the possibility of different mechanisms for restriction of RapGEF2 expression in brain and periphery: Astrocytes and oligodendrocytes exclude RapGEF2 production by not expressing either family of transcript, but the NN transcript is present peripherally without protein production. We have produced NS1 cells in which the Rapgef2 gene is mutated, preventing protein production (Xu et. al., 2021, JNE). PiggyBac-mediated expression of NCS or NN Rapgef2 in these cells will clarify the role of the transcript variants in protein production and stability.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NSF IOS-1755004

Title: Ror2 homodimerization activates a neuronal wnt signaling cascade to increase trafficking of nmdars

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[2]Physiol. & Biophysics, [1]Univ. of Washington, Seattle, WA; [3]Physiol & Biophysics, Univ. Washington, Seattle, WA

Abstract: RoR2 belongs to a conserved family of tyrosine kinase receptors that acts as a Wnt receptor and is involved in developmental processes, angiogenesis, cell movement, and cell polarity. Furthermore, RoR2 remains in the mature Central Nervous System in different areas such as hippocampus and is present in dendrites, regulating neural process and synapses homeostasis. In this case, RoR2 plays an important role in synapse formation in neuronal primary cultures where its downregulation results in decreased synaptic contacts. Depending on the cellular context, RoR2 can exerts its function as homodimer or heterodimer with other Wnt receptors to activate or repress transcription of different proteins. We had described a novel noncanonical Wnt signaling cascade in neurons that requires RoR2. Wnt5a, but not Wnt7a, increases dendritic intracellular Ca2+, activates PKC and JNK kinases in dendrites, and increases trafficking of NMDA-type glutamate receptors to synapses in a RoR2-dependent manner, as its knockdown prevents signaling (McQuate et al. 2017). This increase in synaptic content of NMDARs lowers the threshold for long-term potentiation (LTP), a form of synaptic plasticity underlying memory formation. Whether neuronal RoR2 acts by itself or in association with a co-receptor is not known. Using chemically induced dimerization of recombinant RoR2, we tested whether homodimerization of RoR2 is sufficient to activate the signaling cascade leading to increased trafficking of NMDA receptors. In HEK293 cells RoR2 homodimerization by itself can activate RoR2 autophosphorylation and activates PKC and JNK. Additionally, in dissociated culture neurons RoR2 homodimerization increase cytosolic calcium and increases trafficking of NMDARS. These results suggest that RoR2 homodimerization is sufficient to activate the neuronal Wnt signaling pathway present in the mature CNS.

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Poster

269. Neuropeptides and Other Signaling Molecules

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Title: Anterograde delivery of Rab10-organelles regulates the sorting of internalised TrkB for retrograde axonal transport: a flexibility factor

Authors: *O. LAZO, G. SCHIAVO;
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Abstract: Neurons process real-time information coming from axon terminals to coordinate complex cellular responses, including gene expression, growth and plasticity. Input from distal axons is encoded as a stream of endocytic organelles termed signalling endosomes, which are targeted to the soma. Formation of these organelles depends on target-derived molecules, such as brain-derived neurotrophic factor (BDNF), which is recognised by TrkB receptors on the plasma membrane, endocytosed and transported to the soma along the microtubules network. Notwithstanding its physiological and neuropathological importance, the mechanism controlling the sorting of TrkB to signalling endosomes is currently unknown. In this work, we used primary mouse brain neurons and advanced confocal microscopy to uncover the small GTPase Rab10 as critical for TrkB sorting and propagation of BDNF signalling from axon terminals to the soma. We manipulated the expression and activity of Rab10 in neurons cultured in microfluidic devices, and study axonal transport, sorting of receptors and recruitment of motor proteins. Our data demonstrate that Rab10 defines a class of axonal organelles, which are rapidly mobilised towards the axon terminal upon BDNF stimulation, enabling the axon to fine-tune retrograde signalling depending on BDNF availability at the synapse. These results suggest that Rab10 provides flexibility to the endosomal system and help to clarify the neuroprotective phenotype recently associated to Rab10 polymorphisms in Alzheimer's disease, providing novel therapeutic targets for neurodegenerative conditions.

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Poster

270. Potassium Channels

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Support: NIH NINDS grant F31NS110192
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Title: Modulating Hippocampal Glutamate Release with Kv2-Mediated Endoplasmic Reticulum/Plasma Membrane Junctions

Authors: *L. PANZERA[1], B. JOHNSON[2], I. CHO[1], M. TAMKUN[3], M. HOPPA[1];
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Abstract: Endoplasmic reticulum/plasma membrane (ER/PM) junctions are subcellular hubs important for mediating calcium homeostasis, protein localization and lipid exchange. Although a number of ER proteins can interact with lipids on the PM, specific proteinaceous PM anchors that form stable ER junctions have remained elusive. The voltage-gated potassium channel Kv2.1 is the first PM protein found to engineer ER/PM junctions through interaction and recruitment of the transmembrane ER protein VAMP-associated protein (VAP). While Kv2.1 has a canonical conducting role in shaping electrical activity in the somatodendritic compartment, a non-conducting population of Kv2.1 channels form ER/PM junctions occupying ~10% of the neuronal soma. These sites are involved in delivery of membrane proteins to the PM in addition to being sites of neuron-glia contact. Here, we present data in cultured hippocampal neurons that loss of Kv2.1 decreases the ability of the somatic ER to fill with calcium during electrical stimulation. More importantly, and in contrast to Kv2.1 dogma, we go on to demonstrate that Kv2.1 is enriched in synaptic terminals where it exclusively engages in a non-conducting role, significantly altering both ER calcium handling and synaptic function. Using noninvasive optical indicators, we reveal that genetic knockdown of Kv2.1 impairs stimulation-evoked calcium uptake into the ER and decreases synaptic vesicle exocytosis. Further, localization of Kv2.1 as well as VAP recruitment to terminals were found to be important for maintaining the unique role of these ER/PM junctions with regards to important aspects of synaptic physiology.

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Poster

270. Potassium Channels

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Program #/Poster #: 270.02

Topic: B.03. Ion Channels

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Title: Differential expression of electrically silent KvS subunits creates diversity in Kv2 channels in brain

Authors: *M. J. FERNS[1], D. VAN DER LIST[2], N. C. VIERRA[2], E. BLANK[2], J. S. TRIMMER[2];
[1]Anesthesiol. & Pain Med. and Physiol. & Membrane Biol., [2]Physiol. and Membrane Biol., Univ. of California Davis, Davis, CA

Abstract: Voltage-gated K+ channels of the Kv2 family are highly expressed in brain and play dual roles in regulating neuronal action potentials and membrane excitability, and in organizing specialized endoplasmic reticulum - plasma membrane (ER-PM) junctions on neuronal cell bodies and proximal dendrites. Numerous studies have defined the expression, localization and function of the Kv2.1 and Kv2.2 subunits which form homo- or hetero-tetrameric channels in brain neurons. However, studies suggest that these Kv2 subunits may also co-assemble with a related family of "electrically silent" (KvS) subunits. Most notably, when co-expressed in heterologous cells KvS subunits co-assemble with Kv2 subunits to form hetero-tetrameric channels with unique biophysical properties, and KvS subunit mRNA expression overlaps with that of Kv2.1 and Kv2.2 in brain neurons. To begin to define the contribution of KvS subunits in mammalian brain we characterized their protein expression, localization and contribution to Kv2 channels in rodent brain. In mass spectrometry-based proteomic experiments, we identified four KvS subunits present in Kv2.1 complexes immunopurified from mouse brain, with Kv5.1 being the most abundant (~16% of spectral abundance of Kv2.1). Focusing on Kv5.1, we found that it co-immunoprecipitated together with Kv2.1 and Kv2.2 from brain lysates, being a component of >15% of Kv2.1 and 6% of Kv2.2-containing channels. That Kv5.1 protein levels are decreased by 70% in Kv2.1 knockout (KO) mice and 95% in Kv2.1/2.2 DKO mice supports that Kv5.1 expression in brain is dependent on its co-assembly into hetero-tetrameric Kv2 channels (Kv2.1 > Kv2.2). Multiplex immunofluorescent labelling of rodent brain sections revealed that Kv5.1 is highly expressed in neocortex, where it is present in a large percentage of Kv2-positive neurons in layer 2/3, and in a smaller percentage of neurons in layers 5 and 6. Kv5.1 is extensively colocalized at the cellular level with both Kv2.1 and Kv2.2, however the ratios of Kv2.1, Kv2.2 and Kv5.1 immunolabeling vary widely between neurons even in the same cortical layer. At the subcellular level, Kv5.1 is co-clustered with Kv2 subunits at ER-PM junctions in cortical neurons, Finally, we found that Kv5.1 plasma membrane localization and clustering is significantly reduced in cortical neurons in Kv2.1 KO mice, providing further evidence that Kv2.1/Kv5.1 hetero-tetrameric channels predominate over Kv2.2/5.1 channels in rodent cortex. Together, these findings demonstrate that KvS subunits create an unappreciated level of diversity in Kv2 channels in brain, and likely regulate Kv2 channel function in a neuron-specific manner.

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Poster

270. Potassium Channels

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Support: R21NS101648
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Title: SPHKAP links depolarization to activation of type I PKA at neuronal ER-PM junctions formed by Kv2.1 channels

Authors: *N. C. VIERRA[1], M. KIRMIZ[1], L. RIBEIRO-SILVA[1], D. VAN DER LIST[1], P. BHANDARI[2], O. A. MACK[3], J. CARROLL[3], S. A. AICHER[3], R. SHIGEMOTO[2], J. S. TRIMMER[1];
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Abstract: Spatial and functional segregation of intracellular signaling machinery is critical for protein kinase A (PKA) signaling, which regulates diverse neuronal processes including synaptic transmission, gene expression, and electrical excitability. However, specific mechanisms organizing PKA in the aspiny regions of neurons, especially the soma, are poorly understood. Contacts between the endoplasmic reticulum (ER) and the plasma membrane (PM) provide platforms that facilitate compartmentalized signaling in all eukaryotic cells. Prominent ER-PM junctions are formed in many brain neurons by Kv2.1, a voltage-gated potassium channel whose C-terminus tethers the PM to the ER. We used mass spectrometry-based proteomics to define the molecular composition of Kv2.1-mediated ER-PM contacts as a starting point to identify their specific neuronal functions. We previously showed that these sites on neuronal somata regulate local membrane lipid turnover and form unique calcium signaling microdomains that couple firing to gene expression. Here, we show that type I PKA is also highly concentrated on ER cisterns at Kv2.1-mediated ER-PM junctions, recruited by the type I PKA-specific anchoring protein SPHKAP (SPHK1 interactor, AKAP domain containing protein). Immunolabeled brain sections from mice and rats of both sexes and cultured rat hippocampal neurons display robust co-clustering of Kv2.1, SPHKAP, and type I PKA. Immuno-electron microscopy revealed SPHKAP as part of the dense matrix found between and surrounding subsurface ER cisterns in neuronal somata. Reciprocal regulation of PKA and calcium signaling machinery is a key mechanism to coordinate their signaling pathways, and we find that L-type calcium channels (LTCCs) and ryanodine receptors co-localize with type I PKA, SPHKAP, and Kv2.1. shRNA knockdown of SPHKAP in hippocampal neurons reduced the amount of PKA molecules near LTCCs, impaired depolarization-evoked calcium influx, and decreased depolarization-induced activation of CREB and c-Fos, transcription factors involved in learning and memory. Moreover, SPHKAP-dependent anchoring of type I PKA was critical for LTCC-triggered PKA substrate phosphorylation. Proteomic analysis of phosphorylated PKA substrates immunopurified from cultured neurons showed that depolarization triggers PKA phosphorylation of targets involved in homeostatic plasticity. Together, these data reveal that SPHKAP targets type I PKA to Kv2.1-mediated ER-PM junctions where it participates in a novel complex that compartmentalizes PKA signaling in the neuronal soma to link depolarization-induced calcium entry, PKA activity, and gene expression.

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Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.04

Topic: B.03. Ion Channels

Title: Dehydroepiandrosterone sulfate (DHEAS) is an endogenous Kv7 channel modulator

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Abstract: Kv7 potassium channels are low voltage activated potassium channels. One of important physiological roles of Kv7 channels is reversible increase of excitability of neurons or myocytes by suppression of Kv7 currents upon activation of Gq-coupled receptors. The pathway toward Kv7 current suppression involves phospholipase C and an essential co-factor, phosphatidylinositol 4,5-bisphosphate (PIP2). PIP2 is required to maintain the ion-conducting conformation of Kv7 channels. Loss of PIP2 from Kv7 subunit is the major mechanism for Kv7 current suppression.
However, whether responsiveness of Kv7 channel is dynamically regulated or not remains unknown. We found that 10 μM DHEAS, a steroid hormone, reduces Kv7 current suppression induced by activation of m1 muscarinic receptors, when assessed by Kv7.2 or Kv7.5 homomeric channels. Ci-VSP induced PIP2 depletion experiments suggested that this reduced responsiveness of Kv7 channels is due to stabilization of the PIP2-bound conformation of Kv7 channels. *In silico* modeling suggests that high affinity binding of DHEAS to Kv7 channel is an underlying mechanism for this modulation. Exogenous DHEAS administration (10 mg/kg, i.p.) in mice attenuated the late phase nociception in formalin. These results suggest that modulation of Kv7 channel responsiveness can modify nociception.

Disclosures: **N. Hoshi:** None. **L. Alhassen:** None. **O. Civelli:** None.

Poster

270. Potassium Channels

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.05

Topic: B.03. Ion Channels

Support: 1K99GM146028
NIDDK-KUH R25, San Antonio Program for Undergraduate Research in Renal Science (SPURRS)

Title: Characterization of PIP2 Binding Sites and Affinity and Phosphorylation Modulation of Calmodulin and PIP2 Binding on Kv7.2 Ion Channel

Authors: *D. NYANCHO[1], K. CAO[2], C. ARCHER[3];
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Abstract: Kv7 channels are voltage-gated K^+ membrane channels expressed throughout the body. In the brain, they are critical for modulating neuronal excitability. Structural studies of Kv7 channels show that the channel is a tetramer of varying combinations of subunits Kv7.1-5. Kv7 channels are regulated by G protein-coupled receptors by altering levels of three key signaling molecules: Ca^{2+}- regulated calmodulin (CaM), phosphatidylinositol 4,5-bisphosphate (PIP2), and protein kinase C (PKC). The regulatory domain (Kv7RD) of Kv7 channels is the proximal carboxyl terminus region containing binding or action sites for these key signaling molecules. PIP2 has three binding sites on Kv7RD: S6Jx, AB linker, and B-Ext. Also within the Kv7RD, CaM has two binding domains called the A and B helices. PIP2-binding to Kv7 channels is associated with channel opening, whereas Ca^{2+}-CaM and PKC binding are associated with decreased channel activity. Kv7.2 is the subunit of interest in this study due to more than 80 mutations mapped to its gene resulting in neonatal epilepsies and encephalopathies. These mutations lie within the binding domains for PIP2, CaM, and PKC. PKC action on Kv7.2 is different from other subunits in that the phosphorylation site is a serine rather than threonine at residue 527 (S527), and Kv7.2 has an additional phosphorylation site at S520. Our preliminary data showed that phosphorylation by PKC reduced the affinity of Kv7.4 for CaM and PIP2. We hypothesize that phosphorylation of Kv7.2 will have the same effect. This study seeks to establish whether CaM and PIP2 compete for binding to Kv7.2 and to determine phosphorylation's role in modulating CaM and PIP2 to Kv7.2. The CaM binding sites have been well characterized for Kv7.2, however the PIP2 binding sites have are not fully characterized outside of functional patch-clamp studies. We investigated PIP2 affinity for Kv7.2 utilizing short synthetic peptides and purified Kv7.2RD fused to His-MBP. Microscale thermophoresis and fluorescence polarization assays showed that PIP2 directly binds multiple sites within the Kv7.2RD. We used a similar approach to show how phosphorylation of S527 and S520 impact CaM and PIP2 binding affinities to the Kv7.2RD. Following these assays, we used a structural biophysics approach to explore further how phosphorylation affects the ability of CaM to compete with PIP2 for binding the Kv7.2RD. Since Kv7 channels help regulate neuronal excitability, the results of this study will contribute to our understanding of how Kv7 channels are regulated. This can translate to improved therapies for seizures, stroke, pain, and traumatic brain injuries.

Disclosures: **D. Nyancho:** None. **K. Cao:** None. **C. Archer:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.06

Topic: B.03. Ion Channels

Support: NIH 1R01-NS073875

Title: Cloperastine reduces morphine-induced respiratory depression in rats: Potential mechanisms for GIRK channel inhibition in locus coeruleus neurons

Authors: *H. YAO, H. XING, N. CUI, C. M. JOHNSON, C. JIANG;
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Abstract: Background: Respiratory depression is the main cause of death in opioid drug misusage. Currently the only available therapeutic is naloxone that also blocks certain desired opioid effects. Here we show evidence of alleviating the respiratory depression with the antitussive cloperastine (CPS). Studies were performed in conscious rats in vivo and in brains slices in vitro. Results: In plethysmography, CPS (30 mg/kg, s.c.) alleviated, but not eliminated, morphine-induced breathing depression. CPS oral treatment (30 mg/kg/day, 7-day) shifted LD50 of morphine (i.p.) from 106 mg/kg to 130 mg/kg. Morphine increased K+ current in GIRK2 / μ-opioid receptor (MOR) double transfected HEK cells, which was reversed by 5μM CPS. Morphine hyperpolarized locus coeruleus (LC) neurons, raised their input resistance and decreased firing activity, all of which were reversed by CPS. Removal of neuronal firing activity with 0.5 μM TTX reduced the cellular responses of both morphine and CPS. In the presence of 0.5 μM TTX, the morphine-induced hyperpolarization can be eliminated by CPS, suggesting that both pre- and postsynaptic mechanisms are involved. The presynaptic cells are likely to be AMPA glutamatergic as CNQX had a similar effect to TTX. The presynaptic mechanisms involved GIRK channels in synaptic terminals, as CPS increased mEPSCs amplitude and frequency in LC cells to the same degree as the metabotropic glutamate receptor blocker LY341495, and had no further effect after LY341495. Conclusion: CPS alleviates morphine-induced breathing depression, blocks MOR-mediated GIRK2 channel activation, and improves morphine LD50. In LC neurons, CPS seems to counteract morphine via blocking GIRK channels 1) in the LC cells, 2) in presynaptic glutamatergic neurons, and 3) in glutamatergic synaptic terminals, all of which have excitatory effects on LC neurons in contrast to the inhibitory effects of morphine. Therefore, it is likely that CPS interacts with morphine signaling at multiple acting sites.

Disclosures: H. Yao: None. **H. Xing:** None. **N. Cui:** None. **C.M. Johnson:** None. **C. Jiang:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.07

Topic: B.03. Ion Channels

Support: FRM

Title: Synaptic and intrinsic potentiation in O-LM interneurons is induced by theta patterns of stimulation

Authors: *M. SAMMARI, Y. INGLEBERT, N. ANKRI, M. RUSSIER, S. INCONTRO, D. DEBANNE;
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Abstract: Oriens lacunosum-moleculare (O-LM) interneurons display a non-conventional form of long-term synaptic potentiation (LTP) conferred by calcium-permeable AMPA receptors (CP-AMPAR). So far, this form of LTP has been induced in O-LM cells by physiologically unrealistic protocols. We report here the induction of both synaptic and intrinsic potentiation in O-LM interneurons following stimulation of afferent glutamatergic inputs in the theta (θ) frequency range. LTP is induced by synaptic activation of CP-AMPAR whereas long-term potentiation of intrinsic excitability (LTP-IE) results from the mGluR1-dependent down-regulation of Kv7 voltage-dependent potassium channel and hyperpolarization activated and cyclic nucleotide-gated (HCN) channel through the depletion of phosphatidylinositol-4,5-bi-phosphate (PIP2). LTP and LTP-IE are reversible, demonstrating that both synaptic and intrinsic changes are bidirectional in O-LM cells. We conclude that physiological stimuli such as θ patterns induce synaptic and intrinsic potentiation in O-LM interneurons.

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Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.08

Topic: B.03. Ion Channels

Support: 1F31AG074665-01
R01NS114130

Title: Kv1.3 potassium channels exhibit domain specific protein interactions in activated microglia.

Authors: *C. BOWEN[1], Y. LIN[1], H. M. NGUYEN[3], P. BAGCHI[2], D. DUONG[2], P. KUMAR[1], C. ESPINOSA-GARCIA[1], A. NATU[1], H. WULFF[3], N. T. SEYFRIED[2], S. RANGARAJU[1];
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Abstract: Alzheimer's Disease (AD) is characterized by progressive Aβ pathology and neuroinflammation. Disease-activated microglia in the brain, with potential contributions from peripheral T cells, promote to neuroinflammation in AD. The Kv1.3 potassium channel is highly expressed on proinflammatory microglia as well as memory T cells. Prior work showed that

blockade of Kv1.3 reduces Aβ pathology and decreases the proinflammatory phenotype of microglia. The molecular mechanisms regulated by Kv1.3 channels in microglia and T cells remain incompletely understood. A better understanding of Kv1.3-regulated mechanisms and pathways can be obtained by identifying proteins that interact with N and C terminal cytosolic domains of Kv1.3 channels. We utilized TurboID, a biotin ligase that biotinylates proteins within a 10nm proximity, fused to Kv1.3 and validated Kv1.3-TurboID fusion constructs in HEK Cells. We created three constructs, where TurboID was fused to the N terminus, C terminus, and a truncated Kv1.3, where the PDZ-binding domain is removed. We transduced these constructs into BV2 cells, a murine microglial cell line, and Jurkat T-cells, a human T-cell line, to determine potential immune interactors with Kv1.3. BV-2 and Jurkat T-cell stable cell lines were created and confirmed via qPCR and electrophysiology. Western Blot and Flow cytometry confirm TurboID biotinylates proteins. Mass spectrometry (MS) of biotinylated proteins was performed to identify proteins within proximity to both domains of Kv1.3. BV2 biotinylated proteomes identified by MS revealed distinct N terminal and C terminal Kv1.3 interactors. Many of the Kv1.3 interactors overlapped between the N and C terminus in the presence or absence of LPS inflammatory stimulation. The N terminus interacts with translation (e.g. Rpl10 and Eef1a1), plasma membrane proteins (e.g. Calr1 and Psma1), and mitochondrial tracking proteins (e.g. TIMM23), while the C terminus interacts with immune response associated proteins (e.g. Cd68, Tlr2, and Csf1). With the removal of the C terminal PDZ-binding domain, we observed reduced immune response and inflammatory proteins (e.g. Tmem106b, Larp1, and Gbp2) interacting with Kv1.3. This indicates that immune interactors with Kv1.3 likely depend on the C terminal PDZ domain while the primary function of the N terminus is protein processing and transport to the plasma membrane. Overall, this data identifies strong candidates for potential interactors with Kv1.3 and provides insight on how Kv1.3 may be influencing microglial and leukocyte immune function in AD.

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Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.09

Topic: B.03. Ion Channels

Support: NIH Grant DC01919

Title: Modulation of Potassium Channels in Auditory Neurons Preserves Temporal Fidelity

Authors: *L. K. KACZMAREK;
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Abstract: Potassium channels in auditory neurons are rapidly modified by changes in the auditory environment. In response to elevated auditory stimulation, short-term mechanisms such as protein phosphorylation and long-term mechanisms linked to channel synthesis increase the activity of channels that promote high frequency firing. It has been suggested that this allows neurons to fire at high rates in response to high sound levels. We have now used simple simulations of cochlear hair cells and postsynaptic neurons to demonstrate that the amplitudes of potassium currents in neurons required for optimal encoding of a low-level auditory signal differs substantially from that for louder sounds. Specifically, the cross correlation of the output of a neuron with an auditory stimulus is increased by increasing potassium currents as sound amplitude increases. This correlation is, however, entirely independent of firing rate because combinations of currents that maximize firing to a stimulus provide very poor temporal fidelity. The simulations provide an explanation for the modulation of the intrinsic excitability of auditory brainstem neurons by changes in environmental sound levels. We carried out further simulations using much slower patterns of simulations that are unrelated to auditory stimuli but match those expected in other brain regions and sensory modalities. These simulations also demonstrated the same phenomenon, that temporal information in a weak pattern of synaptic inputs is most faithfully preserved with low amplitude potassium currents but that potassium currents must be increased for fidelity at high rates of synaptic stimulation. These findings suggest that modulation of potassium currents to preserve temporal information as the intensity of stimulation changes may be a general rule in the nervous system.

Disclosures: L.K. Kaczmarek: None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.10

Topic: B.03. Ion Channels

Support: NIH Grant DC01919
NS111242

Title: A Kv3.3 channel mutant that fails to trigger actin nucleation impairs intrinsic excitability and firing patterns in the cerebellum

Authors: *Y. WANG[1], Y. ZHANG[3], X.-B. GAO[4], M. C. CRAIR[2], T. L. HORVATH[5], L. K. KACZMAREK[3];
[1]Yale Univ., [2]Dept. of Neurosci., Yale Univ., New Haven, CT; [3]Yale Univ. Sch. Med., New Haven, CT; [4]Yale Univ. Schoo of Med., New Haven, CT; [5]Comparative Med., Yale Med. Sch., New Haven, CT

Abstract: The Kv3.3 voltage-dependent potassium channel is expressed at high levels in Purkinje cells of the cerebellum, as well as in several auditory brainstem nuclei. This channel

activates and deactivates rapidly in response to depolarization and plays an important role in neuronal firing. In addition to its role in intrinsic neuronal excitability, Kv3.3 also binds several cytoplasmic proteins, including Hax-1 and TBK1, to stimulate actin nucleation at the plasma membrane. Recent work, using auditory and hippocampal neurons, has shown that the presence of Kv3.3 adjacent to presynaptic release sites is required for both slow and rapid endocytosis of synaptic vesicles and for normal recovery from synaptic depression after repetitive firing. Mutations in gene for Kv3.3 gene lead to Spinocerebellar Ataxia type 13(SCA13), a neurodegenerative movement disorder. One such mutation, Kv3.3-G592R, produces channels that have normal conduction properties but fail to nucleate actin filaments. To test the effects of this mutation on excitability we have begun to carry out patch clamp recordings of Purkinje neurons in Kv3.3- G592R knock-in mice, and to evaluate global patterns of cerebellar activity using wide field imaging. Using patch clamp recordings, we found that the amplitude of voltage-gated K^+ currents of Purkinje cells in acute cerebellar slices medium in normal extracellular medium was not significantly different between wild-type and Kv3.3- G592R mice. In the presence of a low concentration of TEA (1 mM) sufficient to block Kv3 currents, however, we found that residual K^+ currents were significantly larger at positive potentials in neurons from Kv3.3- G592R mice than in those of wild-type neurons. To determine whether global calcium dynamics are also abnormal in the mutant cerebellar Purkinje neurons, we carried wide field calcium imaging in both wild type and G592R Kv3.3 knock-in mice cerebellum. AAV9-Syn-GCaMP6s virus was injected into both wild type and mutant mice at age P0-P1. Three weeks after injections, cerebella were sliced and calcium activity was measured in Purkinje cells. Preliminary data showed the following: 1. Spontaneous bursts were more frequent in the control compared to the knock-in. 2. Average event durations were smaller in the control. 3. dF/F peak magnitudes were similar in two groups. These results suggest that the Kv3.3- G592R point mutation undermines the excitability of cerebellar Purkinje neurons, resulting in decreased burst frequency and disrupted calcium dynamics during individual events, as evidenced by the prolonged duration of the bursts in Purkinje cells expressing the disease-causing mutation.

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Poster

270. Potassium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 270.11

Topic: B.03. Ion Channels

Support: NIH grant DC01919 to LKK
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Autifony Therapeutics Ltd

Title: Regulation of axonal outgrowth by the Kv3.4/PCDH9 protein complex is mediated by the WAVE complex

Authors: *Y. ZHANG[1], M. CHATTERJEE[1], N. DELUCA[1], J. WU[1], W. LAM[1], S. KUMAR[1], G. LIN[2], D. NAVARATNAM[1], L. SONG[2], L. K. KACZMAREK[1];
[1]Yale Univ. Sch. Med., New Haven, CT; [2]Shanghai Ninth People's Hosp., Shanghai, China

Abstract: Kv3.4 is a voltage-dependent potassium channel that plays an important role in neuronal growth cone guidance and pathfinding during embryonic development. It remains highly expressed in subsets of neurons in the adult nervous system, including granule cells of the cerebellum. In this study, we investigated the mechanism of how Kv3.4 channels regulate neurite outgrowth. A yeast two- hybrid screen using the Kv3.4 C-terminus as bait found the major Kv3.4- interacting protein to be protocadherin 9 (PCDH9), a calcium-dependent cell-cell adhesion molecule that is known to be linked to the WAVE actin-nucleating complex. This was confirmed by co-immunoprecipitation in both Kv3.4 expressing cell lines and mouse brain tissue. In contrast, no interaction with PCDH9 was found for the closely related channel subunit Kv3.3. We found that expression of Kv3.4 protein and current absolutely requires its interaction with PCDH9. Expression and currents of Kv3.4 were markedly reduced or abolished when the PCDH9 was knocked down from Kv3.4 expressing cells but recovered on overexpression of human PCDH9 into the PCDH9 knock-out cells. To determine whether the Kv3.4-PCDH9 interaction alters the actin cytoskeleton of cells, we stained F-actin filament with phalloidin. We found the length of filopodia containing F-actin was longer in Kv3.4 expressing CHO cells compared those in untransfected CHO cells or in Kv3.4 cells with PCDH9 knockdown. In cultured cerebellar granular cells from Kv3.4 knockout mice, both the length and number of branches of neurites were reduced compared to those in the wild type. The same effect was observed in cerebellar granule neurons when PCDH9 was knocked down in neurons from wild type mice. In addition, the pattern of migration of cerebellar granule cells and Purkinje neurons were delayed in the cerebellum of the Kv3.4 knockout mice. Because PCDH9 contains a WIRS (Wave Regulatory complex Interacting Sequence), we infected cerebellar granule cells with a peptide containing the PCDH9-specific WIRS sequence to disrupt the link between PCDH9 and the WAVE complex. We found that the length and branches of cerebellar granule cells were reduced in wild type mice to a much greater degree than in Kv3.4 knock out mice. Our results strongly suggest that the normal function of Kv3.4 channel requires its binding to PCDH9 and that the effects of this protein complex on neurite outgrowth are mediated by the WAVE complex.

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Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.12

Topic: B.03. Ion Channels

Title: Kv4.2 channel regulate synaptic strength via Ca^{2+}- dependent signaling mechanism in dentate granule cells

Authors: *S. LEE[1], W.-K. HO[2];
[1]Physiol., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [2]Physiol., Seoul Natl. Univ. Col. Med., Seoul, Korea, Republic of

Abstract: A-type voltage-gated K^+ currents (I_A), mediated by Kv4 family channels, are known to play key roles in regulating neuronal excitability. In mature granule cells (GCs) of the dentate gyrus, Kv4.1, Kv4.2 and Kv4.3 are known to be expressed and these channels show distinct subcellular distribution. Previously, we reported that Kv4.1, which is highly expressed in soma of granule cell, is a key player in regulating firing frequency in mature GCs. Unlike Kv4.1, Kv4.2 is highly expressed in dendrites of mature GCs as well as in CA1 pyramidal neurons, suggesting its role in regulating dendritic excitability and synaptic integration. In the present study, we investigated the contribution of Kv4.2 to regulating synaptic integration in GCs by blocking Kv4.2 channels using Kv4.2 antibody in pipette solutions. We stimulated lateral perforant pathways at 10 s interval for 30 minutes, and recorded excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs) from the same cells. Changes in EPSP and EPSC amplitudes were monitored while antibodies were diffused into the cells, and the amplitudes obtained 5 min and 10 min after patch break-in were regarded as control and Kv4.2 block condition, respectively. Kv4.2 antibody induced potentiation of EPSC amplitude (142 ± 5.3%) as well as EPSP amplitude (160± 8.5%). However, Kv4.2 antibody did not affect NMDAR-mediated EPSC or inhibitory postsynaptic potentials in GCs. These results suggest that EPSP potentiation by Kv4.2 inhibition is largely mediated by potentiation of AMPA currents and increased dendritic excitability play a minor role. We demonstrated that the increase in EPSC by Kv4.2 antibody was abolished by 10 mM BAPTA in intracellular solution and PKC inhibitor (GF109203X) or calmodulin inhibitor (calmidazolium) preincubation in extracellular solution. These results suggest that Kv4.2 inhibition induces AMPA receptor recruitment in synaptic sites in Ca^{2+}- and PKC/CaM-dependent manners. Kv4.3 antibody did not induce significant change in EPSC nor EPSP amplitude. Collectively, this study demonstrates specialized role of Kv4.2 in regulating synaptic strength at excitatory synapses in mature granule cells.

Disclosures: **S. Lee:** None. **W. Ho:** None.

Poster

270. Potassium Channels

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Program #/Poster #: 270.13

Topic: B.03. Ion Channels

Support: Brains and Behavior Fellowship
NIH Grant NS116327

Title: Pka-mediated phosphorylation at s552 blocks pias3-mediated sumoylation at k579 when kv4.2 is in the ternary complex

Authors: *L.-A. JANSEN[1], L. A. MIDDLETON[2], M. A. WELCH[3], D. J. BARO[4];
[1]Georgia State Univ., Loganville, GA; [2]Biol., [3]Georgia State Univ., Atlanta, GA; [4]Dept Biol, Georgia State Univ. Dept. of Biol., Atlanta, GA

Abstract: Kv4.2 is a voltage-activated K^+ channel that contributes to the transient outward current I_A in the brain and I_{tof} in the heart. In native tissue,Kv4.2 forms a ternary complex with the K^+ Channel Interacting Protein 1-4 (KChIP1-4) and the dipeptidyl peptidase-like protein 6/10 (DPP6/10). These proteins exert influence over channel gating, trafficking and the effects of post-translational modifications. In hippocampal neurons, PKA-mediated phosphorylation of Kv4.2 at S552 reduces I_A by inducing clathrin-mediated internalization of the channel. A recent study on channels comprising only Kv4.2 and KChIP2 suggested that S552 phosphorylation caused dissociation of Kv4.2-KChIP2 interaction in lipid rafts. It is not clear that the same is true for the ternary complex. Downstream of this PKA site, is a known SUMO site, K579. When Kv4.2 is expressed in the ternary complex, enhanced SUMOylation at K579 augments rab11a-dependent recycling of endocytosed channels back to the plasma membrane, thereby enhancing surface expression and I_A maximal conductance (Gmax). This effect was not seen when Kv4.2 was expressed alone, and it was not studied in channels comprising only Kv4 and KChIP2 subunits. Phosphorylation can play a permissive role in SUMOylation, and if S552 phosphorylation blocked K579 SUMOylation, then it would increase channel internalization by reducing the recycling of endocytosed channels. This hypothesis was tested using HEK cells overexpressing the ternary complex (Kv4.2, KChIP2a, DPP10). SUMOylation was enhanced by co-expressing the E3 SUMO ligase, PIAS3 (aka KChAP). PIAS3 produced a 58% rab11a-dependent increase in I_A Gmax (139 ± 14.95nS vs 87.72 ± 6.158nS, t-test, p=0.0008). The effect of PIAS3 was blocked by superfusion of the PKA activator, 8-Bromo-cAMP (95.35 ± 9.731nS vs 139.0 ± 14.95nS, t-test, p=0.0231). In order to determine if phosphorylation at S552 blocked the effect of PIAS3, site-directed mutagenesis was used to create phosphodeficient (S552A) and phosphomimetic (S552E) Kv4.2 mutants. HEK cells expressing mutant ternary complexes were transiently transfected with PIAS3 and superfused with 8-Bromo-cAMP. The effect of 8-Bromo-cAMP on the PIAS3 induced increase in I_A Gmax was blocked by S552A (141.3 ± 14.69nS) and was mimicked (93.77 ± 5.124nS) and occluded (108.5 ± 6.982nS) by S522E (one way ANOVA, $F(6,56) = 6.190$, p=<0.0001). These data are consistent with the hypothesis that S552 phosphorylation blocks K579 SUMOylation when Kv4.2 exists in a ternary complex, and the effect of S552 phosphorylation varies according to the make-up of the Kv4.2 channel.

Disclosures: **L. Jansen:** None. **L.A. Middleton:** None. **M.A. Welch:** None. **D.J. Baro:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.14

Topic: B.03. Ion Channels

Support: American Heart Association Grant 13SDG16150007
National Ataxia Foundation Grant YI-SCA
NIH Grant 4R33NS101182–03

Title: Genetic Mutations of $K_{Ca}2.3$ and $K_{Ca}3.1$ Channels Affect Calcium Sensitivity

Authors: *R. ORFALI, Y.-W. NAM, M. ASIKUR RAHMAN, M. DOWNEY, G. YANG, M. ZHANG;
Sch. of Pharm., Chapman Univ., Irvine, CA

Abstract: Small- and intermediate-conductance Ca^{2+}-activated potassium ($K_{Ca}2.x$ and $K_{Ca}3.1$, also called SK and IK) channels are voltage-independent. They are activated exclusively by intracellular Ca^{2+}. Heterozygous genetic mutations of $K_{Ca}2.3$ channels have been associated with Zimmermann-Laband syndrome (ZLS) and idiopathic noncirrhotic portal hypertension (INCPH), while $K_{Ca}3.1$ channel mutations were reported in hereditary xerocytosis (HX) patients. We measured the apparent Ca^{2+} sensitivity of $K_{Ca}2.3$ and $K_{Ca}3.1$ heterologously expressed in HEK293 cells, using inside-out patch clamp recordings. Wild-type $K_{Ca}2.3$ channels have a Ca^{2+} EC_{50} value of ~0.3 μM, while the apparent Ca^{2+} sensitivity of wild-type $K_{Ca}3.1$ channels is ~0.27 μM. ZLS and INCPH-related $K_{Ca}2.3$_S436C and $K_{Ca}2.3$_V450L channels with mutations in the $S_{45}A/S_{45}B$ helices exhibited increased Ca^{2+} sensitivity. The corresponding mutations in $K_{Ca}3.1$ channels also elevated the apparent Ca^{2+} sensitivity. HX-related $K_{Ca}3.1$_S314P, $K_{Ca}3.1$_A322V and $K_{Ca}3.1$_R352H channels with mutations in the HA/HB helices are hypersensitive to Ca^{2+}, whereas $K_{Ca}2.3$ channels with the corresponding mutations are not. The different effects of the equivalent mutations in the HA/HB helices on the apparent Ca^{2+} sensitivity of $K_{Ca}2.3$ and $K_{Ca}3.1$ channels may imply distinct modulation of the two-channel subtypes by the HA/HB helices. AP14145 reduced the apparent Ca^{2+} sensitivity of the hypersensitive mutant $K_{Ca}2.3$ channels, suggesting the potential therapeutic usefulness of negative gating modulators.

Disclosures: R. orfali: None. **Y. Nam:** None. **M. Asikur Rahman:** None. **M. Zhang:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.15

Topic: B.03. Ion Channels

Title: Computational Modelling of the Dopamine Neuron Reveals Cocaine Exposure Modulates the Firing Patterns by Inhibiting Small Conductance Calcium Activated Potassium Current

Authors: *C. MAHAPATRA[1], A. KAUR[2];
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Abstract: Objective The neurological reason behind the loss of dopamine neurons causing Parkinson's disease (PD) is an unsolved mystery. **Background** As PD is identified as a classic disorder of "brain arrhythmias", several pharmacological targets are under clinical trial to rejuvenate neurons for evoking normal firing patterns. With experimental evidence, this in silico study investigates action potential (AP) oscillation patterns of dopamine neurons towards cocaine exposure. **Methods** This single compartmental in silico model comprises the inward rectifier ion channels, voltage-gated sodium channel, voltage-gated potassium channel, L-type calcium channel, large-conductance calcium-dependent potassium (BK) channel, small conductance calcium-dependent potassium (SK) channel, and calcium diffusion mechanisms. All ion channels are expressed by the conventional Hodgkin-Huxley formalism. Cocaine exposure (1mg/kg to 10mg/kg) profile is reflected as the conductance of SK channel is mimicked by changing the maximum conductance of SK channel in dopamine neuron. **Results** After injecting a current stimulus (Istim) of varying magnitude (0.1-0.6nA) and duration (1-5ms), APs are reproduced by the whole-cell model. The modulating effects of cocaine exposure on dopamine neurons' electrophysiological properties are investigated in two folds. First, we simulated the current-voltage profile of the SK ion channel with respect to multiple doses of cocaine under the voltage clamp protocol. It showed the continuous decrease of outward current because of multiple doses of cocaine from 1mg/kg to 10mg/kg. Then, the altered SK ion channel outward current is incorporated into the whole-cell model to investigate the AP firing patterns. The frequency of the firing patterns is elevated for the cocaine dose of 10mg/kg when the cell in injected by the current stimulus. **Conclusions** Cocaine works on a membrane receptor pathway to inhibit the extracellular calcium entry into the cell. As a result, a few SK ion channels are activated across the membrane and it reduces the whole-cell outward current. The reduced outward current elevates the cell's excitability for AP generation. Our in-silico study interprets a sub-cellular mechanism linking cocaine-evoked altered ion channel activity to neuronal firing patterns, shedding light on novel pharmacological targets for PD.

Disclosures: C. Mahapatra: None. **A. Kaur:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 270.16

Topic: B.03. Ion Channels

Support: NINDS R21NS125503
NINDS U54NS108874

Title: Maladaptive Compensation in KCNQ2-Related Neurodevelopmental Epilepsy in Patient-Specific iPSC-Derived Neurons

Authors: *D. SIMKIN[1], M. GHARIB[1], K. A. MARSHALL[1], A. L. GEORGE, Jr.[2], E. KISKINIS[1];
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Abstract: Heterozygous loss-of-function mutations in *KCNQ2*, which encodes a voltage-gated K^+ channel subunit responsible for neuronal M-current, have been associated with neonatal developmental and epileptic encephalopathy (KCNQ2-DEE). This complex disorder manifests as severe early-onset seizures and impaired neurodevelopment. While the effects of *KCNQ2* mutations have been studied extensively in heterologous expression systems, their effects on the inherent properties of human neurons are poorly understood. Specifically, what remains unclear is how defects in M-current affect the electrophysiological properties of human neurons during a critical neurodevelopmental maturation period. Using induced pluripotent stem cells (iPSCs) and CRISPR/Cas9 gene editing we have established KCNQ2-DEE disease model systems and recently demonstrated that neurons derived from a patient with KCNQ2-DEE (carrying a R581Q pathogenic variant) exhibit progressively enhanced burst-suppression-like firing, as neurons mature on multi-electrode arrays (MEAs), in contrast to isogenic mutation-corrected controls (PMID 33544076). This maladaptive maturation time course is associated with transcriptional and functional upregulation of Ca^{2+}-activated K^+ channels leading to faster action potential (AP) repolarization and larger post-burst after-hyperpolarizations (AHP) over several weeks in culture.
Here, we examined the broader relevance of our findings by establishing five additional KCNQ2-DEE patient-specific and isogenic mutation-corrected control iPSC-derived excitatory neuron pairs (*KCNQ2* pathogenic variants: R207W, H228R, T274M, F305del and P335L), and investigating whether maladaptive homeostatic responses are characteristic of KCNQ2-DEE. Although the patient mutations lead to different levels of channel loss-of-function, we find that all five additional patient-specific iPSC-derived neuronal lines exhibit enhanced bursting propensity when compared to their isogenic controls on MEAs. Gene expression studies revealed several upregulated Ca^{2+}-activated K^+ channel genes across patient lines. Furthermore, we find that this aberrant bursting phenotype is specific to chronic M-current blockade rather than hyperactivity through inhibition of other voltage-gated K^+ channels. We hypothesize that upregulation of K^+ channels associated with this bursting phenotype is a specific response to M-channel dysfunction. Because KCNQ2-DEE presents in the first days of life, targeting the underlying cause (i.e. *KCNQ2*) may be challenging thus, identifying novel downstream targets may enable alternative therapeutic strategies.

Disclosures: D. Simkin: None. **M. Gharib:** None. **K.A. Marshall:** None. **A.L. George:** None. **E. Kiskinis:** None.

Poster

270. Potassium Channels

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Program #/Poster #: 270.17

Topic: B.03. Ion Channels

Support: U54 NS108874
R01 NS101596

Title: Kcnq2 gain-of-function variants: are they acting as gain-of-function in neurons?

Authors: *N. VARGHESE, K. SPRINGER, A. TZINGOUNIS;
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Abstract: A major breakthrough over the last few years has been the identification of *de novo* pathogenic variants that underlie neurodevelopmental disorders. For example, both loss-of-function (LOF) and gain-of-function (GOF) variants in KCNQ potassium channel family members lead to developmental and epileptic encephalopathy. This is particularly striking for KCNQ2, in which GOF variants represent more severe forms of KCNQ2 encephalopathy than LOF variants and increase the likelihood of death later in life. Work in heterologous cells has shown that KCNQ2 GOF variants often neutralize the 2nd voltage-sensor arginine of KCNQ2 (KCNQ2^{R201}) leading to greater potassium channel activity at resting membrane potentials and membrane hyperpolarization. Currently, it is unknown whether KCNQ2 GOF variants dampen or increase the firing activity of neurons, raising the question on whether they act as GOF or LOF in regards to neuronal activity. Using conditional *Kcnq2*^{R201C} knockin mice, we found that selective expression of*Kcnq2*^{R201C} in forebrain excitatory neurons led to a decrease in excitability and spike frequency adaptation in hippocampal CA1 pyramidal cells, consistent with expression of KCNQ2 GOF variant in these neurons. This decrease in excitability was observed independent of the radial location of CA1 pyramidal cells (deep vs superficial) and stimulation protocol (step, ramp, cosine). In contrast, recordings from Layer 2/3 pyramidal neurons of the somatosensory cortex displayed an increase in their firing activity, akin to what we previously observed in *Kcnq2* null neurons. Thus, our findings suggest that whether potassium channel variants act as LOF or GOF in neurons would vary depending on cell type and brain subregion.

Disclosures: N. Varghese: None. **K. Springer:** None. **A. Tzingounis:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.18

Topic: B.03. Ion Channels

Support: F31-HL156470
R01HL137094

Title: Expression of an encephalopathy-associated Kcnq2 gain-of-function mutation in Phox2b neurons suppresses respiratory activity.

Authors: *J. SOTO[1], C. M. CLEARY[1], D. MULKEY[1], A. TZINGOUNIS[2];
[1]Physiol. and Neurobio., Univ. of Connecticut, Storrs, CT; [2]Physiol. and Neurobio., university of connecticut, Storrs, CT

Abstract: KCNQ channels are key determinants of neuronal activity, and gain-of-function mutations in KCNQ2 are associated with developmental and epileptic encephalopathy. In particular, patients that express the recurrent KCNQ2 gain-of-function mutation R201C exhibit a severe hypoventilation phenotype. This got our attention because KCNQ channels regulate activity of neurons in the retrotrapezoid nucleus (RTN) that control breathing in response to changes in CO2/H+ (i.e., function as respiratory chemoreceptors). Therefore, we hypothesize that KCNQ2 channels regulate activity of RTN chemoreceptors and respiratory behavior. To test this, we first characterized Kcnq transcript expression by chemosensitive RTN neurons identified based on location in the ventral parafacial region and expression of Phox2b. We found that chemosensitive RTN neurons preferentially express Kcnq2 but minimally express other Kcnq subtypes. Next, we conditionally expressed Kcnq2 R201C in chemosensitive RTN neurons ($Phox2b^{Cre/+}$::$Kcnq2^{R201C/+}$) and characterized respiratory function at the cellular and whole animal levels. At the whole animal level, $Phox2b^{Cre/+}$::$Kcnq2^{R201C/+}$ mice hypoventilate under room air conditions (23% decrease in minute ventilation) and show a blunted ventilatory response to graded increases in CO2 compared to control (32% decrease in minute ventilation). Consistent with this, we found at the cellular level that chemosensitive RTN neurons in slices from $Phox2b^{Cre/+}$::$Kcnq2^{R201C/+}$ mice are less excitable under control conditions (37% decrease in baseline activity) and show a 44% decrease in CO2/H+-responsiveness. Further, bath application of a selective Kcnq2 blocker (ML252; 10 uM) increased activity of RTN neurons in control and $Phox2b^{Cre/+}$::$Kcnq2^{R201C/+}$ tissue and negated differences between genotypes. We also found that conditional Kcnq2 knockout mice ($Phox2b^{Cre/+}$::$Kcnq2^{fl/fl}$) hyperventilate under room air conditions (24% increase in minute ventilation) but show a hypercapnic ventilatory response similar to control mice. These results suggest Kcnq2 channels regulate activity of RTN neurons and respiratory behavior.

Disclosures: J. Soto: None. **C.M. Cleary:** None. **D. Mulkey:** None. **A. Tzingounis:** None.

Poster

270. Potassium Channels

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Program #/Poster #: 270.19

Topic: B.03. Ion Channels

Support: NIH Grant NS102239

Title: Kna1.1 antagonism with PRX2904 suppresses epileptiform activity due to a heterozygous mutation in Kcnt1

Authors: *I. H. QURAISHI[1], M. PEDRAM[2], M. BROMWICH[2], K. M. KAHLIG[3], L. K. KACZMAREK[2];
[1]Neurol., [2]Pharmacol., Yale Univ. Sch. of Med., New Haven, CT; [3]Praxis Precision Medicines, Boston, MA

Abstract: Epilepsy of infancy with migrating focal seizures (EIMFS) is an epileptic encephalopathy associated with highly intractable seizures and devastating neurodevelopmental disability. EIMFS has been associated with heterozygous gain-of-function variants in the Na-activated K channel Slack (KNa1.1), which is encoded by KCNT1. Previously available Slack/KNa1.1 antagonists have been limited in clinical use by poor specificity and a small therapeutic window. A specific KNa1.1 antagonist PRX2904 was recently discovered and shown to suppress spikes and seizures in a homozygous mouse model of KCNT1 epilepsy (Griffin et al., ACS Med Chem Lett. 2021). Human KCNT1 epilepsy is a heterozygous condition, however, so we evaluated the effects of this compound on an epileptic mouse model carrying the mutation $Kcnt1^{+/R455H}$. These mice have spontaneous seizures, interictal epileptiform discharges, and decreased thresholds for induced seizures. $Kcnt1^{+/R455H}$ mice were tested with pentylenetetrazole (PTZ) 50 mg/kg IP administered 1 or 2 hours after administration of either PRX2904 75 mg/kg SC or vehicle control. Seizures induced by PTZ were scored using a modified Racine scale, and the latency to convulsive seizures was compared between drug and vehicle groups (log rank test). Next, $Kcnt1^{+/R455H}$ mice were implanted with screw electrodes for continuous monitoring. After 72 hours of baseline EEG, PRX2904 75 mg/kg was administered, followed by an additional 72 hours of recording. Epileptiform discharges were manually identified, and rates compared between the baseline period and the 24 hours after drug delivery (Wilcoxon matched rank pairs test). Following 1 hour of pretreatment, 1/16 animals delivered PRX2904 had PTZ-induced convulsive seizures, as compared with 6/15 animals given the vehicle control ($p=0.037$, Fisher's exact test); and after 2 hours of pretreatment 1/20 animals delivered PRX2904 had convulsive seizures as compared with 13/20 animals given vehicle control ($p=0.0001$). Latency to seizures was also prolonged by the drug ($p=0.025$ for 1 hour pre-treatment, $p=0.0001$ for 2-hour pretreatment). Baseline epileptiform spike rates on EEG were variable between animals, but there was a marked per-animal reduction in the 24 hours following PRX2904 administration ($p=0.005$, $N=12$). These results suggest that KNa1.1 antagonists may be an effective treatment for KCNT1 epilepsy.

Disclosures: I.H. Quraishi: None. **M. Pedram:** None. **M. Bromwich:** None. **K.M. Kahlig:** A. Employment/Salary (full or part-time):; Praxis Precision Medicines. **L.K. Kaczmarek:** 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.; Praxis Precision Medicines.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

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Program #/Poster #: 270.20

Topic: B.03. Ion Channels

Support: NIH grant NS102239 to LKK

Title: Positive cooperativity in gain-of-function Slack (KCNT1) mutations is mediated by the cytoplasmic C-terminus

Authors: I. H. QURAISHI[1], ***H. WANG**[2], T. MALONE[2], H. MCCLURE[2], J. KRONENGOLD[2], L. K. KACZMAREK[3];
[1]Dept. of Neurol., [2]Dept of Pharmacol., Yale Univ., New Haven, CT; [3]Yale Univ. Sch. Med., New Haven, CT

Abstract: Gain-of-function mutations in the Slack (KCNT1, KNa1.1) sodium-activated potassium channel are associated with early-onset epilepsy and severe intellectual disability. Electrophysiological recordings in cRNA-injected oocytes have shown increases in evoked current ranging from two-fold to over 20-fold for different mutations. One of the highest gain-of-function mutations, and one most highly represented in the patient population is KCNT1 R474H. While many mutations alter sensitivity to internal Na concentrations or voltage-dependence, recordings of single channels in isolated patches do not typically show the same degree potentiation as whole oocyte macroscopic currents. Previous work comparing open probability in patches containing one or two channels with that in clusters of four or more channels suggested positive cooperativity between individual channels, which was greatly increased by gain-of-function mutations. We have now more robustly demonstrated that positive cooperativity provides a good fit to multi-channel Slack recordings. Markov models simulating single-channel recordings show that cooperativity is sufficient to explain observed recordings, whereas alternative mechanisms, such as the existence of unobserved channels or cluster-dependent phosphorylation, cannot explain observed recordings. Because channel-channel interactions are likely mediated by the large cytoplasmic C-terminal domains of Slack, we generated and tested two expression constructs related to this region. One construct, Slack-CT, contained this C-terminal domain alone. Such isolated cytoplasmic domains would be expected to compete with intact channels for interacting sites on neighboring channels, reducing the likelihood of cooperative interaction. Co-expression of Slack-CT with the full-length Slack-R474H gain-of-function mutant channel produced a greater than 60% reduction in potassium current. In contrast, co-expression of Slack-CT with wild-type Slack did not produce a significant reduction of total current. The second construct we evaluated was a truncation mutation Δ804 Slack in which the distal C-terminal region was deleted. Currents from a patch containing multiple truncated channels could be explained by a linear combination of single channel activity from patches with a small number of channels, indicating that cooperativity was lost by deletion of the C-terminus. Experiments are underway to test the specificity of the C-terminal domain. The findings further confirm the hypothesis that Slack channels gate cooperatively and that human disease-causing mutations are associated with a higher degree of cooperativity.

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Poster

270. Potassium Channels

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Topic: B.03. Ion Channels

Support: NIH grant NS102239

Title: Activation of Slack (KCNT1) channels is coupled to the stimulation of β-actin translation in cortical neurons

Authors: T. J. MALONE[1], Y. ZHANG[2], J. WU[2], R. CHEN[3], P. LICZNERSKI[3], ***M. PEDRAM**[2], S. NAHIYAN[2], E. A. JONAS[3], L. K. KACZMAREK[2];
[1]NINDS, Natl. Inst. of Neurolog. Disorders and Stroke, Rockville, MD; [2]Yale Univ. Sch. Med., New Haven, CT; [3]Intrnl. Medicine/Section Endocrinol., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The Slack channel is a large conductance sodium-activated potassium channel expressed in neurons of the cerebral cortex, as well as in numerous other brain regions. Human gain-of-function Slack mutations are associated with early-onset epilepsy and severe intellectual disability. The cytoplasmic C-terminal of Slack channels has been shown to interact with two regulators of mRNA translation, Fragile X Mental Retardation Protein (FMRP) and Cytoplasmic FMRP-Interacting Protein 1 (CYFIP1) and can be co-immunoprecipitated with several neuronal mRNAs. Thus, improper regulation of mRNA translation may be a possible contributor to the intellectual disability associated with the human mutations. To investigate this possibility, we have measured rates of mRNA translation in cell lines expressing either wild type Slack or the disease-causing mutation Slack-R455H, as well as in neurons from wild type mice and those with the Slack-R455H mutation. We first transfected HEK cells or cortical neurons in primary culture with a fluorescent Dendra-2 reporter construct in which the coding region was flanked by the 5' and 3' UTR of β-actin. Using FRAP to visualize protein in real-time synthesis and immunoblots to measure total and translating protein levels, we found that activation of wild type Slack channels triggers increased synthesis of the reporter protein in both HEK cells and neurons. In cells expressing the Slack-R455H mutant, however, both total protein and translation levels of the reporter construct were markedly increased over those in cells with the wild-type channel, even in the absence of channel stimulation. This gain-of-function in translation was found to require the β-actin UTRs. To determine whether the effect of the mutation extends to translation of native β-actin, we carried out a proximity ligation assay with β-actin and puromycin to detect sites of β-actin translation in cortical neurons in culture. The number of positive puncta was very markedly enhanced by the Slack-R455H mutation in the dendrites but not the somata of the neurons. Experiments are currently in progress to test the effect of pharmacological inhibitors of Slack to determine whether ion flux is required for the observed gain-of-function in translation. The findings suggest that disease-causing mutations in Slack disrupt normal activity-dependent translation in neurons and that this underlies the resultant intellectual disability.

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Poster

270. Potassium Channels

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Program #/Poster #: 270.22

Topic: B.03. Ion Channels

Support: NS102239

Title: Paradoxical effects of KCNT1 channel gain-of-function on neuronal excitability in a mouse model of childhood epilepsy

Authors: *J. WU, Y. ZHANG, I. H. QURAISHI, M. BROMWICH, M. PEDRAM, L. K. KACZMAREK;
Yale Univ. Sch. Med., Yale Univ. Sch. Med., New Haven, CT

Abstract: Autosomal dominant pathogenic variants in the gene encoding the Na^+-activated K^+ channel KCNT1 (Slack, $K_{Na}1.1$) have emerged as an important cause of epilepsy and intellectual disability (ID). These variants have been shown to increase peak potassium current magnitude and produce gain-of-function (GOF). However, the molecular mechanisms of KCNT1 channel GOF in network hyperexcitability and seizures, leading to epilepsy and ID have yet to be determined. In a genetic mouse model of epilepsy expressing the *Kcnt1-R455H* GOF mutation, voltage-clamp studies revealed that Na^+-activated K^+ currents (K_{Na}) were increased in both excitatory glutamatergic and inhibitory GABAergic neurons of the cerebral cortex. Current-clamp studies revealed that the excitability and action potential (AP) generation of excitatory neurons were enhanced by the *Kcnt1-R455H* mutation with decreased AP half width and increased afterhyperpolarizations. Paradoxically, the excitability of inhibitory interneurons in the same mutant animals is suppressed, with increased rheobase and reduced input resistance. These results suggest that the *Kcnt1-R455H* GOF variant leads to network hyperexcitability and produces early-onset seizures by enhancing excitation in excitatory neurons and suppressing excitability in inhibitory interneurons. Two potential molecular mechanisms are being tested to explain why the increased K_{Na} current has opposite effects on the excitability of excitatory and inhibitory neurons. The first potential explanation is that different Kcnt1 isoforms may expressed in these neurons. Previous work has shown that alternative splicing of Kcnt1 RNA results in either a rapidly-activating Slack-A channel or a slowly-activating Slack-B channel. Our ongoing in situ hybridization and co-immunolocalization experiments aim to test if excitatory neurons have the A-splice isoform channels while inhibitory neurons express the B-isoform channels. The other potential explanation is that proximity of K_{Na} channels to sodium channels (Na_V channels) differs in the two types of cells. Our ongoing co-immunolocalization and proximity ligation assay experiments aim to test if the sodium channels are tightly coupled to K_{Na} channels

in the excitatory neurons, resulting in rapid repolarization of action potentials and increased firing rates. Conversely, Na_V channels may be decoupled from the K_{Na} channels in the inhibitory neurons, leading to slow afterhyperpolarizations during repetitive firing. Our study may lead to novel methods and targets of treating epilepsy and neurodevelopmental disorders in patients with *KCNT1* GOF mutations.

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Poster

270. Potassium Channels

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Program #/Poster #: 270.23

Topic: B.03. Ion Channels

Title: Characterisation of cell lines expressing the lysosomal ion channel TMEM175 for use in high-throughput electrophysiology and fluorescence-based screening platforms

Authors: *R. BURLEY, S. MAIDMENT, X. KODJI, A. HYMAN, K. KUAN, S. WILLIAMS, J. KAMMONEN;
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Abstract: TMEM175 is a K^+ channel involved in the regulation of lysosomal pH necessary for effective autophagic clearance. It is believed that TMEM175 provides counter ions to achieve the acidic pH that is necessary for the optimal activity of lysosomal enzymes such as cathepsin D. Variants of TMEM175 with loss-of-function are associated with an increased incidence of Parkinson's disease and Lewy Body dementia, with earlier age of onset, while gain-of-function variants reduce the risk. Activators of TMEM175 are therefore potential therapeutic agents for the treatment of Parkinson's disease and are the subject of intense interest as they may encourage the breakdown of protein aggregates formed by α-synuclein. We describe the creation and characterization of TMEM175 expressing cell lines and the development of a 384 well FLIPR Thallium flux assay and a 384 well plate-based automated patch-clamp assay useful in high-throughput screening for compounds that activate TMEM175. HEK293 cells overexpressing WT TMEM175 and cells overexpressing the M393T and Q65P variants are compared with respect to their electrophysiology and pharmacology using tool activators and blockers. For fluorescence measurements using Tl^+ flux on the FLIPR, 20,000 cells/well were incubated with Molecular Devices Potassium Assay Kit Dye for 60 mins, compounds incubated at room temperature for 30 mins and read for 5 mins after addition of 2 mM Tl^+. The slope of the fluorescence signal was analysed. 30 µM of the Akt/PKB activator SC-79 produced a robust increase in the slope of the curve with an estimated EC_{50} of 4.3 µM. 100 µM 4-AP in the presence of SC-79 reduced the rate of Tl^+ flux compared to wells treated with SC-79 alone. Electrophysiology utilized single hole chips on the Sophion Qube and achieved greater than 80% success rates having >1 GΩ seals. Using a CsHEPES-based intracellular solution and 4 mM extracellular K^+, 1 s voltage ramps

from -100 to +100 mV (V_H=-80 mV) elicited outward currents reversing around -64 mV. Current amplitude measured at +100 mV increased from 280±29 pA (*n*=30) in the presence of DMSO vehicle control to 1,814±221 pA (*n*=30) after application of 30 µM SC-79. The EC_{50} for SC-79 was estimated to be 8.85 µM. Currents were robustly inhibited by 100 µM 4-AP (IC_{50} 43.65 µM). We have developed cell lines capable of generating robust plate-based fluorescence and automated electrophysiology-based readouts. The utilizing of high-throughput screening assays for modulators of TMEM175 will enable the discovery of novel pharmacological tools and potential therapeutics for the treatment of neurological disorders.

Disclosures: **R. Burley:** None. **S. Maidment:** None. **X. Kodji:** None. **A. Hyman:** None. **K. Kuan:** None. **S. Williams:** None. **J. Kammonen:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.24

Topic: B.03. Ion Channels

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MCST R&I grant 2017-029T
RIDT grant E17L077

Title: Locus coeruleus neurons firing pattern is regulated by ERG voltage-gated K+ channels

Authors: *F. HAIDAR[1], S. HASAN[2], F. DELICATA[3], L. GUASTI[4], A. ARCANGELI[4], P. IMBRICI[5], M. PESSIA[3,6], M. VALENTINO[3], M. D'ADAMO[7];
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Abstract: Locus coeruleus (LC) neurons with their extensive innervations throughout the brain control a broad range of physiological processes. Several ion channels have been characterized in LC neurons that control intrinsic membrane properties and excitability. However, ERG (*ether-à-gogo–related gene)* K + channels that are particularly important in setting neuronal firing rhythms and automaticity have not yet been discovered in the LC. Moreover, the neurophysiological and pathophysiological roles of ERG channels in the brain remain unclear despite their expression in several structures. By performing immunohistochemical investigations using brainstem tissue dissected from young (P10) and adult (P60) mice (C57BL/6J), we found that ERG-1A, ERG-1B, ERG-2, and ERG-3 are highly expressed in the LC neurons. To examine the functional role of ERG channels current-clamp recordings were performed on LC neurons in brain slices dissected from adult C57BL/6J male mice (P40±10)

under visual control. Approximately 70% of the recorded LC neurons were responsive to ERG channel block by WAY-123,398 a class III anti-arrhythmic agent. ERG channel blockade increased spontaneous firing activity and discharge irregularity of LC neurons. In this study, and for the first time we prove the presence of distinct ERG channel subunits in the LC where they play an imperative role in modulating neuronal discharge patterns. We thus propose ERG channels as important players behind the changes in, and/or maintenance of LC firing patterns that are implicated in the generation of different behaviors as well as in different diseases.

Disclosures: F. Haidar: None. **S. Hasan:** None. **F. Delicata:** None. **L. Guasti:** None. **A. Arcangeli:** None. **P. Imbrici:** None. **M. Pessia:** None. **M. Valentino:** None. **M. D'Adamo:** None.

Poster

270. Potassium Channels

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 270.25

Topic: D.05. Auditory & Vestibular Systems

Support: NIH grant R01 DC012347 (RAE, AL)
NSF GRF (HRM)

Title: The potassium channel subunit $K_V1.8$ (Kcna10) differentially shapes gain, tuning, and timing of receptor potentials in type I and II vestibular hair cells

Authors: *H. MARTIN[1], A. LYSAKOWSKI[2], R. EATOCK[1];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Anat. and Cell Biol., Univ. of Illinois At Chicago, Chicago, IL

Abstract: Vestibular hair cells (HCs) convert head motions into receptor potentials that drive synaptic transmission, enabling rapid gaze- and posture-stabilizing reflexes, spatial navigation, and gravity sensing. Type I and II HCs (HC-I and HC-II) are contacted by calyceal and bouton terminals, respectively, and express different voltage-gated K+ conductances with specialized biophysical properties that differently tune receptor potentials and synaptic transmission: in HC-Is, a large, low-voltage-activated K+ conductance ($g_{K,L}$) is open at resting potential; in HC-IIs, an A-type conductance (g_A) is rapidly activated then inactivated by depolarizing receptor potentials. These biophysical differences are thought to affect the tuning and even the nature of transmission onto calyceal and bouton terminals.
We studied the K_V conductances in HC-Is and HC-IIs from utricles of mice wildtype, heterozygous, and null for the pore-forming subunit, $K_V1.8$ (Lee et al. 2013, Hearing Research 300:1-9). $g_{K,L}$ and g_A were both absent in $K_V1.8$-null mice. Because the residual K_V conductance was similar in HC-Is and HC-IIs (a smaller delayed rectifier in the K_V7 family), their different receptor potential tuning reflects the different kinetics of their KV1.8-containing channels, $g_{K,L}$ and g_A. By comparing receptor potential properties in $K_V1.8$-control and -null HCs, we show

here the impact of $K_V1.8$ expression.
In HC-Is, the presence of $K_V1.8$ reduced input resistance ~20-fold and raised the lowpass corner frequency (f_C) of receptor potentials re: transduction currents >12-fold (from ~25 Hz to >300 Hz), well above the physiological range of head motions (~0-20 Hz, Carriot et al. 2017, J Physio 595:2751-2766). This may be important for the speed of transmission with the calyceal afferent terminal on HC-Is.
In HC-IIs, the presence of $K_V1.8$ also reduced input resistance and raised lowpass f_C, although by less: from ~2300 MΩ ($K_V1.8$-null) to ~1700 MΩ (control) and from ~17 Hz (null) to ~70 Hz (control). Some $K_V1.8$-null HC-IIs were electrically resonant such that current injection evoked voltage oscillations. By reducing electrical tuning, g_A improves representation of the stimulus time course by the receptor potential.
We propose that $K_V1.8$ is a pore-forming subunit of $g_{K,L}$ in HC-Is and g_A in HC-IIs, with marked differences in voltage dependence and inactivation arising from cell type-specific factors, such as other K_V1 subunits, accessory proteins, or second messengers. We are interested in what factors act in each hair cell type to produce such different effects on the gain, tuning, and timing of their receptor potentials.
We thank S. Jones and T. Friedman for sharing $K_V1.8$ mutant mice.

Disclosures: H. Martin: None. **A. Lysakowski:** None. **R. Eatock:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.01

Topic: B.05. Synaptic Plasticity

Support: ERC Grant 802354

Title: Determinants of short-term synaptic plasticity at a high release probability synapse

Authors: *D. N. CHIU, B. C. CARTER;
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Abstract: The layer 4 to layer 2/3 synapse in the rodent whisker somatosensory cortex is a glutamatergic synapse notable for its initial high probability of release (P_r; ~0.8). Most high P_r synapses exhibit short-term synaptic depression, presumably due to the depletion of vesicles following initial release. While L4-L2/3 synaptic responses depress for most interstimulus intervals (ISIs) up to 5 s, as measured by the paired pulse ratio ($EPSC_2/EPSC_1$), two observations seem to contradict the depletion model: first, depression is absent at ISIs of 10-20 ms; and second, depression is not maximal at the shortest ISIs, but rather 'peaks' at ~200 ms. If a transient lack of release-ready vesicles is responsible for short-term synaptic depression, it follows that depression should be greatest at shorter intervals and recover monotonically. What, then, can account for the fact that responses first facilitate, then depress, then recover? To

understand the mechanism(s) underlying the biphasic time course of short-term plasticity at this synapse, we used whole-cell electrophysiology and two-photon calcium imaging in acute slices from juvenile mice of either sex. We tested several candidate mechanisms including neuromodulation, postsynaptic receptor desensitization, use-dependent changes in presynaptic AP-evoked calcium, and heterogeneity of release among stimulated synapses. We found that, at single synapses, P_r varies as a function of ISI, giving rise to the short-term plasticity time course. Furthermore, the higher-than-expected P_r at short ISIs depends on expression of synaptotagmin 7. Our results show that the pattern of short-term plasticity at this synapse arises from high initial P_r coupled with two distinct processes: 1) a rapid decrease in P_r following vesicle release that recovers slowly (τ = ~2 s); and 2) a transient, synaptotagmin 7-dependent increase in P_r following an AP (τ = ~100 ms). We thus reveal a mechanism by which synapses can maintain a very high probability of neurotransmission for multiple action potentials within a short time frame.

Disclosures: D.N. Chiu: None. **B.C. Carter:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.02

Topic: B.05. Synaptic Plasticity

Support: NSERC Discovery Grant
CIHR Foundation Grant

Title: Exercise and highly palatable food erase stress-induced synaptic priming in CRH^{PVN}neurons

Authors: *M. ROJAS-CARVAJAL, T. FÜZESI, D. V. BAIMOUKHAMETOVA, N. DAVIU, J. S. BAINS;
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Abstract: Neuroendocrine and autonomic responses to stress are critical for survival. Stress also imprints the brain. This may promote adaptations to future stressors but may also contribute to maladaptive responses involved in the emergence of neuropsychiatric diseases. Multiple plasticity mechanisms have been described, but little is known about how these processes might be reversed. Intriguingly, people use various strategies to buffer stress. Two popular approaches include exercise or consumption of highly palatable food, and here we tested the effects of these distinct stress buffering strategies on synaptic metaplasticity induced by acute stress in corticotropin release hormone cells in the paraventricular nucleus of the hypothalamus (CRH^{PVN}). We examined the effects of exercise or consumption of palatable food on short-term potentiation (STP) of glutamate synapses on CRH^{PVN} neurons. We obtained whole-cell patch clamp recordings from mouse CRH^{PVN} neurons in hypothalamic slices and evaluated the effects

of running for 1h or consuming highly palatable food after acute stress on STP. Following footshock, high frequency stimulation of glutamate synapses on CRH^{PVN} elicited STP (142 ± 10% of baseline EPSC, n=17, *P*<.0001). By contrast, STP was blunted if footshock was immediately followed by exercise (101 ± 7.0% of baseline EPSC, n=12, *P*=.81) or consumption of highly palatable food (101 ± 6.6% of baseline EPSC, n=11, *P*=.50). To further investigate how exercise and highly palatable food affect CRH^{PVN} in vivo, we expressed a genetically encoded Ca reporter (GCaMP6f) in CRH^{PVN} neurons and used fibre photometry to record population activity in vivo. Unstressed mice were subjected to exercise or allowed to consume highly palatable food. The latter caused a reduction in CRH^{PVN} activity while exercise had the opposite effect. Since these conditions induced opposite effects on CRH^{PVN} activity, we investigated the effects of exercise and highly palatable food on downstream neuroendocrine signaling by evaluating circulating corticosterone (CORT) - the main stress hormone in rodents. In all groups, CORT levels increased 15 min after stress (1963 ± 160 ng/ml; N=27, *P*<.0001); exercise resulted in a further increase in CORT (2977.45 ± 168 ng/mL, n=9, *P*<.0001) but highly palatable food had no effect in CORT (1892.24 ± 320 ng/mL, n=8, *P*=.76). Our findings demonstrate that although exercise and highly palatable food have opposing effects of CRH^{PVN} activity, they have similar effects on reducing STP after stress.

Disclosures: **M. Rojas-Carvajal:** None. **T. Füzesi:** None. **D.V. Baimoukhametova:** None. **N. Daviu:** None. **J.S. Bains:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.03

Topic: B.05. Synaptic Plasticity

Title: Hippocampal GABAergic impairments revealed by baclofen in the $SOD1^{G93A}$ rat model of amyotrophic lateral sclerosis (ALS)

Authors: S. R. ANDREASEN[1], K. H. ANDERSEN[1,2], M. S. JENSEN[1], ***M. M. HOLM**[1]; [1]Dept. of Biomedicine, Aarhus Univ., Aarhus C, Denmark; [2]Dept. of Clin. Med. - Nuclear Med. and PET, Aarhus Univ., Aarhus, Denmark

Abstract: Excitatory-inhibitory imbalances are known to contribute to disease manifestations in ALS. Our lab has previously demonstrated excitatory-inhibitory imbalances in the wobbler mouse model of ALS (Andreasen et al., *Neuroscience Letters*, 2017). Here we employed another established ALS model, the $SOD1^{G93A}$ rat, which overexpresses human superoxide dismutase 1 (SOD1) harboring a glycine to alanine substitution ($SOD1^{G93A}$). To evaluate alterations in the inhibitory system, we employed the $GABA_B$ receptor agonist baclofen, a well-known tool to study GABAergic mechanisms.
We prepared acute coronal brain sections from deeply anaesthetized female wild-type and female $SOD1^{G93A}$ overexpressing rats. We delivered paired-pulse stimulations of Schaffer collaterals

while recording field excitatory postsynaptic potentials (fEPSPs) in the CA1 synapses. Paired-pulse ratios were calculated by normalizing the slope of the second fEPSP to the slope of the first fEPSP. Recordings were performed in standard artificial cerebrospinal fluid (ACSF) and in the presence of 10 µM baclofen. Baclofen is known to activate presynaptic $GABA_B$ receptors, inhibiting vesicle release and resulting in increased paired-pulse ratios upon paired stimulations. Testing standard 50 ms interstimulus intervals in wild-type slices; the paired-pulse ratio increased significantly from 1.36 ± 0.044 (n = 18) in ACSF to 1.60 ± 0.059 (n = 9) in the presence of 10 µM baclofen at ($P < 0.005$). Interestingly, when testing 50 ms intervals in $SOD1^{G93A}$ slices we instead observed similar ratios at 1.43 ± 0.047 (n = 18) in ACSF and 1.52 ± 0.11 (n = 8) in the presence of 10 µM baclofen ($P > 0.05$). Testing very short intervals (20 ms) revealed baclofen effects in both genotypes.
To evaluate possible changes when challenging the synapses at longer interstimulus intervals, we also tested 500 ms intervals. In wild-type slices, the ratio significantly increased from 0.94 ± 0.023 (n = 18) in ACSF to 1.03 ± 0.022 (n = 9) in the presence of 10 µM baclofen ($P < 0.05$). Confirming our findings at the 50 ms intervals; in $SOD1^{G93A}$ slices we observed that the paired-pulse ratio was 0.93 ± 0.027 (n = 18) in ACSF and 0.93 ± 0.061 (n = 8) in the presence of 10 µM baclofen ($P > 0.05$).
We conclude that hippocampal networks in $SOD1^{G93A}$ rats are less responsive to the $GABA_B$ receptor agonist baclofen as compared to wild-types, thereby documenting GABAergic impairments. These studies add further details to disease mechanisms underlying ALS and may ultimately contribute to better treatment strategies for this disorder.

Disclosures: S.R. Andreasen: None. **K.H. Andersen:** None. **M.S. Jensen:** None. **M.M. Holm:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.04

Topic: B.05. Synaptic Plasticity

Title: Plasticity of feedforward thalamocortical circuits during sensory learning

Authors: *J. A. CHRISTIAN, E. PARK, A. L. BARTH;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Sensory learning can drive changes in synaptic function within the neocortex, but the input- and target-specificity of this plasticity is not well-understood. Because neocortical circuits are composed of a molecularly and anatomically diverse set of neurons, it will be critical to evaluate changes at discrete nodes of the network to further understand how this plasticity is both initiated and can alter principles for information processing. Prior studies from our lab and other have shown that in tactile and auditory neocortex, higher-order thalamic inputs are potentiated in the early stages of training in sensory association task (Audette et al 2019, Williams and Holtmat

2019, Pardi et al 2020). Here we investigated whether fast, feedforward inputs from the first-order thalamocortical and intracortical pathways were altered during learning. Channelrhodopsin was expressed in ventro-posterior medial (VPM) thalamus and animals were trained to associate a whisker-stimulus with a water reward. Recordings in acute brain slices showed that photo-stimulation evoked quantal excitatory postsynaptic currents from VPM thalamus onto layer 4 (L4) excitatory neurons in the barrel cortex were not rapidly potentiated at the onset of training. Channelrhodopsin expression in L4 excitatory neurons also enabled pathway-specific analysis of an intracortical feedforward pathway across the early training period. Our experiments will test the hypothesis that fast, feedforward sensory pathways in sensory cortex are less plastic than higher-order feedback pathways.

Disclosures: **J.A. Christian:** None. **E. Park:** None. **A.L. Barth:** None.

Poster

271. Short-Term Synaptic Plasticity

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Topic: B.05. Synaptic Plasticity

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Title: Simultaneous but not delayed repetitive paired cortical and neuromuscular electrical stimulations effectively facilitate corticospinal excitability

Authors: *N. CAO[1,2], A. SASAKI[1,3,2], A. YUASA[4], M. MILOSEVIC[3], K. NAKAZAWA[1]; [1]The Univ. of Tokyo, Tokyo, Japan; [2]The Japan Society for the Promotion of Sci., Tokyo, Japan; [3]Osaka Univ., Tayonaka, Japan; [4]Fujita Hlth. Univ. Sch. of Med., Aichi, Japan

Abstract: Previously our group tested the combination of cortical repetitive transcranial magnetic stimulation (rTMS) and neuromuscular electrical stimulation (NMES) sequentially. It showed that facilitatory rTMS priming can make the subsequent NMES more responsive on central nervous system excitability. However, the precise timing of activation of pairing rTMS and NMES for neuromodulation needs further investigation. The purpose of this study was to examine corticospinal excitability changes after short-duration simultaneous or delayed paired stimulation applied using cortical rTMS and peripheral activation using NMES (n = 11). In this protocol, intermittent theta burst stimulation (iTBS) was applied to on the hot spot of upper-limb extensor capri radialis (ECR) muscle for totally 600 pulses as an established cortical facilitation

rTMS protocol, while NMES was applied to activate the ECR muscle at 50 Hz frequency. Especially, both cortical stimulation (iTBS) and peripheral muscular stimulation (NMES) were delivered with the rhythm by 2 s with stimuli and 8 s without stimuli for totally 192 s. Two interventions were compared on effects of timing (simultaneous or delayed): (1) iTBS with simultaneous NMES; (2) iTBS with 5 s delayed NMES. Single-pulse motor evoked potential (MEP) responses elicited by transcranial magnetic stimulation of the primary motor cortex and maximum motor (Mmax) responses elicited by radial nerve stimulation were compared before and after each intervention on ECR muscle. Our results showed that corticospinal excitability (MEP/Mmax) was only facilitated after simultaneous but not delayed intervention ($p<0.05$). This result demonstrates that simultaneous repetitive paired stimulation could effectively evoke rapid corticospinal excitability change within short-time application, especially depend on the timing of activation on both cortical and sensorimotor networks. These results may have implications for rehabilitation or treatment of motor function after neurological injuries.

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Poster

271. Short-Term Synaptic Plasticity

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Program #/Poster #: 271.06

Topic: B.05. Synaptic Plasticity

Support: DBT BT/PR12255/MED/122/8/2016
NCBS-TIFR
DBT-NaMoR, INDIA

Title: Circuit and synaptic mechanisms of E-I balance and Short-term plasticity gating probed using repeated pulses of spatially patterned input

Authors: *A. ASOPA, U. S. BHALLA;
Natl. Ctr. for Biol. Sci., Tata Inst. of Fundamental Res., Bengaluru, India

Abstract: Hippocampal CA3-CA1 circuit represents multiple modalities including time, space, and orientation. But for CA1 cells to represent information, they must overcome the precise and tight balance between excitation and inhibition (Bhatia et al 2019), cross the threshold and fire. We study how the E-I balance in CA1 pyramidal neurons evolves with changing firing rates in CA3 layer cells, and what conditions result escape from this balance and firing. We performed in vitro whole cell patch clamp recordings from CA1 cells in acute mouse hippocampal sections (male and female, 2-3 months of age). We simultaneously stimulated Channelrhodopsin (ChR2) expressing CA3 pyramidal cells with 13um x 8um spots of light using a digital projector (Mightex Polygon 400). We created 5-spot and 15-spot patterns from a set of 45 spots. These patterns were used to stimulate CA3 cells at multiple frequencies (8 pulses at 20, 30, 40, 50, and

100Hz). Current clamp recordings were made to record suprathreshold responses and voltage clamp recordings were made to record E and I synaptic conductances resulting from these stimuli. Responses to single spots that form the patterns were also recorded to determine the expected subthreshold responses. Gabazine was used for no-inhibition control. We measured the responses of CA1 cells to these stimuli in CA3 to find which conditions induce spiking and corresponding E-I dynamics. We find that spiking in CA1 is modulated by tight E-I balance, degree of convergence of inputs at interneurons and CA1 pyramidal cells, and short-term plasticity at E and I synapses. We found that ratios of response of first pulse to a) the second pulse (PPR) and b) the average of last three pulses (STPR) do not significantly change across activation of different subsets of CA3 networks at all the measured frequencies. This may imply that higher frequency bursts in input layer or prior weight tuning might be needed for CA1 cells to overcome E-I balance. Moreover, the E-I currents measured in voltage clamp under short-term plasticity also continue to remain balanced. This may mean that external inputs or neuromodulatory influences like acetylcholine could play a role in spiking of CA1 neurons. Based on the E-I post-synaptic currents data we have developed a stochastic chemical kinetics model of synaptic release and short-term plasticity to better understand the interplay of E-I synaptic dynamics. We propose that these short-term E-I dynamics resulting in activity gating may be relevant to understanding the formation and representation of place and time cell sequences and have implications for understanding diseases that arise from E-I imbalance.

Disclosures: **A. Asopa:** None. **U.S. Bhalla:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.07

Topic: B.05. Synaptic Plasticity

Title: Etomidate suppresses both short term and long term potentiation induced by a weak theta-burst stimulus paradigm

Authors: *M. DREGER, R. PEARCE;
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Abstract: We recently reported that 1 µM etomidate suppresses LTP of the Schaffer Collateral pathway through modulation of β2-GABAARs (Figueroa et al., J Neurophysiol, 2021). In that study we used a theta-burst stimulus (TBS) protocol consisting of 10 bursts of 4 pulses each (TBS40) to induce LTP. Here, we sought to develop a protocol that would elicit LTP with fewer stimuli, and to test whether LTP elicited by fewer stimuli could be blocked by a lower concentration of etomidate, closer to the concentration that impairs memory *in-vivo*.
Coronal hippocampal brain slices (400 µm) prepared from 2–4-month-old C57BL/6J mice were perfused in a high flow chamber (Scientific Systems Design) at 16 mL/min with aCSF (32°C). 0.5 µM ETOM was added during the one-hour room temperature recovering period and

circulated throughout the experiment. Extracellularly recorded field EPSPs (fEPSPs) were measured every 20 sec in *stratum radiatum* in response to SC stimulation at the CA1/CA2 border. The stimulus intensity at which the fEPSP slope was half maximum was used throughout the 30-min pre-TBS baseline and 60-min post-TBS recordings. A TBS train consisting of three bursts of four pulses each, with an interstimulus interval of 10 ms and an interburst interval of 200 ms (TBS12) was used to induce LTP. Statistical comparisons were made using linear mixed effects modeling implemented in the lmerTest package in R Studio, based on the model EPSPslope ~ Drug + (1|SliceID) + (1|Time:SliceID).
Under saline control conditions, fEPSP slope between 50-60 minutes following TBS12 was increased to 132.4±3.9% of pre-TBS baseline ($t(14.2)=33.8$, $p<0.0001$). In the presence of 0.5% ETOM, the increase in fEPSP slope was modestly but significantly reduced by -2.6±0.7% compared to saline ($t(471)=-3.3$, $p=0.008$). Transient short term potentiation (STP, 0-2 minutes following TBS12) was also seen in slices perfused by saline, with an increase of 226±15% over baseline ($t(14.2)=15$, $p<0.0001$). In the presence of 0.5 μM ETOM, STP was significantly reduced by 107±8% compared to saline ($t9(46.5)=-12.4$, $p<0.0001$).
We conclude that 0.5 μM etomidate modestly reduces LTP in response to a weak theta-burst protocol. However, it produces a much greater reduction in STP following that same stimulus. Our findings suggest that effects of etomidate on memory might reflect modulation of short term as well as long term potentiation.

Disclosures: M. Dreger: None. **R. Pearce:** None.

Poster

271. Short-Term Synaptic Plasticity

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Program #/Poster #: 271.08

Topic: B.05. Synaptic Plasticity

Support: KAKENHI 21K06434

Title: Simulation analysis of use-dependent modification of presynaptic Ca^{2+} entry at hippocampal mossy fiber synapse

Authors: *H. KAMIYA;
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Abstract: Ca^{2+} ions are essential for the transmitter release from the presynaptic terminals and modification of the Ca^{2+} entry critically determines the synaptic strength. So far, we have developed a simple and sufficiently realistic model of the hippocampal mossy fibers with *en passant* structure as well as ionic conductance with the experimentally determined properties of the axonal Na^{+}-, K^{+}-, and Ca^{2+}-channels by direct recordings from the axon terminals. In this study, it was attempted to explore the use-dependent modification of presynaptic Ca^{2+} entry into the hippocampal mossy fiber terminals (Kamiya *et al.*, 2002) by using this numerical model. For

quantitative evaluation of each elementary process of synaptic transmission, axonal action potentials, as well as presynaptic Ca^{2+} current, were calculated using the simulation. At a frequency of 10-50 Hz, presynaptic Ca^{2+} entry by propagating action potentials increased gradually upon repetitive stimulation. Since this use-dependent increase of presynaptic Ca^{2+} entry was much reduced by exchanging the model to that lacking inactivation of K^+ channels, it was suggested that accumulated inactivation of K^+ channels during repetitive stimulation and broadening of action potentials may account for the use-dependent increase in the presynaptic Ca^{2+} entry at the mossy fiber-CA3 synapse. The wide dynamic range of facilitation at this synapse may partly be mediated by the accumulated inactivation of K^+ channels and subsequent use-dependent enhancement of the Ca^{2+} entry into the presynaptic terminals.

Disclosures: H. Kamiya: None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.09

Topic: B.05. Synaptic Plasticity

Support: ERC Dyn-Syn-Mem 787340
CIHR postdoctoral fellowship 158090

Title: The role of AMPA receptor surface mobility in high-frequency short-term synaptic plasticity

Authors: *A. NOWACKA, A. M. GETZ, C. BREILLAT, S. DABURON, D. BESSA-NETO, C. LEMOIGNE, M. SAINLOS, D. CHOQUET;
Interdisciplinary Inst. for Neurosci., Univ. de Bordeaux / CNRS, Bordeaux, France

Abstract: Activity-dependent plasticity of synaptic transmission is a key mechanism underlying learning and memory. During high-frequency short-term synaptic plasticity (HF-STP) the amplitude of synaptic responses changes upon presynaptic stimulation on a timescale of seconds. HF-STP is important for information processing in the brain, serving particularly for temporal integration. However, the precise functions of HF-STP, and its impact on information processing, remain unknown. It is generally accepted that HF-STP is regulated primarily by presynaptic mechanisms. However, postsynaptic mechanisms have been shown to regulate HF-STP, although their role here remains to be fully understood. In 2002 we demonstrated that AMPA receptors (AMPARs), the main excitatory receptors in the brain, are trafficked in and out synapses by surface diffusion that complements endo- and exocytosis. Here, we study the functional role of AMPAR surface diffusion in HF-STP in integrated slice tissue models with intact synaptic connectivity. We use the AP-GluA2 knock-in (KI) mouse model, developed in the lab, where GluA2 subunits of AMPARs are tagged with a 15 amino acid biotinylation acceptor peptide (AP-tag) and can be specifically biotinylated when co-expressed with an

endoplasmic reticulum resident biotin ligase ($BirA^{ER}$), and immobilized on the cell surface with a biotin-binding protein NeutrAvidin. With this we show that immobilization of endogenous AMPARs modulates HF-STP by increasing synaptic depression in the Schaffer collateral-CA1 synapse of organotypic hippocampal slices. This effect is reversed when AMPAR desensitization blockers are applied, suggesting that the modulation of HF-STP is achieved by preventing the replacement of desensitized AMPARs in the synapse. Moreover, with iGluSnFr imaging we find no change in presynaptic glutamate release upon AMPAR immobilization. Altogether this strongly suggests a postsynaptic contribution of AMPAR mobility in regulating HF-STP. We aim to determine exhaustively the respective contributions of presynaptic transmitter release and postsynaptic AMPAR biophysics and mobility in HF-STP and to identify physiological processes which act upon AMPAR kinetics and mobility to regulate HF-STP in the brain. Moreover, AMPAR cross-link is a promising tool to achieve cell-specific blockade of HF-STP that may allow the transition from modeling-based evidence of HF-STP roles in brain function to experimental evidence.

Disclosures: **A. Nowacka:** None. **A.M. Getz:** None. **C. Breillat:** None. **S. Daburon:** None. **D. Bessa-Neto:** None. **C. Lemoigne:** None. **M. Sainlos:** None. **D. Choquet:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.10

Topic: B.05. Synaptic Plasticity

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ONR N00014-18-1-2114
ONR N00014-21-1-2940
NIH NIDA RHD089491A

Title: Marked differences in signal processing across nodes of the hippocampal circuit

Authors: *J. QUINTANILLA, B. G. GUNN, J. C. LAUTERBORN, A. A. LE, C. M. GALL, G. LYNCH;
Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: Within different hippocampal subregions, the manner in which responses to behaviorally relevant rhythms of input are processed is surprisingly not well understood. Specifically, how inputs arriving at theta (5Hz), beta (20Hz), and gamma (50Hz) frequencies are transformed, filtered, or amplified across hippocampal synapses is not known. Using adult mouse (C57/BL6) hippocampal slices, the present studies measured synaptic responses across three nodes of the hippocampal circuit: the lateral perforant path (LPP) projections from lateral entorhinal cortex to dentate gyrus (DG), the medial perforant path (MPP) projections from medial entorhinal cortex to DG, and the Schaffer commissural, *CA3-CA1 projections*. Ten-pulse

trains arriving at theta, beta, and gamma frequencies all produced marked within-train facilitation of dendritic responses at *CA3-CA1* synapses. However, similar stimulation of the *MPP-DG* connections resulted in a marked within-train depression at all three frequencies. Interestingly, theta stimulation of the LPP produced a modest facilitation of dendritic responses recorded in the outer molecular layer of the DG, whereas gamma activation resulted in an initial facilitation followed by an increasingly greater depression to below baseline levels. Treatment with the $GABA_AR$ antagonist, picrotoxin, had no influence on the depression seen with gamma stimulation. However, with reduced extracellular calcium levels gamma stimulation of *LPP-DG* system elicited frequency facilitation comparable to that observed for *CA3-CA1*. Thus, the unusual filtering properties of *LPP-DG* terminals were associated with presynaptic elements relating to release probability, as opposed to postsynaptic features. One such presynaptic element is the vesicular glutamate transporter 2 (VGLUT2), which is associated with high release probability synapses. VGLUT2 levels were found to be much greater at LPP synapses as compared to *CA3-CA1* terminals. Together, the unusual properties of the *LPP-DG* function as a low-pass filter that is largely associated with presynaptic factors. However, the LPP also terminates on the distal apical dendrites of field CA3. Intriguingly, the unusual *LPP-DG* filtering was not found at *LPP-CA3* synapses and instead gamma input resulted in an initial facilitation with responses returning to near baseline levels later in the train. These observations demonstrate target specification of synaptic properties across two terminals of the same axon (*LPP-DG* & *LPP-CA3*) and suggest that high frequency information (gamma rhythms) arriving from the lateral entorhinal cortex is preferentially routed through *LPP-CA3* rather than *LPP-DG*.

Disclosures: J. Quintanilla: None. **B.G. Gunn:** None. **J.C. Lauterborn:** None. **A.A. Le:** None. **C.M. Gall:** None. **G. Lynch:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.11

Topic: B.05. Synaptic Plasticity

Support: NRF Grant 2019M3C7A1031742
NRF Grant 2020R1A2C2005868

Title: Altered short-term synaptic plasticity in the PVN of the FKBP5-deficient mice

Authors: *S. ZHANG, C. CHUNG;
Dept. of Biol. sciences, Konkuk Univ., Seoul, Korea, Republic of

Abstract: FKBP5 is one of negative modulators of glucocorticoid receptors (GR) and certain FKBP5 SNPs are clinically known to be highly related with psychiatric disorders, including anxiety disorders and depression. FKBP5 overexpressing mice are reported to show increased anxiety and depression-like behaviors whereas FKBP5-deficient mice exhibit stress-resilient

phenotypes such as faster stress copying behavior and anxiolytic behavior. Moreover, the paraventricular nucleus of the hypothalamus (PVN)-specific deletion of FKBP5 mice showed increased GR activity. PVN is the key modulator of the HPA axis, governing stress responses in the body through a well-known short-term plasticity (STP) mediated by the inhibition of NMDARs. We investigated the impact of FKBP5 loss on STP of the PVN and found that stress did not induce STP in the PVN of FKBP5-deficient mice, leaving NMDAR-mediated spontaneous transmission intact. Additional blockade of TrkB signaling allows stress-induced STP in FKBP5 lacking PVN neurons. Our observations suggest that BDNF-TrkB signaling may contribute to the stress resilience of FKBP5-deficient PVN, thereby providing a useful coping strategy against stress with clinical relevance.

Disclosures: S. Zhang: None. **C. Chung:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.12

Topic: B.05. Synaptic Plasticity

Support: European Research Council ERC 692692
Fond zur Förderung der Wissenschaftlichen Forschung Z 312-B27

Title: Nanotopographical alterations in hippocampal mossy fiber bouton synapses after chemical plasticity induction

Authors: *O. KIM, Y. OKAMOTO, R. SHIGEMOTO, P. JONAS;
Inst. of Sci. and Technol. Austria (ISTA), Klosterneuburg, Austria

Abstract: Structural and functional synaptic plasticity plays a key role in storage and processing of information in the brain. However, the link between structure and function remains enigmatic. Although recent studies elucidated a potential correlation between physiological and morphological properties of synapses during synaptic plasticity (Vandael et al., 2020, Neuron 107: 509-521), the corresponding molecular mechanisms remain unknown. Hence, to pinpoint changes in the molecular architecture at hippocampal mossy fiber boutons (MFBs) during plasticity, we combined paired recording of MFB-CA3 pyramidal neuron (PN) synapses, freeze-substitution of acute hippocampal slices, and freeze-fracture replica immunolabeling (FRIL) of calcium channels and synaptic proteins (Munc13-1 and Munc13-2) in mouse hippocampus. Chemical short-term potentiation (cSTP) was induced by the adenylyl cyclase activator forskolin (FSK, 50 µM) and measured 5 min after FSK application. This approach allowed us to correlate structural and physiological data, thus, establishing the mechanisms governing cSTP. First, we found that during cSTP both readily releasable pool (RRP) and release probability (Pr) increased (RRP: control - 1.05 ± 0.12 nA vs. cSTP - 2.31 ± 0.28 nA, $p < 0.01$, here and below Wilcoxon signed-rank test; Pr: control - 0.17 ± 0.02 vs. cSTP - 0.24 ± 0.03, $p < 0.05$; $n = 8$ pairs).

Similarly, we found that the docked vesicle pool increased after FSK application and cSTP induction (number of docked vesicles per 100 nm active zone (AZ) profile length: control - 0.8 ± 0.06 (n = 70 AZs) vs. cSTP - 1.1 ± 0.07 (n = 64 AZs), $p < 0.01$). Finally, FRIL revealed that the mean number of clusters of Munc13-1 in the MFB AZs significantly increased from 2.4 ± 0.1 in control to 3.3 ± 0.2 clusters during cSTP (90 and 51 AZs respectively, $p < 0.01$). In addition, the mean nearest-neighbor distance (NND) between calcium channels and Munc13-1 proteins decreased after FSK application (control - 47.4 ± 2.2 nm vs. cSTP - 39.2 ± 2.7 nm; $p < 0.01$). Still, the magnitude of change was smaller than the increase in the number of Munc13-1 clusters during cSTP, similar to the effects of FSK on RRP and Pr observed in electrophysiology. In contrast, both the distribution of Munc13-2 and the NND between calcium channels and Munc13-2s remained unchanged during cSTP, suggesting an isoform-dependent difference in protein functions. Altogether, our results indicate a marked correlation between the size of RRP, the docked vesicle pool, and the number of clusters of the priming protein Munc13-1 at MFBs. Further, we show that exactly this expansion of releasable vesicles and to a lesser extent increase in Pr dictates cSTP at MFB-CA3 PN synapses.

Disclosures: O. Kim: None. **Y. Okamoto:** None. **R. Shigemoto:** None. **P. Jonas:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.13

Topic: B.05. Synaptic Plasticity

Support: R00/K99
Startup Funds

Title: Synaptic plasticity of cocaine seeking medium spiny neurons utilizing cFos-Trap2 mice after self-administration training

Authors: *L. FLOM, S. HODGINS, L. VACCARO, J. CROUSE, A. BOBADILLA;
Pharm., Univ. of Wyoming, Laramie, WY

Abstract: Substance use disorder, a disease characterized by uncontrollable cravings and drug-seeking behaviors, causes a decrease in social, financial, and emotional function. Understanding substance-induced changes to the nucleus Accumbens core (NAcore), a key integration center for the brain reward system, is crucial for development of therapeutics for substance use disorder. One area of interest is examining small active subpopulations of neurons, also known as neuronal ensembles, which have been linked to seeking psychoactive substances. Our research is examining changes specific to this subpopulation of neurons. We are documenting the synaptic plasticity of ensemble medium spiny neurons (MSNs) in the NAcore during cocaine seeking. Cocaine seeking neurons were identified using male and female c-Fos^{iCreERT2}/Ai14 transgenic mice. Mice underwent cocaine self-administration followed by extinction training and cue-

induced reinstatement to induce cocaine seeking. Tagging active MSNs in the NAcore was followed with analysis of the dendritic spines (spine diameter and density) in animals seeking cocaine (n=7) and controls (n=8). Analysis showed a change to spine head density but no significant change to spine head diameter. Understanding changes to the synaptic plasticity of MSNs in the NAcore expands our knowledge of drug relapse and contributes to developing therapeutic strategies for treating substance use disorder.

Disclosures: L. Flom: None. **S. Hodgins:** None. **L. Vaccaro:** None. **J. Crouse:** None. **A. Bobadilla:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.14

Topic: B.05. Synaptic Plasticity

Title: Synaptotagmin-3 is required for facilitation and CDR

Authors: *D. J. WEINGARTEN, A. SHRESTHA, S. L. JACKMAN;
Vollum Inst., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: The strength of synaptic connections between neurons is dynamically modulated by short-term plasticity (STP). Individual synapses often express multiple forms of STP, which makes synaptic transmission activity-dependent and highly non-linear. This is hypothesized to dramatically affect the flow of information through neural circuits but testing this hypothesis has been difficult without knowledge of the underlying molecular mechanisms. We recently showed that a neuronal calcium sensor called Synaptagmin-7 (SYT7) is the mechanism for a form of STP called facilitation, which boosts neurotransmitter release during high-frequency activity. Here we use patch-clamp electrophysiology in transgenic mice to show that a closely-related calcium sensor, Synaptotagmin-3 (SYT3), acts as the mechanism for a second form of STP termed Calcium-dependent Recovery from Depression (CDR) in the calyx of Held. We ablate each synaptotagmin isoform to explore how facilitation and CDR interact to regulate synaptic transmission in the hippocampus and cerebellum. We find that SYT3 and SYT7 serve partially overlapping functions in boosting neurotransmitter release, but the two isoforms are activated by different neuronal firing frequencies. Computational modeling suggests that both forms of STP are driven by an increase in the number of releasable vesicles in the presynaptic terminal. These results provide new insight into the molecular steps that trigger neurotransmitter release and offer new tools to investigate the role of STP in neural circuit function.

Disclosures: D.J. Weingarten: None. **A. Shrestha:** None. **S.L. Jackman:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.15

Topic: B.05. Synaptic Plasticity

Title: Tetraphenylphosphonium inhibits persistent activity during trace fear conditoning.

Authors: *H.-R. LEE, S.-H. LEE;
Seoul Univ., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Post-tetanic potentiation (PTP) was proposed as short-term plasticity that may mediate the generation of persistent activity during working memory. Persistent activity (PA) in prelimbic cortex (PL) is essential for acquisition of trace fear memory. However, the neurophysiological mechanisms underlying PA are poorly understood. We examined the neurobiological mechanism of PTP in PL, and tested whether PTP plays a role in PA and trace fear conditioning. Using optogenetic stimulation, we stimulated afferent fibers to layer 5 (L5) pyramidal neurons (PNs) in cell-type and layer-specific manner. We found that PTP was induced at the synapses onto L5 corticopontine (Cpn) PNs from L2/3 PNs and L5 commissural (COM) PNs, but not at synapses onto L5 COM PNs. While PTP at both synapse types onto Cpn cells was inhibited by protein kinase C inhibitor, tetraphenylphosphonium (TPP), a mitochondrial NCLX blocker, suppressed PTP only at L2/3-to-Cpn synapses. Studying the effect of TPP infusion into the PL on trace fear conditioning, we found that TPP did not affect the trace memory formation per se, but reduced the maintenance of the fear memory during fear memory extinction test. In vivo recordings revealed that c.a. 10% of PL-PNs exhibited PA after conditioning stimulus (CS, tone). The TPP infusion abolished such post-CS PA during both conditioning and extinction training. These results imply that PTP at L2/3-to-Cpn synapses is required for post-CS PA during trace fear conditioning, and plays a role in maintenance of trace fear memory.

Disclosures: H. Lee: None. **S. Lee:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.16

Topic: B.05. Synaptic Plasticity

Support: NSERC of Canada 04617

Title: 17β-estradiol facilitates excitatory synaptic transmission in layers II and III of the entorhinal cortex via suppression of synaptic inhibition.

Authors: *A. A. BATALLÁN BURROWES[1], E. MOISAN[2], C. LA RUE[3], C. A. CHAPMAN[4]; [1]Concordia Univ., Concordia Univ., Montréal, QC, Canada; [2]Psychology, Concordia Univ., Montreeal, QC, Canada; [3]Psychology, [4]Concordia Univ., Concordia Univ., Montreal, QC, Canada

Abstract: Acute estrogen supplementation can have rapid effects on rodent behavior and cognition through the rapid modulation of synaptic transmission and intracellular signaling mechanisms. Estrogen receptor (ER) activation can inhibit GABAergic neurons, modulate excitatory NMDA and AMPA currents, and reversibly increase CA1 pyramidal neuron dendritic spine density. The entorhinal cortex is a significant source of cortical associational input to the hippocampus. Previous work has shown that 20-minute application of 17β-estradiol (E2) can facilitate excitatory postsynaptic field potentials in the lateral entorhinal cortex through activation of the G protein-coupled estrogen receptor-1 (GPER1). The current study tested whether this facilitation is induced via E2-induced changes in APMA- or NMDA-glutamate receptor-mediated excitatory postsynaptic currents (EPSCs), or GABA-mediated inhibitory postsynaptic currents (IPSCs) in principal cells of layers II and III of the rat entorhinal cortex. On postnatal day (PD) 63, female Long-Evans rats were ovariectomized and implanted with a subdermal E2 capsule to maintain constant low levels of circulating E2. Electrophysiological recordings were collected from horizontal brain slices between PD70 and PD91. Pharmacologically isolated whole-cell EPSCs and IPSCs were recorded from principal neurons in layers II and III in response to electrical stimulation of layer I afferents. After stable baseline recordings, 10 nM E2 was bath-applied for 20 min, followed by a 30-min washout period. Application of E2 resulted in no significant changes in isolated AMPA-mediated EPSCs. The amplitude of NMDA-mediated EPSCs was reduced during the application of E2, and remained reduced during the washout period. However, E2 resulted in a marked reduction in the amplitude of GABA-mediated IPSCs that reversed during the 30-min washout period. These results indicate that the facilitation of excitatory synaptic transmission induced by E2 in the entorhinal cortex, which may contribute to the cognitive effects of estrogen in the hippocampal region, is largely due to a reduction in the strength of the inhibitory synaptic transmission.

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Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

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Program #/Poster #: 271.17

Topic: B.05. Synaptic Plasticity

Support: FONDECYT Grant Nº 120–0474
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Title: Chronic ketamine administration reduces phasic dopamine release and impairs short-term excitatory synaptic plasticity in nucleus accumbens of adult male rats.

Authors: *V. B. VELÁSQUEZ[1,2], C. ESTAY-OLMOS[1,2], C. BONANSCO[1], M. FUENZALIDA[1], R. SOTOMAYOR-ZARATE[1];
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Abstract: Ketamine is a phencyclidine derivative which is used therapeutically as a dissociative anesthetic in the treatment of pain and lately used in resistant depression treatment with great outcomes. In addition, ketamine has been used as a recreational drug specially in Asia, Europe, North America and recently in South America. Pharmacologically, ketamine is considered a non-competitive antagonist of the NMDA receptor and recent investigations showed that ketamine has an inhibitory effect in voltage dependent Na^+ and K^+ channels and inhibits dopamine (DA) reuptake in several brain areas such as prefrontal cortex, hippocampus and nucleus accumbens (NAc). However, the chronic effects of ketamine on brain areas such as NAc, a structure involved in drug reward have been poorly studied. The aim of this work was to determine whether ketamine administration for 10 days affect DA release and excitatory transmission in NAc. Adult male Sprague-Dawley rats (3 - 4 months old) were injected with ketamine (30 mg/kg, i.p.) or saline (control group; 1 mL/kg i.p.) for 10 consecutive days. During this protocol we measured locomotor activity on the 1^{st}, 5^{th} and 10^{th} day of treatment. One day after last dose of ketamine (11^{th} day) animals were euthanized and used for fast-scan cyclic voltammetry (FSCV) and electrophysiological experiments to evaluate tonic and phasic DA release, short-term excitatory plasticity and the passive properties of the medium spiny neurons (MSNs) in the NAc core, respectively. Our results show that chronic ketamine administration increases locomotor activity on 5^{th} and 10^{th} day, reduces the phasic DA release and increases the probability of glutamate release in NAc core. These findings suggest that repetitive ketamine use may lead to pathological consequences by producing neurochemical and synaptic function changes in NAc, a key nucleus involved in reward and drug addiction.

Disclosures: V.B. Velásquez: None. **C. Estay-Olmos:** None. **C. Bonansco:** None. **M. Fuenzalida:** None. **R. Sotomayor-Zarate:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 271.18

Topic: B.05. Synaptic Plasticity

Support: CIHR Grant 702GrantRN38364
NSERC PGSD scholarship
Neurasmus EMJMD 703 scholarship

Title: Functional subtypes of synaptic dynamics revealed by model-based classification

Authors: *J. BENINGER[1], J. ROSSBROICH[2], K. TOTH[1], R. NAUD[1];
[1]Dept. of Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada; [2]Friedrich Miescher Inst. for Biomed. Res., Basel, Switzerland

Abstract: Synapses show preferential responses to particular temporal patterns of activity. Across synapses, however, there is a large degree of response heterogeneity that is routinely and tacitly separated into classes. Here we combined a kernel-based model and machine learning techniques to infer functionally distinct classes of short-term plasticity in data from the Allen Institute Synaptic Physiology data set. First, we fitted our computational model to rodent synapses in the database, which reduced the dynamics of each synapse to a small number of parameters. Then, we found that better prediction of transcriptomic cell type can be achieved by using model parameters than by using standard electrophysiological features. To identify the presence of classes of synaptic dynamics, we compared three approaches: 1) observing functional classes that arose in the prediction of transcriptomic cell class using machine learning, 2) unsupervised clustering of model parameters, and 3) averaging the model parameters belonging to the same cell class. In rodent data, we found a remarkable convergence onto four functional classes. These groups were labelled “strongly facilitating”, “depressing”, “facilitating then depressing” and “no plasticity”. Application of the same clustering methods in human data inferred the same classes. We propose that these functional types of synaptic dynamics shape the connectivity of the brain and that, paired with our model fits, will be useful as readily available, biologically plausible building blocks for computational simulations.

Disclosures: J. Beninger: None. **J. Rossbroich:** None. **K. Toth:** None. **R. Naud:** None.

Poster

271. Short-Term Synaptic Plasticity

Location: SDCC Halls B-H

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Program #/Poster #: 271.19

Topic: B.05. Synaptic Plasticity

Support: R37NS045876
R01NS112706-01
James G. Hirsch, MD Endowed Medical Student Research Fellowship
Yale School of Medicine Fellowship for Medical Student Research

Title: Presynaptic plasticity in a mammalian synapse involves actin remodeling and vesicle recruitment

Authors: *S. SUBRAMANIAN[1], K. SAN AGUSTIN RUIZ[2], A. SHTEYMAN[1], L. K. KACZMAREK[1,2], E. A. JONAS[1];
[1]Yale Univ. Sch. Med., New Haven, CT; [2]New York Univ., New York, NY

Abstract: Post-tetanic potentiation (PTP) is a form of short-term plasticity that is induced by repeated stimulation. One way in which PTP occurs is through an increase in the number of vesicles at the active zone. The calyx of Held is a large, rapidly firing synapse involved in sound localization. The presynaptic terminal of the calyx of Held contains a dense layer of filamentous actin (F-actin) that is organized in part by the potassium channel Kv3.3 in order to facilitate endocytosis of vesicles and replenishment of the readily releasable pool, functions critical to maintaining synaptic transmission at high frequencies. In this study we used three-dimensional super resolution microscopy techniques to determine if repeated stimulation of the calyx of Held synapse would result in reorganization of the actin cytoskeleton to allow the accumulation of additional vesicles at the active zone. We found that repeated stimulation results in the bundling of actin filaments with a coincidental increase in the number of vesicles at the active zone, suggesting that actin is cleared from sites of vesicle accumulation. Mitochondria fuel the energy-demanding process of synaptic transmission. Given the close association of mitochondria with the cytoskeleton and vesicle pools, we used electron microscopy to analyze whether repeated stimulation would alter mitochondrial structure or localization with respect to active zones. We found an increase in electron density of mitochondria located near active zones, indicative of ATP synthase activity. Our findings indicate that synaptic activity induces dynamic structural and metabolic changes in the presynaptic terminal that enable short-term plasticity, adding to the emerging links between the cytoskeleton, mitochondrial metabolism, and neurodegenerative disease.

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Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.01

Topic: B.07. Network Interactions

Support: European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3)

Title: Nitric Oxide production and diffusion model in a cerebellar spiking neural network.

Authors: *A. TRAPANI[1], B. GAMBOSI[1], A. ANTONIETTI[2], G. NALDI[3], E. D'ANGELO[2], A. PEDROCCHI[1];
[1]Politecnico di Milano, Milano, Italy; [2]Univ. di Pavia, Pavia, Italy; [3]Univ. di Milano, Milano, Italy

Abstract: Since the discovery of Nitric Oxide (NO) acting as an intracellular messenger in the brain, there is growing evidence that NO is responsible for the coordination of synaptic activity,

both excitatory and inhibitory. Cellular types that can produce NO molecules have been found in the cerebral cortex, hippocampus and cerebellum. As NO synthesized in response to an external stimulus diffuses freely across the cell membrane, spreading rapidly in the extracellular space, it can provide a type of neural communication that goes beyond the anatomical synaptic connection. An increasing number of studies suggested that a closely-packed group of neurons containing neuronal NO synthase (nNOS) enzyme may generate a diffuse cloud of NO when stimulated, thus acting as a volume transmitter with a relatively large area of influence. Given NO's relevance in the cerebellar granular layer, we focused on simulating NO produced in the synapses between mossy fibres and granule cells. We developed a model able to simulate the production and diffusion of NO molecules and integrate it into a 3D scaffold model of the cerebellar microcircuit. We modelled the dynamics of NO production with two differential equations: one describing the Ca2+/calmodulin binding and the other representing the activation of the nNOS enzyme. To model the NO diffusion, we used the heat diffusion equation modified to take into account the activity of the NO source and the NO consumption in the extracellular space. To validate the single-source model, we compare our results with simulations performed in NEURON (Reaction&Diffusion module), where all the steps in the biochemical reactions involved in the NO synthesis are implemented. Unlike the complex reaction cascade simulated in NEURON, our production function can replicate the synthesis process in two steps, following the timing of spike events. Starting from this single-source model, we implemented a Python simulator for "diffusive communication" in large spiking neural networks. We integrated it with the Brain Scaffold Builder (BSB), which enables spatially, topologically and morphologically detailed neural network simulations. Thus, we can compute the NO concentration in given points of the network as the sum of the diffusion profile from surrounding active sources, and we can replicate *in vitro* experimental protocol performed in the granular layer where the NO concentration is measured after an electrical stimulation on the mossy fibres. Further developments will include a model to simulate the dependency between NO concentration and changes in the synaptic intrinsic conductances of the granule cells to investigate NO's role in plasticity mechanisms.

Disclosures: **A. Trapani:** None. **B. Gambosi:** None. **A. Antonietti:** None. **G. Naldi:** None. **E. D'Angelo:** None. **A. Pedrocchi:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

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Program #/Poster #: 272.02

Topic: B.07. Network Interactions

Support: Canadian Institutes of Health Research
Natural Sciences and Engineering Research Council
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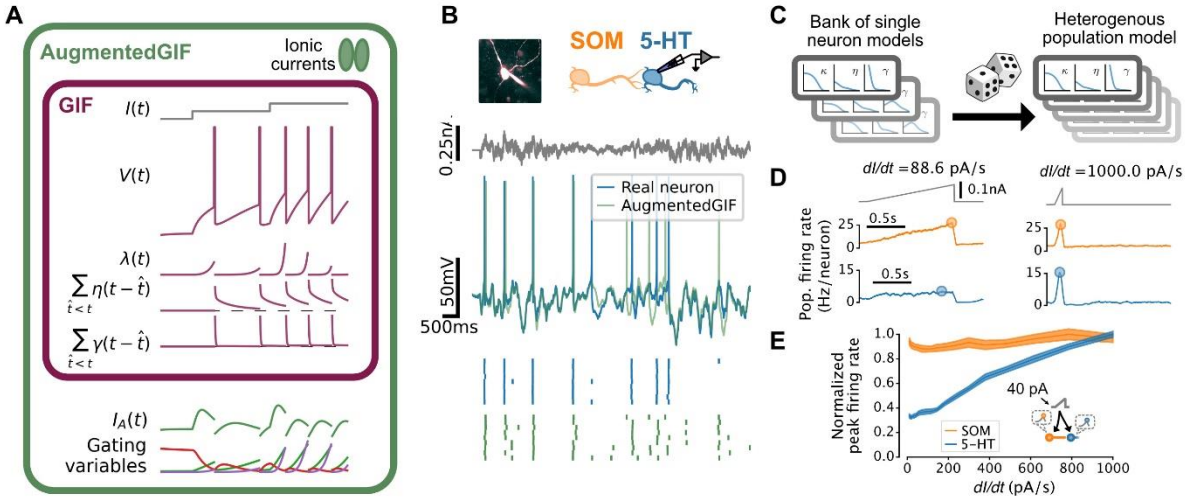
Brain Canada
Krembil Foundation

Title: Temporal derivative computation in the dorsal raphe network revealed by an experimentally-driven augmented integrate-and-fire modeling framework

Authors: ***E. F. HARKIN**[1], A. PAYEUR[2], M. B. LYNN[1], J.-F. BOUCHER[1], L. CAYA-BISSONNETTE[1], D. CYR[1], C. STEWART[3], A. LONGTIN[1], R. NAUD[1], J.-C. BÉÏQUE[1]; [1]Ctr. for Neural Dynamics, Univ. of Ottawa, Ottawa, ON, Canada; [2]Mila, Univ. de Montréal, Montréal, QC, Canada; [3]Dept. of Biol., McGill Univ., Montréal, QC, Canada

Abstract: By means of an expansive innervation, the relatively few phylogenetically-old serotonin (5-HT) neurons of the dorsal raphe nucleus (DRN) are well-positioned to adaptively regulate behaviour via coordinated modulation of neural circuits across the brain. The anatomy of DRN afferents has been dissected in detail, yet the computations performed within the DRN itself are still unknown. To gain insight into the connection between its physiological form and computational function, we developed a new class of experimentally-constrained spiking neuron model that combines the realism of Hodgkin-Huxley models with the simplicity and predictive power of generalized integrate-and-fire (GIF) models. Our augmented GIF models and whole-cell electrophysiological recordings of DRN 5-HT and somatostatin (SOM) neurons revealed key excitability features of each cell type: a large A-type potassium current, potent after-hyperpolarization currents, and dynamic spike threshold in 5-HT neurons, and weaker adaptation and a high degree of cell-to-cell variability in SOM neurons. Simulating spiking networks composed of models fitted to individual cells of each type led to three predictions about the computational effects of these features: First, the substantial heterogeneity of DRN SOM neurons causes feed-forward inhibition of 5-HT neurons to be divisive rather than subtractive. Second, previously-described endocannabinoid modulation of feed-forward inhibition has a multiplicative effect on 5-HT output. Third, the potent adaptation mechanisms found in 5-HT neurons cause their population-level firing rate to powerfully report the temporal derivative of their input. By applying a new bottom-up biological neural network modelling approach to the DRN, this work gives insight into the computational function of the 5-HT system. As the search for a behavioural correlate of DRN 5-HT output continues, our results suggest that the DRN is particularly sensitive to how its inputs change over time, reflecting one of the salient emerging computations that dominate its output to regulate behaviour.



Disclosures: **E.F. Harkin:** None. **A. Payeur:** None. **M.B. Lynn:** None. **J. Boucher:** None. **L. Caya-Bissonnette:** None. **D. Cyr:** None. **C. Stewart:** None. **A. Longtin:** None. **R. Naud:** None. **J. Béïque:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.03

Topic: B.07. Network Interactions

Support: NCBS-TIFR

Title: Computational analysis of organized synaptic input in networks with random connectivity

Authors: *B. P. SOMASHEKAR[1], U. S. BHALLA[2];
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Abstract: Much of the information that the brain processes consists of stimuli which are organized in time. The ability to relate different entities, learn ordering relationships between them and predict future events is vital for tasks such as perception, communication and context discrimination. Several studies have reported links between behavioral activity and spatio-temporally ordered neural activity in the form of cell assemblies. How is information from neuronal assemblies decoded by neurons downstream?
Dendritic computations via clusters or sequences have been indicated to play a vital role in processing inputs and enhancing neuronal coding capacities. Dendrites achieve this feat with the help of nonlinearities that occur in the form of specific types of channels or chemical mechanisms. However dendritic computations require precise convergence of correlated inputs onto short segments of dendrites. Can clustered and sequential convergence arise even in networks with random connectivity or do they require special connectivity rules? What is the effect of background activity on these computations? We used analytical equations and numerical simulations of connectivity based on network statistics data from the rat hippocampus and cortex to tackle these questions. With the help of subcellular electrical and chemical models, we explored the role of background activity on sequence discrimination and cluster selectivity. Our results indicate that sequential and clustered convergence of 3-6 inputs is likely even in networks with random connectivity. However, discrimination of different patterns via clustered selectivity becomes difficult in networks with random connectivity. This occurs because combinatorics also supports the convergence of a large number of alternate clusters containing repeating inputs from ensembles. This invokes the need for selective wiring or special plasticity rules to enable this computation. Sequences may be less affected by such effects, depending on the degree of selectivity offered by the mechanism at play. Sequence selectivity via electrical mechanisms is more sensitive to ectopic inputs than chemical mechanisms as inputs arriving in flanking regions of the sequence could also affect selectivity. Our work suggests that sequential and clustered convergence of 3-6 inputs is likely even with random connectivity, and sequence

discrimination computation is best suited for low noise networks operating with mechanisms that provide good physiological selectivity such as the hippocampal-CICR and hippocampal-electrical configurations.

Disclosures: **B.P. Somashekar:** None. **U.S. Bhalla:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.04

Topic: B.07. Network Interactions

Support: KAKENHI 18H05213 from JSPS
KAKENHI 19H04994 from JSPS

Title: Spontaneous replay of priors for causal inference in recurrent network models

Authors: *T. ASABUKI[1], T. FUKAI[2];
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Abstract: The brain learns an internal model of the environment to improve performance in various cognitive behaviors such as perception and inference. Several recent studies suggest that these internal representations, or optimal priors of the environment, are maintained in spontaneous activity. For instance, repeated exposure to visual stimuli generates spontaneous activity of visual cortical neurons that reactivates the stimulus-evoked activity patterns. However, the underlying mechanisms for learning such priors remain unclear. Here, we developed a recurrent spiking neural network model that remembers the statistical structure of sensory events. Our model automatically segments repeated patterns in input to encode them into cell assemblies by minimizing the errors in probability structure between sensory-evoked and internally-driven activities. Thus trained model spontaneously replays these cell assemblies with frequencies proportional to the occurrence probabilities of the corresponding sensory events. To our surprise, our model accurately replicates the behavioral biases observed in monkeys performing a sensory decision-making task.

Disclosures: **T. Asabuki:** None. **T. Fukai:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

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Program #/Poster #: 272.05

Topic: B.07. Network Interactions

Support: HFSP RGP 0019/2018

Title: Synaptic Plasticity Induces Sleep-wake Transitions in Large-scale Computational Models

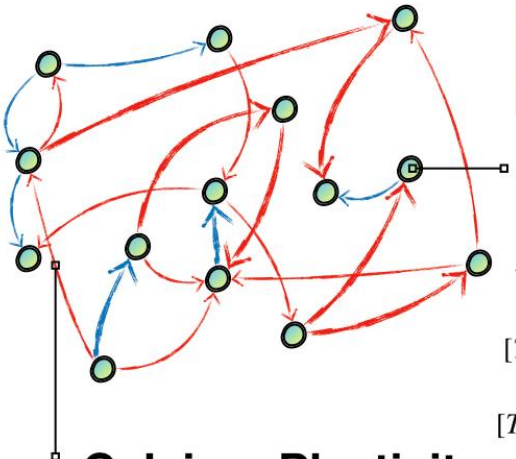
Authors: *G. SUN[1], D. B. FORGER[2];
[1]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [2]Univ. Michigan, Ann Arbor, MI

Abstract: Understanding sleep has been a central topic in sleep research. Researchers have raised different hypotheses and computational models to explain sleep and sleep-wake transitions. However, most of these models achieve sleep-wake transitions by directly changing the electrophysiology of neurons and ignore many electrophysiological or synaptic processes that are known to affect sleep dynamics.
Using a newly built simulation platform that can parallelly simulate hundreds of thousands of neurons with full electrophysiology as fast as in real time, we simulate a network of spiking cortical neurons that include calcium signaling. In this network, we also simulate a calcium synaptic plasticity model that regulates the synaptic weights between the neurons. Surprisingly, we observe that the spontaneous fluctuating calcium dynamics can affect the synaptic weight distribution, which further causes the whole network to transition between sleep and wake. Alternating extracellular calcium concentrations can also induce sleep-wake transitions of the network, a phenomenon that has been known from past experiments.

Model Summary

1.A spiking neuron model with Calcium Signaling

2.A Calcium Plasticity Rule that governs the synaptic weights



AN Neuron

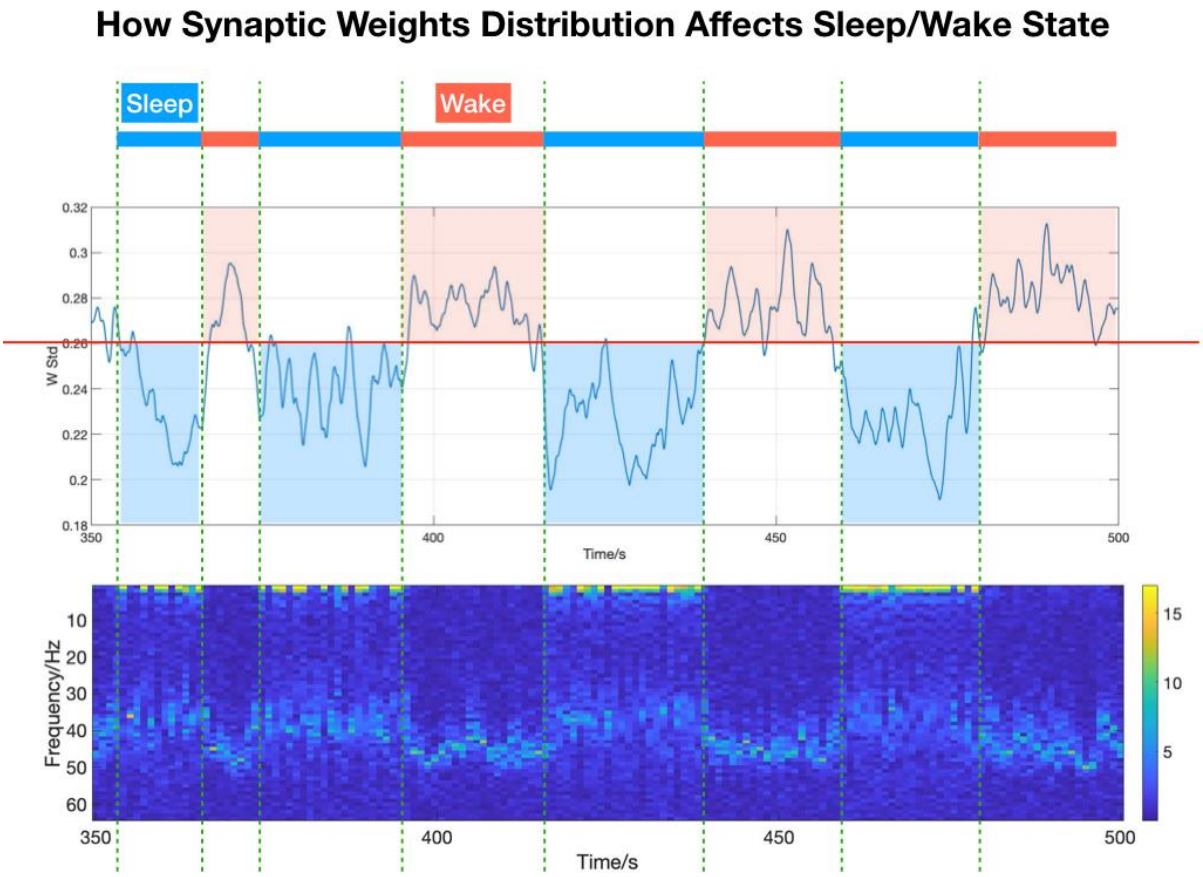
$$\frac{dy_s}{dt} = a_r[T_{in}]_j - \frac{y_s}{\tau_s}, s = NMDA \ldots$$

$$[T_{in}]_j = \frac{1}{N_i}\sum_i w_{ij}[T_{out}]_i$$

$$[T_{out}]_i = \frac{T_{max}}{1 + exp(-(V_i - V_T)/K_p)}$$

Calcium Plasticity

$$\tau\frac{dw_{ij}}{dt} = \gamma_p(w_{max} - w_{ij})H[c(t) - \theta_p] - \gamma_d(w_{ij} - w_{min})H[c(t) - \theta_d]$$



Disclosures: **G. Sun:** None. **D.B. Forger:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.06

Topic: B.07. Network Interactions

Support: NIH R01EY026924
NIH R01NS113073

Title: A computational model approach to account for phase-dependent recruitment of sensory areas during working memory

Authors: ***W. H. NESSE**[1], I. VANEGAS[2], M. PARTO, Sr.[4], K. CLARK[5], B. NOUDOOST[3]; [1]Univ. of Utah, Univ. of Utah, Salt Lake Cty, UT; [2]Ophthalmology and Visual Sci., [3]Univ. of Utah, Univ. of Utah, Salt Lake City, UT; [4]Iran Univ. of Sci. and Technol. (IUST), Iran Univ. of

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Abstract: Working memory (WM) has been shown to induce changes in local field oscillations in extrastriate visual cortex, particularly in the Beta frequency range (12-30 Hz). These WM-dependent oscillations are also accompanied by changes in spike timing relative to this oscillatory phase, allowing sensory information to be represented in the phase of spikes. Moreover, WM has been shown to increase the response gain in visual areas, as reflected in their enhanced firing rate in the presence of visual stimuli. Our preliminary experimental results show WM-dependent changes in firing rate are correlated with WM-dependent oscillatory changes on a trial by trial basis. This raised the hypothesis that WM-dependent changes in response gain could be a byproduct of WM-induced oscillatory changes in a network. In order to test this idea, we developed a spiking network model that can account for WM-induced changes observed in visual areas. The network is capable of representing stimulus input in its average spike count (rate code), as well as in terms of oscillatory phase of spikes (phase code). Consistent with experimental findings, we found that our proxy WM input signal increased the frequency and amplitude of network oscillations in a stimulus dependent manner, while preserving the number of spikes per oscillation and strengthening spike phase locking. This corroborates our experimental findings regarding the relationship between WM-dependent changes in oscillation and response gain. In order to test whether response gain is a byproduct of oscillatory changes, we developed a modified version of our model with increased inhibitory gain. This manipulation could eliminate the changes in stimulus-dependent oscillation frequency, as well as stimulus-dependent changes in average spike count. However, the relative timing of spikes to the phase of the oscillation could still reflect the stimulus information. Thus, the capacity of the rate code to reflect stimulus strength in this network seems to rely on changes in the frequency of oscillation, while the phase coding capacity seems to be independent of these alterations in rate and oscillatory frequency. The model simulation results also showed that the phase code provides a more reliable representation of the input compared to the rate code. These results suggest that WM recruits sensory areas primarily via a phase code and provides an network-level description of this recruitment.

Disclosures: W.H. Nesse: None. **I. Vanegas:** None. **M. Parto:** None. **K. Clark:** None. **B. Noudoost:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.07

Topic: B.07. Network Interactions

Support: DIRP, NIMH, USA, ZIAMH002797
BRAIN initiative Grant U19 NS107464-01

Title: Brain-inspired reservoir computing

Authors: *K. SRINIVASAN[1,2], D. PLENZ[1], M. GIRVAN[2];
[1]Section on Critical Brain Dynamics, Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Physics, Univ. of Maryland, Col. Park, College Park, MD

Abstract: Artificial recurrent neural networks (RNNs) comprise a large and diverse class of machine learning models that are designed by a more or less detailed analogy with real brain networks. Reservoir Computing is one such machine learning framework that has been garnering a lot of attention. Recent work with reservoir computers has demonstrated excellent performance in time-series forecasting as well as other practical tasks like speech recognition. While traditional reservoir computers (RCs) use a randomly generated neural network, various alternate systems have been suggested for the "reservoir" component, including small-world networks, spiking neural networks, and even physical reservoirs such as photonic cavities and field programmable gate arrays (FPGAs). By using a brain-inspired network model instead of a traditional RC we hope to identify the salient features that contribute to the information processing capacity of neural networks. One such key feature is the interplay of excitation and inhibition (E/I). Through our RC model, we demonstrate that networks having inhibitory neurons have greatly improved performance over their purely excitatory counterparts. Even moderate inhibition can make the network more robust and maintains better performance over a wider range of RC parameters. Another important feature that contributes to the performance of neural networks is criticality. Through our analysis, we show that RCs have enhanced performance in the vicinity of a critical point. Our results suggest that reservoirs composed of critical, E/I units achieve better and more robust performance in essential information processing metrics like (i) **Memory** - How long the RC can remember an input, (ii) **Separability** - How effectively the RC can differentiate 2 inputs in its phase space and (iii) **Dynamic Range** - What range of input amplitudes the RC can handle. Overall, our brain-inspired computational models provide crucial insights into our understanding of information processing in the brain using RCs with E/I balanced networks close to criticality.

Disclosures: **K. Srinivasan:** None. **D. Plenz:** None. **M. Girvan:** None.

Poster

272. Computational Modelling of Synaptic Networks

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Program #/Poster #: 272.08

Topic: B.07. Network Interactions

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EU/FP7 Moving Beyond grant ITN-No-316639 grant agreement no. 604102 (HBP)
EU/Horizon 2020, no.945539 (HBP SGA3)
EU-project euSNN (MSCA-ITN-ETN H2020-860563)

Title: The substantia nigra pars reticulata in vitro and in silico

Authors: *W. S. THOMPSON[1], J. J. J. HJORTH[2], A. KOZLOV[2], G. SILBERBERG[1], J. HELLGREN KOTALESKI[1,2], S. GRILLNER[1];
[1]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Dept. of Computat. Sci. and Technology, Sci. for Life Lab., Royal Inst. of Technol., Stockholm, Sweden

Abstract: The substantia nigra pars reticulata (SNr) constitutes the largest output nucleus of the basal ganglia. It plays an important role in motor control, integrating multiple inputs and sending organised projections to a range of downstream targets. Recent experimental work has provided evidence for fine-grain functional subdivisions through this basal ganglia circuit, suggesting a sophisticated pattern of information processing. Here we present a detailed model of the SNr and its afferents. The electrophysiological and morphological properties of neurons in the mouse SNr are characterized in acute brain slices via whole cell patch-clamp and morphological reconstruction. Furthermore, a novel cortico-nigral connection is functionally characterized with virally targeted optogenetics administered to motor cortex. Using reconstructed morphologies, multicompartmental single-cell models are instantiated within the NEURON simulation environment and simulated with known ion channels. In order to accurately recapitulate the neuronal physiology observed experimentally, model parameters are optimized with a genetic algorithm. The unique synaptic properties of the different input sources are captured, with spatially organised connections established via touch-detection, using the Snudda framework. The model integrates with a large-scale model of the dorsal striatum that features detailed models of striatal projection neurons and interneurons (Hjorth et al., 2020). By recreating the precise topography of these pathways in silico we have established a data-driven platform to investigate information flow through the basal ganglia in virtual experiments that would otherwise be impossible to conduct.
Reference: Hjorth et al., 2020, Proc. Natl. Acad. Sci. U. S. A. 117(17):9554-9565.

Disclosures: W.S. Thompson: None. **J.J.J. Hjorth:** None. **A. Kozlov:** None. **G. Silberberg:** None. **J. Hellgren Kotaleski:** None. **S. Grillner:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.09

Topic: B.07. Network Interactions

Support: Science Foundation Ireland Grant Number 18/CRT/6049

Title: Neural desynchronization via targeted removal of connections in a model of the parkinsonian basal ganglia

Authors: *C. MCLOUGHLIN, M. LOWERY;
Electronic and Electrical Engin., Univ. Col. Dublin, Dublin, Ireland

Abstract: Synchronous activity in the subthalamic nucleus (STN) is correlated with motor symptom severity in Parkinson's disease. Reductions in synchrony associated with deep brain stimulation are correlated with symptom improvement. Changes in coupling strength and neural properties influence the emergence of pathological synchrony within the cortico-basal ganglia, however the effects of changes in connection the pattern between neurons are less clear. We have shown how network structure impacts synchrony in a model of the STN-GPe (Globus Pallidus externus), and how theoretical methods developed for coupled systems can approximately predict the level of synchrony. Extending this approach, here we explore how synchrony may be reduced via structural network changes involving targeted removal of connections. Our model consisted of 200 Hodgkin Huxley neurons coupled by excitatory (STN-GPe) and inhibitory (GPe-STN, GPe-GPe) synapses. Networks were generated using random (RD), small world (SW), scale free (SF), or spatial (SP) rules. Synchrony was estimated using a variance based measure averaged across each population. The targeted removal (TR) algorithm treats the STN-GPe as a single population, with neurons defined in terms of their connections (edges). Each neuron is assigned to one of 4 groups in a process that minimises edges between groups. Edges not within a group or going to an adjacent group are then removed. From coupled oscillator theory, maximising the ratio of within to between group edges should reduce global synchrony by creating 4 out of phase populations, with the phase relation promoted by the feedforward connections between groups. The efficacy of TR was assessed by comparing the resulting synchrony with that obtained following random removal of the same number of edges. TR reduced synchrony for SW by 36.5%, SP by 32.7%, RD by 30.3% and SF by 63.1%. The proportion of edges removed varied with topology: SW and SP required the removal of the fewest edges at 8.6% and 12.9% while RD and SF required 44.7% and 47.2%. In contrast, random removal of the same number of edges reduced synchrony for SW by 3.17%, SP by 8.89%, RD by 19.9%, and SF by 76.6%. In summary TR performed well for SW and SP networks, achieving large synchrony reductions with minimal edge removal, but the approach requires the removal of almost half the edges in RD and SF networks. In addition, TR was less effective for SF networks compared to random removal. These results show how targeted decoupling of neurons through novel neuromodulation or similar interventions may off a less intrusive method for reducing pathological synchrony in certain neuronal topologies.

Disclosures: C. McLoughlin: None. **M. Lowery:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.10

Topic: B.07. Network Interactions

Title: Anatomical and computational investigation of ventral pallidal modulation of the basolateral amygdala

Authors: *T. BANKS[1], T. TUNA[3], C. SEVINÇ[4], G. UNAL[5], S. S. NAIR[2];
[2]Electrical & Computer Engin., [1]Univ. of Missouri, Columbia, MO; [3]Texas A&M University; Bogazici Univ., College Station, TX; [4]Cognitive Sci., Bogazici Univ., İstanbul, Turkey; [5]Psikoloji Bolumu, Bogazici Univ., Istanbul, Turkey

Abstract: In addition to its dense cholinergic innervation, the basal forebrain (BF) provides substantial GABAergic, glutamatergic and peptidergic inputs to various limbic structures. The role of these projections in hippocampus-dependent cognitive processes are relatively well-studied. BF cholinergic and GABAergic projections to the hippocampus differentially contribute to the local network activity such as the generation of hippocampal theta rhythm. Several BF nuclei send similar cholinergic and GABAergic projections to the basolateral amygdala (BLA), but their localization and function on amygdala-dependent affective processes are not fully understood. We utilized a two-fold strategy to identify BF neuronal groups that target the BLA and reveal their potential role in local network activity. We combined retrograde tract-tracing with fluorescent immunohistochemistry in Wistar rats to reveal cholinergic and parvalbumin (PV) or calbindin (CB)-immunoreactive putative GABAergic BF neurons projecting to the lateral (LA), basolateral (BL) nuclei of the BLA as well as the central nucleus and the bed nucleus of the stria terminalis. Once we identified ventral pallidum (VP) projections to the BLA as the densest source of non-cholinergic BF innervation of the amygdala, we developed a BLA network model incorporating BF cholinergic and GABAergic inputs. Retrograde tracer injections into the LA and BL produced the densest labeling in the VP, substantia innominata, and horizontal diagonal band, collectively constituting more than 75% of the labeled BF neurons. Of all BLA-projecting VP neurons, 28.6% were CB+ and 7.3% were PV+ putative GABAergic cells. In order to test the potential network function of the revealed ventral pallidal projections in the amygdala, we developed a 1000-neuron BLA network model that has principal neurons (PNs) as well as PV+, somatostatin+, and calretinin+ BLA interneurons, connected using AMPA, NMDA, and GABA synapses. Extrinsic inputs comprised constant excitatory thalamic/cortical inputs, rhythmic BF GABAergic projections, and modulatory BF cholinergic input. To mimic phase-dependent disinhibition, 90% of the BF GABAergic input was provided to the PV+ interneurons while 10% was directed to the PNs. As hypothesized, local theta oscillations arose when both the cholinergic and the GABAergic input from the BF were present in addition to the excitatory thalamic/cortical input. Removal of the cholinergic or the GABAergic BF input alone decreased BLA theta power, and the oscillation ceased completely when the GABAergic input is cut together with either the cholinergic or the thalamic/cortical input.

Disclosures: T. Banks: None. **T. Tuna:** None. **C. Sevinç:** None. **G. Unal:** None. **S.S. Nair:** None.

Poster

272. Computational Modelling of Synaptic Networks

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.11

Topic: B.07. Network Interactions

Support: NIH MH122023
NSF OAC-1730655

Title: Self-learning neurophysiology tutorials in the medical curriculum

Authors: *S. NAIR[1], G. GLICKERT[2], M. WALKER[3], S. S. NAIR[4];
[1]Med. School, Psychiatry, American Univ. of the Carribean, Columbia, MO; [2]Neural Engin., Univ. of Missouri, Columbia, Wildwood, MO; [3]Biomed. Sci., Univ. of Missouri-Kansas City, Kansas City, MO; [4]Electrical & Computer Engin., Univ. of Missouri, Columbia, MO

Abstract: Computational tools are increasingly being used in the medical sciences for both research and instruction. The new area of computational psychiatry, for instance, aims to provide a stronger neurobiological foundation for psychiatry with the goal of improving diagnosis by highlighting the cellular and circuit correlates of neuropsychiatric disorders. According to NIMH, the new field will "... foster a novel biologically-based computational framework to identify and validate biomarkers and novel treatment targets relevant to the prevention, treatment, and recovery of psychiatric disorders". Computational tools designed for self-learning will benefit students, residents and researchers from Neurology and Psychiatry who may be interested in this new and growing area. Herein, we report a curriculum for neurology and psychiatry students, residents and researchers that comprises self-learning tools such as slides, videos, and freely available software tutorials (virtual labs) that can be run on any browser without the need for install. The self-learning tutorials cover both fundamental concepts in neurobiology as well as fear and extinction learning concepts, all of which form the foundations for computational psychiatry. We use the NEURON programming language to model neurobiology phenomena and use Python for graphing and visualization of the models. In a series of six browser-based Google Colab notebooks, we build from single cell mechanisms to small circuits. Another similar set of six tutorials cover the basics of the mammalian fear circuit and the formation of rodent fear memory after Pavlovian tone-shock conditioning. Each notebook contains interactive controls that allow students to work with the simulations with no prior programming experience. We have observed that these simulations (that include assignments) facilitate student engagement and increase confidence toward computational tools. Herein we outline how the tutorials can be used with slides and instructional videos to form self-learning packages that can be incorporated into instructional materials for neurology and psychiatry students and residents, or as stand-alone learning packages for medical researchers.

Disclosures: S. Nair: None. **G. Glickert:** None. **M. Walker:** None. **S.S. Nair:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.12

Topic: B.07. Network Interactions

Title: Eeg biomarkers of reduced inhibition in human cortical microcircuits in depression

Authors: *F. MAZZA[1,2], T. A. VALIANTE[5,6,3,7], J. D. GRIFFITHS[4], E. HAY[1,4,2];
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Abstract: Reduced cortical inhibition by somatostatin-expressing (SST) interneurons has been strongly associated with treatment-resistant depression. However, whether the effects of reduced SST interneuron inhibition on microcircuit activity have signatures detectible in electroencephalography (EEG) signals remains unknown. We simulated resting-state activity and EEG using detailed models of human cortical microcircuits with normal (healthy) or reduced SST interneuron inhibition (depression). Healthy microcircuit models showed emergent key features of resting-state EEG, and depression microcircuits exhibited increased theta, alpha and low beta power (4 - 15 Hz). The changes in depression involved a combination of an aperiodic broadband, and periodic theta and low beta components. We then demonstrated the specificity of the EEG signatures of reduced SST interneuron inhibition by showing they were distinct from those corresponding to reduced parvalbumin-expressing (PV) interneuron inhibition. Our study thus links SST interneuron inhibition level to distinct features in EEG simulated from detailed human microcircuits, which can serve to better identify mechanistic subtypes of depression using EEG, and non-invasively monitor modulation of cortical inhibition.

Disclosures: **F. Mazza:** None. **T.A. Valiante:** None. **J.D. Griffiths:** None. **E. Hay:** None.

Poster

272. Computational Modelling of Synaptic Networks

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Program #/Poster #: 272.13

Topic: B.07. Network Interactions

Support: Yuet Ngor Wong Award
Krembil Foundation
CAMH Discovery Fund
Kavli Foundation

Title: α5-$GABA_A$ receptor modulation recovers impaired stimulus processing in simulated human cortical microcircuits in depression

Authors: *A. GUET-MCCREIGHT[1], H. M. CHAMEH[3], T. D. PREVOT[4,2], T. A. VALIANTE[3,5], E. SIBILLE[6,2], E. HAY[1,7];
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Abstract: Recent studies implicate cellular and circuit mechanisms in depression, and reduced inhibition by somatostatin-expressing interneurons is a key component associated with treatment-resistant depression. Administration of positive allosteric modulators of $GABA_A$ receptor α5 subunits (α5-PAM) that selectively target and recover this lost inhibition, exhibit antidepressant, anxiolytic, and pro-cognitive effects in rodents. However, the functional effects of this drug on human cortical activity *in vivo* are unknown, and currently cannot be readily assessed experimentally. We modeled the effect of α5-PAM on tonic inhibition recorded in human neurons, and then tested α5-PAM effects on cortical processing using detailed data-driven computational models of human cortical microcircuits in health and depression. Our simulations show that α5-PAMs efficaciously and robustly recovered cortical processing, as quantified by stimulus detection metrics, back to healthy levels. We then characterized the effects of α5-PAMs on simulated EEG signals and identified power spectral biomarkers that can be used for assessing drug efficacy. By comparison, simulation of non-selective PAMs did not recover cortical function and had different EEG signatures. Our results and predictions serve to de-risk and facilitate α5-PAM drug translation, identify biomarkers for monitoring, and provide an *in silico* framework for assessing novel pharmacology.

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Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 272.14

Topic: B.07. Network Interactions

Support: ONR N00014-22-1-2453
N00014-21-1-2290
The JPB Foundation
The Baszucki Brain Research Fund

Title: A new model predicts the physiology of cortico-striatal computations in category learning

Authors: A. PATHAK[1], S. BRINCAT[2], S. SENNEFF[3], D. HOFMANN[3], H. H. STREY[3], L. R. MUJICA-PARODI[3], E. K. MILLER[2], *R. GRANGER[1];
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Abstract: Brain circuits acquire, retrieve, and use information, all via physiological operation within anatomical structures. Models typically study either computations (learning, deciding) or

physiologies (dynamics; spiking patterns); how does the latter give rise to the former? Incorporating both requirements adds valuable constraints to models, to help rule out inconsistent hypotheses. Reciprocally, omitting these conditions adds degrees of freedom to models, rendering them even more underconstrained. We demonstrate candidate models of primate visual category learning in which the physiology performs the computations, such that the former is constrained by empirical spiking and LFP data, and the latter by behavioral data. Several findings suggest testable predictions as well as avenues for further study. Distinctive cortical and striatal circuitry incorporates differential excitatory-inhibitory numbers and axon radii; lateral inhibition; transmitters and their time courses; transmission lag time across structures; three distinct localized forms of synaptic change (theta-gamma cortico-cortical LTP; theta-spike LTP reversal; cortico-striatal potentiation via synapse-specific DA and ACh afferents from SNc and TANs respectively). Model rhythmic activity is driven in part via ascending ACh (basal forebrain), NE (locus coeruleus), and DA (VTA) afferents to particular subclasses of cortical cells. Behavioral stimuli are used directly from primate experiments. We identify several initial results:

1. The model captures both quantitative and qualitative learning rates and variability seen in behavior and neurophysiology.

2. Like the animals, the model learns visual categories at different rates. Sometimes only one is learned, turning the two-category problem (A v. B) into a one-category task (A v. not A). These results were not initially expected in advance of model analysis.

3. As in the physiological data, the model developed separate beta-band networks for the two categories, driven by a combination of local and systems-level rhythmic activity.

4. The level of activity in striatal tonically active neurons (TANs) predicted striatal variability to given cortical inputs. DA-driven inputs to TANs learn over time, transitioning from initial high striatal MSN variability to less in later trials.

Disclosures: **A. Pathak:** None. **S. Brincat:** None. **S. Senneff:** None. **D. Hofmann:** None. **H.H. Strey:** None. **L.R. Mujica-Parodi:** None. **E.K. Miller:** None. **R. Granger:** None.

Poster

272. Computational Modelling of Synaptic Networks

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: B.07. Network Interactions

Support: NIH R56-AG072473
Emory Alzheimer's Disease Research Center Grant 00100569
NIH K08NS105929
CURE Epilepsy

Title: Ultrafast Simulation of Large-Scale Neocortical Microcircuitry with Biophysically Realistic Neurons

Authors: *M. J. M. ROWAN[1], N. P. PEDERSEN[2], V. OLAH[1];
[1]Emory Univ. Sch. of Med., Atlanta, GA; [2]Neurol., Emory Univ., Atlanta, GA

Abstract: Understanding the activity of the mammalian brain requires an integrative knowledge of circuits at distinct scales, ranging from ion channel gating to circuit connectomics. Computational models are regularly employed to understand how multiple parameters contribute synergistically to circuit behavior. However, traditional models of anatomically and biophysically realistic neurons are computationally demanding, especially when scaled to model local circuits. To overcome this limitation, we trained several artificial neural net (ANN) architectures to model the activity of realistic multicompartmental cortical neurons. We identified an ANN architecture that accurately predicted subthreshold activity and action potential firing of several different cortical pyramidal cell types. The ANN could correctly generalize to previously unobserved synaptic input, including in models containing nonlinear dendritic properties. When scaled, processing times were orders of magnitude faster compared with traditional approaches, allowing for rapid parameter-space mapping in a circuit model of Rett syndrome. Thus, we present a novel ANN approach allowing for rapid, detailed network experiments using inexpensive and commonly available computational resources.

Disclosures: M.J.M. Rowan: None. **N.P. Pedersen:** None. **V. Olah:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

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Program #/Poster #: 272.16

Topic: B.07. Network Interactions

Support: NS116589

Title: Creation of neuronal ensembles and cell-specific homeostatic plasticity through chronic sparse optogenetic stimulation

Authors: *B. LIU, M. J. SEAY, D. V. BUONOMANO;
UCLA, UCLA, Los Angeles, CA

Abstract: Cortical computations emerge from the dynamics of neurons embedded in complex cortical circuits. Within these circuits neuronal ensembles, which represent subnetworks with shared functional connectivity, emerge in an experience-dependent manner. Here, to emulate sensory experience, we chronically optogenetically stimulated sparsely transduced cortical organotypic cultures. Stimulation induced the formation of ensembles, as observed by differential firing rates during spontaneous Up-states, and a decrease in the voltage correlation between the optically stimulated (ChR+) and nonstimulated (ChR-) subpopulations. Paired

whole-cell recordings, revealed that these ensembles were in part formed by a synaptic decoupling between the stimulated and non-stimulated populations—specifically the connection probability between ChR-/ChR- and ChR-/ChR+ pairs was 23% and 10%, respectively, furthermore the mean EPSP amplitude was also smaller between the ChR-/ChR+ pairs. Additionally, these ensemble-specific changes were accompanied by decreases in intrinsic excitability in the stimulated population. We next developed an empirically-based spiking neural network model that exhibited the spontaneous Down<->Up-state transitions observed experimentally. By incorporating the empirically observed changes in intrinsic excitability and connectivity into this model we were able to demonstrate that changes in both intrinsic excitability and connectivity accounted for the experimental results. Our findings establish that chronic patterned stimulation can create ensembles within cortical circuits *ex vivo*. And, importantly, while Up-states are a global network-wide phenomenon, functionally distinct ensembles can preserve their identity during Up-states through differential firing rates and correlations. Finally, at the mechanistic level we show that both changes in intrinsic excitability and connectivity contribute to the formation of ensembles.

Disclosures: B. Liu: None. **M.J. Seay:** None. **D.V. Buonomano:** None.

Poster

272. Computational Modelling of Synaptic Networks

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Topic: B.07. Network Interactions

Support: MRC Sackler PhD fellowship
Wellcome Trust (209164/Z/17/Z)

Title: Non-linear single cell interactions driving whole brain dynamics of epileptic seizures

Authors: *D. BURROWS[1], M. MEYER[2], M. RICHARDSON[1], R. E. ROSCH[1], G. PAO[3]; [1]King's Col. London, [2]King's Col. London, London, United Kingdom; [3]Salk Inst. for Biol. Studies, San Diego, CA

Abstract: Background: Evidence from EEG studies in epilepsy indicate the presence of non-linear interactions between brain areas during seizures. However, the majority of investigations into the network interactions which drive seizures rely on linear analytical techniques. Furthermore, EEG signals represent coarse grained neuronal dynamics, that is averaged fluctuations in postsynaptic potentials which can remove non-linear components of signals. Therefore, in order to understand the origin of such non-linear interactions during seizures, and their contribution towards seizure genesis, we require i) neuron dynamics at single cell resolution, ideally across the entire network, and ii) computational techniques which can account for the non-linear interactions between time series, and are tractable to datasets with tens of thousands of timeseries.

Methods: Here we take advantage of the larval zebrafish, a system which provides access to single neuron resolution across the entire brain, to perform 2 photon calcium imaging during induced seizures. Furthermore, we employ Convergent Cross Mapping (CCM), a computational technique which relies on lagged coordinate embedding to infer the causal relationships between time series, while accounting for non-linear interactions. We take advantage of high performance computing implementations of CCM to perform up to 4x108 comparisons per fish. This is the first time that a causal inference technique has been performed at single cell resolution across the whole brain to examine seizures.
Results: We find that during generalised seizures, alongside an increase in pairwise correlation, the amount of non-linear information between neuron pairs significantly increases. This suggests that seizure dynamics are highly non-linear at the single neuron scale. This increase in non-linear dynamics is largely driven by thalamic nuclei in the zebrafish brain. Interestingly, we show that highly correlated and highly non-linear neurons represent distinct subpopulations, suggesting a spatial structure to seizure evolution involving different interaction types.
Conclusions: In summary, by performing non-linear causal inference at single resolution across the whole brain, we demonstrate a novel pathway for seizure emergence which relies on non-linear signal propagation between neurons. Future work will characterise these non-linear dynamics to understand how they give rise to seizure propagation, and understand whether such dynamics can be used to predict seizures before they happen.

Disclosures: **D. Burrows:** None. **M. Meyer:** None. **M. Richardson:** None. **R.E. Rosch:** None. **G. Pao:** None.

Poster

272. Computational Modelling of Synaptic Networks

Location: SDCC Halls B-H

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Program #/Poster #: 272.18

Topic: B.07. Network Interactions

Support: ERC CoG PrefrontalMap 819496
Israeli Science Foundation 1657/19

Title: Binding with Synaptic clusters on dendrites

Authors: *P. PATIL[1], Y. WASERMAN[1], M. KATKOV[1], O. YIZHAR[1], M. V. TSODYKS[1,2]; [1]Weizmann Inst. of Sci., Rehovot, Israel; [2]Inst. for Advanced Study, Princeton, NJ

Abstract: Binding is the cognitive process in which two or more known entities, features, or modalities, are combined into a single neural representation. For example, the concept of a purple banana comprises the property purpleness and the object banana, formed by the binding of these two features. Although binding is a fundamental process underlying both primary sensory perception (feature binding) and higher-order cognition (abstract reasoning and language comprehension), its neuronal underpinnings and mechanistic implementation in the brain remain

unknown. Previous binding models either lack explicit biological underpinnings or require fast plasticity mechanisms. Our theoretical model implements abstract binding in a network of neurons with dendrites while trying to satisfy multiple biological constraints. The critical insight is that representing a combination of features resembles the logical AND operation. This can be easily implemented by a supralinear response to specific combinations of simultaneous inputs to neighboring synapses in a dendritic compartment. We implemented an associative network with sparse asymmetric connectivity that can bind any pair of items using binary neurons with dendrites. In this model, somatic inhibition mediated by PV neurons is implemented by a dynamic threshold, ensuring that only a fixed sparse fraction of neurons are active at any given time. We find in simulations that any single item and any pair of items (i.e., binding) are attractor states of the network over a wide range of parameters. Analytical calculations in the mean-field limit recapitulate the stability found in simulations. Our model predicts that the binding engram is comprised of clusters of co-active synapses on dendrites of those neurons that represent the bound features. This local dendritic activity might also serve as a marker for future plasticity sites for converting a transient binding representation into a stable, long-term association. We thus show a biologically plausible and testable model for binding memories in cortical circuits.

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Poster

272. Computational Modelling of Synaptic Networks

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Program #/Poster #: 272.19

Topic: B.07. Network Interactions

Support: KAKENHI no. 2 0 K 0 7 7 1 6

Title: An intermediate state between the synchronous and asynchronous states enhances the inter-areal communication with the multiple frequencies: a detailed local circuit model.

Authors: *T. KURIKAWA;
Kansai Med. Univ., Hirakata, Japan

Abstract: An intermediate state between the synchronous and asynchronous states enhances the inter-areal communication with the multiple frequencies: a detailed local circuit model
Dynamic information routing between cortical areas plays a critical role in many cognitive functions. Such dynamic communication is considered to be evoked by coherence between neural activities between the sending and receiving areas with multiple frequencies. In the spatial working memory task, for instance, a specific increase in gamma-range coherence between the hippocampus (HPC) and medial prefrontal cortex (mPFC) is observed. The theta-range coherence between the same areas, in addition, is also observed in the anxiety-evoked task. Thus, the same anatomical circuit, namely HPC and mPFC, is involved in the different cognitive tasks

with multiple-frequency communication. How the multiple-frequency communication in different tasks can be implemented in the neural system is an important question.
Neural dynamics in local circuits are dependent on different types of neurons. Parvalbumin (PV)-expressing neurons are involved in the faster oscillations, whereas somatostatin (SOM)-expressing neurons are involved in the slower oscillations. Vasoactive intestinal peptide (VIP)-expressing neurons are related to the information gating by regulating the excitatory neurons through the dis-inhibitory circuit. Can these different inhibitory neurons generate the multiple-frequency communication in different tasks? If so, what condition is most suitable for the communication?
In this presentation, we develop a detailed neural circuit model including three types of inhibitory neurons (PV, SOM, and VIP neurons) in addition to the excitatory neurons. By using this detailed model, we found that the intermediate state between the synchronous and asynchronous states is suitable for the multiple-frequency communication. The weak connection strength from PV to SOM neurons realizes the intermediate state.

Disclosures: T. Kurikawa: None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 273.01

Title: WITHDRAWN

Poster

273. Microglial Mechanisms of CNS Injury and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 273.02

Topic: B.09. Glial Mechanisms

Support: GR108074

Title: Abnormal synaptic pruning in the developing hippocampus contributes to long-term deficits in hippocampal-dependent function in mice exposed to early adversity

Authors: *S. AHMED, B. POLIS, K. DAYNANDA, R. ISLAM, A. KAFFMAN; Psychiatry, Yale Univ. Sch. of Med., New Haven, CT

Abstract: Stress during specific period of development results in structural and functional hippocampal deficits across diverse mammalian species, including humans and rodents. The

hippocampus undergoes intense microglial-mediated synaptic pruning during the 2nd-3rd weeks of life, a process that is necessary to support normal hippocampal function later in life. Using limited bedding (LB) paradigm, as a model mouse of early adversity, we found a significant impairment in the ability of microglia to engulf synaptic material in vivo and ex vivo in the hippocampus of 17-day old pups. Impairment in this phagocytic activity was closely linked to reduced microglial ramification and lower surface expression of the Triggering Receptor Expressed on Myeloid Cells 2 (Trem2) and was seen in both males and females. Abnormal synaptic pruning was associated with the retention of large number of immature spines and reduced density of functional synapses that persisted in juvenile (P33) mice despite normalization of microglial morphology and capacity to engulf synaptic material. Together, these findings suggest that transient perturbation of microglial-mediated synaptic pruning during the 2nd and 3rd weeks of life contributes to abnormal hippocampal-dependent deficits seen in mice exposed to LB.

Disclosures: S. Ahmed: None. **B. Polis:** None. **K. Daynanda:** None. **R. Islam:** None. **A. Kaffman:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Topic: B.09. Glial Mechanisms

Support: NIH F31 NS120609
NIH R01 EY019277
NIH R21 NS099973
NSF 000497

Title: Microglial surveillance and injury response are controlled by regionally modulated signaling pathways

Authors: *M. B. STOESSEL, R. D. STOWELL, A. K. MAJEWSKA;
Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Synaptic plasticity allows the central nervous system (CNS) to incorporate new sensory experiences and information, and its disruption is associated with many neurological and psychiatric disorders. Much recent work has focused on the contribution of non-neuronal CNS cells, especially microglia, to synaptic plasticity. Though classically thought of in their immune capacities, microglia are vital to many homeostatic processes, including synaptic plasticity of nascent and adult neuronal networks. Despite the emerging consensus that microglial dynamics are critical to brain function during physiological as well as pathological conditions, it is unclear whether these microglial roles and their underlying mechanisms are universal or differ between brain regions. There is a growing body evidence to suggest microglia exhibit a high degree of

regional specialization; existing on a continuum from homeostatic (cortex) to immune vigilant (cerebellum) even in the absence of pathological stimuli. Indeed, cerebellar microglia exhibit unique transcriptional and epigenetic profiles, and distinct functional properties, such as being more phagocytic, morphologically less ramified and less densely distributed. As a consequence, cerebellar microglia survey less of parenchyma than cortical microglia, but compensate for this by undergoing frequent somatic translocations under homeostatic conditions, a phenomenon not observed in cortex. Despite such differences, cerebellar microglia maintain common microglial functions. Two pathways of interest to cortical microglial mediated synaptic plasticity include noradrenergic signaling through the β_2 adrenergic receptor (β_2-AR) and purinergic signaling through the P2Y12 receptor, both of which have been shown to be critically involved in microglial roles in synaptic remodeling and rapid chemotaxis to sites of injury. We used time-lapse *in vivo* imaging through a chronic cranial window preparation to visualize cerebellar microglial surveillance and injury response while β_2-AR and P2Y12 signaling were manipulated. We found β_2-AR stimulation reduced microglial surveillance in both the cortex can cerebellum but slightly increased cerebellar soma migration. We show that β_2-AR stimulation has no effect on the cerebellar focal injury response. Lastly, we demonstrated that P2Y12 deficiency did not alter the response of cerebellar microglia to focal injury, unlike what is seen in cortical microglia. These findings show that cerebellar microglia likely use a distinct set of molecular cues to guide dynamic responses in both homeostatic and pathological conditions.

Disclosures: **M.B. Stoessel:** None. **R.D. Stowell:** None. **A.K. Majewska:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 273.04

Topic: B.09. Glial Mechanisms

Support: StartUp Funds to LKF

Title: Early life immune activation in mice induces behavioral and immune dysregulation that are ameliorated by subsequent inflammatory challenge

Authors: *L. K. DAVIS[1], L. M. INCE[2], S. GULLAPALLI[2], L. K. FONKEN[2];
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Abstract: Neurodevelopmental disorders, such as autism spectrum disorder (ASD), are characterized by aberrant communication, decreased sociability, and increased repetitive behaviors. Although the etiology of ASD is complex and not fully understood, immune dysregulation is implicated. Infants born to mothers who were ill during critical gestational periods are at increased risk for ASD. However, not all mothers who are ill during pregnancy deliver children with ASD, implicating maternal immune activation (MIA) as a disease “primer”, requiring a subsequent stimulus to elicit pathology. To assess this hypothesis, we used a two-hit

model of early life immune activation. C57/Bl6J mouse dams were exposed to polyinosinic:polycytidylic acid (Poly(I:C)) during midgestation to elicit an inflammatory response and a subset of neonates from these dams were then exposed to lipopolysaccharide (LPS) as a secondary immune challenge at postnatal day 4. In adulthood, offspring underwent behavioral testing to assess the presence of ASD-like behaviors or flow cytometry was used to evaluate immune cell populations in the brain and periphery. In agreement with prior work, mice exposed to Poly(I:C) decreased social interactions in the three-chambered sociability test in both sexes and increased repetitive behaviors on the rotarod test in male mice. However, rather than exacerbating behavioral changes, the secondary hit of LPS rescued deficits in social and repetitive behaviors in male, but not in female, mice. Poly(I:C) exposure increased neutrophil populations in the spleens and lymph nodes and microglial populations in the brains of male mice, consistent with our hypothesis that MIA leads to alterations in immunity. In agreement with the behavioral findings, LPS exposure ameliorated increases in immune cell populations in male mice, suggesting that this secondary immune challenge may be protective. Future research aims to examine the mechanisms mediating the protective effects induced by a secondary immune challenge following MIA.

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Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Program #/Poster #: 273.05

Topic: B.09. Glial Mechanisms

Support: 1T32GM136651-01
F30 AG074629-01

Title: Characterizing microglial morphology in medial prefrontal cortex with chronic calcium leak

Authors: *E. WOO[1], A. E. WRONSKA[3], A. MARKS[3], L. H. SANSING[2], A. F. ARNSTEN[2]; [2]Yale Med. Sch., [1]Yale Med. Sch., New Haven, CT; [3]Columbia Univ., New York City, NY

Abstract: Chronic exposure to uncontrollable stress can lead to loss of spines and dendrites in the prefrontal cortex (PFC), a critical region that underlies working memory. The vulnerable circuits in the PFC are normally dependent on elevated calcium signaling to support the persistent firing needed for working memory. However, under various conditions, e.g., aging and chronic stress, these circuits lose calcium-PKA-cAMP regulation, leading to chronically elevated cytosolic calcium. *Ex vivo* studies in aged rhesus macaques and rodents have started to clarify the underlying pathways leading to dysregulated calcium levels, specifically internal calcium release through ryanodine receptor 2 (RyR2) residing on the smooth endoplasmic reticulum (SER). PKA phosphorylation of RyR2 causes calcium “leak,” which occurs in cardiac muscle with stress-

induced heart failure and in postmortem Alzheimer's disease or COVID-19 brain samples (Alzh&Dem 18:955-965, 2022). In rodent PFC, spine loss with chronic stress is prevented by inhibition of PKA signaling, suggesting that microglia mediated synaptic pruning may involve elevated PKA signaling. Consistent with this hypothesis, chronic stress exposure is associated with increased internal complexity of microglia in LII/LIII of rat mPFC (Cereb Crtx 23:1784-1797, 2013). However, there is still much to understand about how the morphological changes of microglia affect function and structure in the PFC. The current research utilized a genetic mouse model, RyR2-S2808D, which causes constitutive calcium leak through a knock-in mutation. We hypothesize that elevated chronic calcium alters microglial morphology and leads to greater surveillance and ability to detect and phagocytose ailing synapses. Immunofluorescence of Iba1 was used to quantify microglial morphology using Image J skeleton and fractal analysis at three months of age in LII/LIII mPFC (n=5 per genotype). Microglial complexity was increased in the RyR2-S2808D mice ($p=0.0009$), consistent with increased ramification of microglial processes. In contrast, cell count ($p=0.7185$), density ($p=0.2892$), and span ratio ($p=0.1573$) did not significantly differ between the two groups. The total endpoint per cell ($p=0.2102$) and total branch length per cell ($p=0.1605$) did not significantly differ between the two groups. Future research can determine how increased calcium leak leads to increased microglial complexity, and whether this phenotype increases synaptic loss which underlies a variety of PFC cognitive disorders.

Disclosures: E. Woo: None. **A.E. Wronska:** None. **A. Marks:** None. **L.H. Sansing:** None. **A.F. Arnsten:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R01AG038961
NIH Grant R01EB009041
Focused Ultrasound Foundation

Title: Assessment of safety and microglia response to short-pulse theranostic ultrasound-mediated blood-brain barrier opening

Authors: *A. J. BATTS, A. R. KLINE-SCHODER, R. L. NOEL, E. E. KONOFAGOU; Biomed. Engin., Columbia Univ., New York, NY

Abstract: Blood-brain barrier opening (BBBO) with focused ultrasound (FUS) and microbubbles (MBs) is a noninvasive, safe, and reversible strategy for targeted drug delivery to the brain. The primary mechanism for FUS-mediated BBBO is MB cavitation, which may be spatially mapped to infer the volume of BBBO (V_{BBBO}) during the procedure. Additionally,

FUS-mediated BBBO has also been shown to induce mild inflammatory responses in the brain depending on the intensity of FUS parameters employed, and the degree of cavitation induced. Using a novel, single imaging array configuration for FUS-mediated BBBO with ultra-short pulse lengths (USPL) and cavitation mapping, called theranostic ultrasound (TUS), we demonstrate the differential immunomodulatory capability of USPL to uncover the role of microglia in BBB repair after opening with TUS. A P4-1 imaging phased array was operated by a research ultrasound system to employ a novel pulse sequence for bilateral BBBO and power cavitation imaging (PCI) (1.0 MPa peak-negative pressure, 1.5-10 cycle USPL, 8×10^8 MBs/mL), which enabled assessment of USPL on microglia stimulation in 8-10-week-old $CX_3CR\text{-}1^{GFP}$ mice, PCI pixel intensity, V_{BBBO} with T_1-weighted MRI, and histological safety via hematoxylin and eosin (H&E) staining in 8-10-week-old C57BL/6J mice. Examination of 10×-magnified images of coronal brain sections from $CX_3CR\text{-}1^{GFP}$ mice sacrificed 24 hours post-TUS BBBO revealed USPL-dependent microglia morphology correlated with PCI signal intensity (Fig. 1A-B) and V_{BBBO} on T_1-weighted MRI acquired 0.5 hr (Fig. 1C-D) and 24 hrs (Fig. 1E-F) after BBBO. Hemispheres sonicated with the 10-cycle USPL elicited congregation of microglia (Fig. 1H) surrounding regions where red blood cell extravasation (RBCE) was observed 24 hours post-TUS on H&E-stained sections (Fig. 1J). No hemispheres sonicated with the 1.5-cycle USPL exhibited RBCE (Fig. 1I). 96 hours post-TUS, no RBCE was detected regardless of USPL (Fig. 1K-L). Analysis of microglia genomic signatures in response to USPL will provide further insight into the effect of TUS on neuroimmune activation.

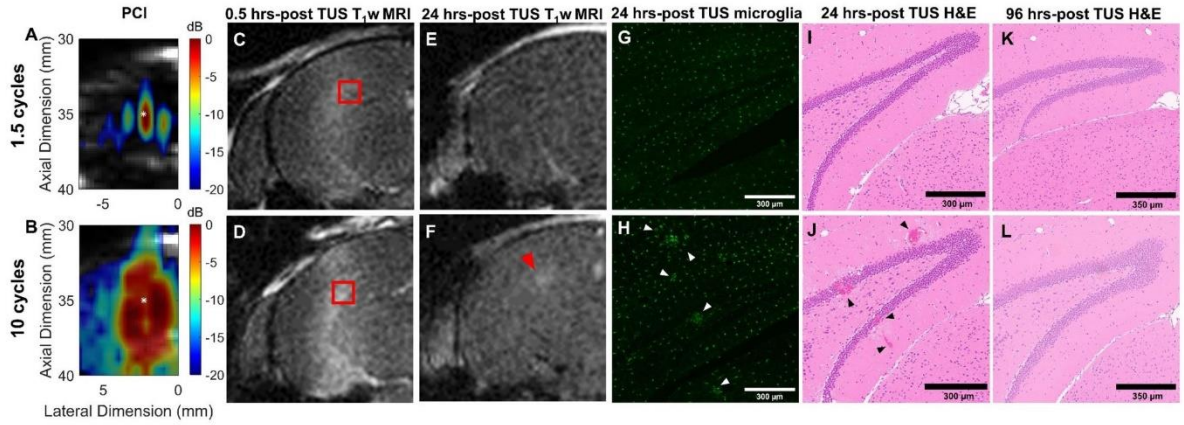

Fig. 1: (A-B) Self-normalized power cavitation images acquired with TUS RASTA overlaid onto a pre-sonication B-mode image of the mouse brain. Average cumulative PCI signal intensity increased 2.66-fold with an increase in USPL from 1.5 to 10 cycles. White asterisks denote the center of the target TUS focal volume. **(C-D)** CE T_1-weighted MRI acquired 0.5 hrs after TUS BBBO. Red square ROIs denote approximate region enlarged in (G-L). **(E-F)** CE T_1-weighted MRI acquired 24 hrs after TUS BBBO. Red arrowhead in (F) denotes remaining permeable area of BBBO where microglia response in (H) was observed. **(G-H)** 10×-magnified images of GFP-fluorescing microglia. White arrowheads in (H) depict congregations of microglia surrounding approximate regions of RBCE in (J). **(I-L)** 10x magnified images of H&E-stained sections from mice sacrificed 24 hours (I-J) or 96 hours (K-L) after BBBO. Black arrowheads in (J) denote regions of RBCE.

Disclosures: A.J. Batts: None. **A.R. Kline-Schoder:** None. **R.L. Noel:** None. **E.E. Konofagou:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Topic: B.09. Glial Mechanisms

Support: NIH Grant 5R01NS088627-10

Title: Cns-associated macrophages and microglia respond differently to mild cortical compression

Authors: *L. WANG[1,2], J. ZHENG[1,2], S. ZHAO[3], L.-J. WU[1,3,4];
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Abstract: Both microglia and CNS-associated macrophages (CAMs) are CX3CR1- and IBA1-positive immune cells in the brain, making it difficult to differentiate these two cell types. Our current study aimed to characterize differences in multiple aspects of these two cell types and their responses to mild brain injury in mice. Firstly, single-cell RNA sequencing data indicated that, in homeostatic conditions CAMs highly expressed Mrc1 gene (encoding CD206 protein), while microglia expressed Tmem119 (encoding TMEM119 protein) and P2ry12 (encoding P2Y12 protein) genes. Secondly, 2-photon *in vivo* imaging of $Cx3cr1^{eGFP/+}$ mouse brain showed that CAMs showed more resistant to PLX3397 ablation compared to microglia. Interestingly, by using the Tmem119-CreER:$Rosa26^{TdT/+}$ transgenic mice to genetically trace and distinguish microglia from CAMs, we found that CAMs could repopulate from residual CAMs but not microglia. Thirdly, CAMs and microglia responded differently to mild cortical compression, where CAMs gradually formed the honey-comb structures, while microglia gradually retracted processes and transformed into the ameboid cells within 24h. Those honey-comb structures which were previously proposed to be formed by microglia were actually formed by CAMs in the superficial area of the brain such as meninges. After 4 weeks brain compression, we surprisingly found many giant cells on the brain surface, which we believed to be CAMs chronically adapted to the continuous cortical compression, while microglia beneath those giant CAM cells have already returned to normal morphology. In summary, our study showed that under physiological conditions, CAMs were molecularly different from microglia, and CAMs pool was not replenished by microglia. After cortical compression, CAMs and microglia reacted morphologically different both in acute and chronic phase, indicating their different functions after brain injuries.

Disclosures: L. Wang: None. **J. Zheng:** None. **S. Zhao:** None. **L. Wu:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 273.08

Topic: B.09. Glial Mechanisms

Support: 5 T32 7278

Title: Microglia calcium influx rate significantly increases during opioid withdrawal

Authors: *D. BERGKAMP, G. D. STUBER, J. F. NEUMAIER;
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Abstract: Microglia are the resident myeloid cells of the CNS, performing many roles including pathogen detection, response to stroke or other physical damage, and elimination of dendritic spines during memory consolidation. In opioid withdrawal, microglia sense ATP released from a myriad of sources via binding to P2Y (metabotropic) and P2X (ionotropic) purinergic receptors. In order to study how calcium influx into microglia changes during opioid withdrawal, we crossed CX3CR1-ERT2Cre mice with FLEX-GCaMP6s mice, allowing for expression of a genetically encoded calcium indicator specifically in microglia following tamoxifen treatment. Mice were then administered fentanyl twice a day for five consecutive days by IP injection in order to induce opioid tolerance. Following the final fentanyl injection, we performed multi-photon imaging of microglia GCaMP6s fluoresence in coronal slices following acute preparation. Compared to opioid naive animals, microglia from animals in the fentanyl treated group showed increased calcium event frequency when slices were treated with naloxone. For future studies, we plan to examine whether multiple cycles of opioid withdrawal significantly change microglia morphology and calcium influx event frequency compared to a single withdrawal experience. Our results suggest that purinergic receptors and purine release generally may both warrant further study as druggable targets in the treatment of opioid use disorder.

Disclosures: D. Bergkamp: None. **G.D. Stuber:** None. **J.F. Neumaier:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 273.09

Topic: B.09. Glial Mechanisms

Support: NIH 5R01DA045649-03

Title: Nicotine Addiction - The Role of IL-18 in the Medial Habenula

Authors: *L. WILLS[1], P. J. KENNY[2], Z. CHEN[3], X. LIU[3];
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Abstract: The habenula-IPn circuit was recently identified as a critical brain system that regulates the motivational properties of nicotine. Our premise is that nicotine-induced alterations in the activity of the habenula-IPn system play a central role in the development and persistence

of the tobacco smoking habit. A unique feature of mHb neurons is their expression of interleukin-18 (IL-18), a cytokine heavily implicated in neurodegenerative processes. IL-18 is induced in mHb, but not in other brain sites, by acute and chronic stress. Given the unique expression of IL-18 in mHb neurons, we hypothesize that this cytokine regulates excitotoxic effects of nicotine. Consistent with this hypothesis, we find that Il18-/- mice are far more sensitive than wild-type mice to excitotoxic effects of self-administered nicotine ($p<0.05$). Furthermore, baseline and nicotine-induced increases in Fos were markedly higher in the IPn of Il18-/- mice compared with wild-type mice ($p<0.01$). As nicotine activates Fos in the IPn by stimulating habenular inputs this suggests that IL-18 deficiency renders mHb neurons hyper-responsive to nicotine. The mechanisms by which IL-18 regulates these changes are unclear. A major action of IL-18 in the brain is to control microglia activity. We find that microglia numbers are far lower in mHb of Il18-/- mice than wild-type mice ($p<0.05$). These data suggest the relationship between IL-18 and microglia may play a role in the development of nicotine addiction.

Disclosures: L. Wills: None. **P.J. Kenny:** None. **Z. Chen:** None. **X. Liu:** None.

Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Program #/Poster #: 273.10

Topic: B.09. Glial Mechanisms

Support: R01AG062716
F31AG072867

Title: Mycobacterium vaccae immunization in aged rats inhibits neuroinflammation via the adaptive and innate immune systems

Authors: *K. SANCHEZ[1], L. M. INCE[1], R. KAKKAR[1], L. K. DAVIS[1], J. S. DARLING[1], R. CHEN[1], S. L. WU[1], A. ZENTAY[1], C. A. LOWRY[2], L. K. FONKEN[1];
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Abstract: Aging is associated with shifts in immune cell populations that traffic to the central nervous system (CNS) via the meninges. The types of immune cells, such as T cells, in the meninges can modulate baseline neuroinflammation through interactions with microglia. With aging, microglia acquire a primed (i.e., sensitized) phenotype that promotes excess neuroinflammation in response to immune stimuli. Prior studies indicate that immunization with the saprophytic bacterium *Mycobacterium vaccae* (*M. vaccae*) in aged rats limits neuroimmune activation. However, whether *M. vaccae*influences T cell populations and migration into the aged brain via the meninges remains unclear. Here, adult (3 mos) and aged (24 mos) Fisher 344 × Brown Norway rats were immunized with *M. vaccae* or vehicle once a week for three weeks. Five days following the final injection, tissue was collected to assess immune regulation

in the periphery, meninges, and brain. Aging led to morphological changes in microglia consistent with a more activated phenotype in the dorsal hippocampus (e.g., greater deramification of microglial processes and swelling of microglial somas). *M. vaccae* treatment ameliorated these age-associated changes in morphology. Additionally, aging led to an increase in evidence of T cells in the CNS as assessed by immunohistochemistry and flow cytometry. Current experiments will determine whether *M. vaccae* shifts T cells populations in the aged brain. Transcriptomic analyses on hippocampal tissue indicated that aging promotes an increase in markers of microglia reactivity (e.g., *Cd68*, *Iba1*, *Tmem119*), which are ameliorated in response to *M. vaccae*. Collectively, these results indicate that *M. vaccae* may quell neuroinflammation and promote a less activated microglial phenotype in the aged brain. Ongoing work will determine the role of T cells in communicating the anti-inflammatory signature induced by *M. vaccae* to the CNS. Taken together, microbial-based approaches could be a promising treatment option for mitigating age-related neuroinflammation and may lessen the risk of developing chronic inflammatory diseases.

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Poster

273. Microglial Mechanisms of CNS Injury and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 273.11

Topic: B.09. Glial Mechanisms

Support: AFAR Grant MCKNIGHT21003

Title: Microglial regulation of the brain extracellular matrix may play critical roles in age-associated memory dysfunction

Authors: *D. T. GRAY[1], A. GUITERREZ[1], C. CHARWAY[1], M. HARATIAN[1], C. A. BARNES[2], L. M. DE BIASE[1];
[1]Physiol., David Geffen Sch. of Med. At UCLA, Los Angeles, CA; [2]Evelyn F. McKnight Brain Inst., Univ. of Arizona, Tucson, AZ

Abstract: The brain extracellular matrix (ECM) is a complex network of proteins and sugars secreted by multiple cell types that provides neurons, glia, and vasculature with structural and biochemical support. Accumulating evidence indicates that microglia, the innate immune cells of the brain, regulate the ECM through release of degradative enzymes, by directly engulfing ECM proteins, and by signaling to other cell types that regulate the ECM. Several studies have shown that aging is accompanied by region-specific changes in the abundance of specialized ECM

structures (e.g., perineuronal nets), and proteins that synthesize ECM scaffold proteins. Our group has shown that microglia also display regional differences in their responses to aging, raising the possibility that these cells play critical roles in determining which populations of neurons become most vulnerable to functional decline across the lifespan. However, whether age-associated changes in microglia, the ECM, or microglial regulation of the ECM have protective or detrimental impacts on cognition later in life has not been clearly determined. Here, we present findings from novel, label-free quantitative proteomics analysis of midbrain (MB) and striatal (STR) tissue from young (n=4, 3mo) and aged (n=4, 24mo) mice that was separated into high- and low-solubility fractions to better isolate ECM proteins. This previously unavailable map of the aging ECM reveals age-associated increases in critical ECM proteoglycans (e.g., aggrecan; MB fold change: 0.7, STR fold change: 4.1), link-proteins (e.g., hyaluronan and proteoglycan link protein 2; MB fold change: 3.3, STR fold change: 8.2), and proteins associated with the vasculature-associated basement membrane (e.g., vitronectin; MB fold change: 2.4, STR fold change: 1.8). These findings suggest that aging is accompanied by dysregulation in the synthesis and/or turnover of ECM proteins and that the magnitude of these changes varies across brain regions. These changes may critically impact brain processes the ECM is known to regulate, including synaptic plasticity and vascular integrity. Next, using both rodent and nonhuman primate models of brain aging, we show that age-related changes in microglia are associated with changes in the ECM in older brains, and that these relationships may negatively impact cognitive function. Finally, we highlight preliminary data and future directions from ongoing experiments that combine behavioral assessments, high-resolution imaging, molecular biology, and proteomic analyses to specifically test the hypothesis that aberrant microglial remodeling of the ECM is a central driver of age-related memory impairments.

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Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Topic: B.09. Glial Mechanisms

Support: NIH Grant RF1 AG064859
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Title: Suppression of microglial p38α MAPK signaling in APPswe/PSENdE9 mice impairs spatial performance but does not alter the pro-inflammatory profile

Authors: D. J. BRAUN[1,2], ***H. N. FRAZIER**[1,3], V. A. DAVIS[1], M. J. COLEMAN[1], L. J. VAN ELDIK[1,2];

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Abstract: While amyloidβ and tau accumulation are the primary pathologies associated with Alzheimer's disease (AD), other pathological processes, such as neuroinflammation, are also involved. The mitogen activated protein kinase p38α (p38α) is expressed in multiple cell-types throughout the brain. Accumulation of amyloid is associated with elevated p38α signaling, which leads to increased tau phosphorylation, excitotoxicity, and altered synaptic plasticity. In microglial cells, amyloid-mediated p38α activity induces pro-inflammatory cytokine release which further elevates p38α signaling in other cell-types. Thus, targeting this pathway may be a relevant therapeutic approach to ameliorate AD-associated neuroinflammation. Here, we investigated the impact of microglial p38α suppression on cognitive function, microglial morphology, and pro-inflammatory cytokine expression in WT (C57BL/6J) and amyloidogenic AD model (APPswe/PSENdE9) mice. Selective knockout (KO) of p38α in microglial cells was induced using a Cre-Lox system under a Cx3Cr1 promoter. Briefly, WT and APPswe/PSENdE9 control ($p38^{+/+}$) and floxed (p38 KO) mice were fed a tamoxifen diet for 28 days beginning around 3 months of age, followed by 3 months of standard chow to allow for replenishment of p38α-expressing peripheral cells and progression of amyloid pathology. Animals then underwent behavioral assessment using the open field (OF), Y-maze novel spatial recognition (NSR), and radial arm water maze (RAWM) tests. Amyloid burden, pro-inflammatory cytokine levels, and microglial morphology in hippocampus and cortex were also assessed. Analysis of OF results showed that APPswe/PSENdE9 mice had increased locomotion compared to WT, and no impact of p38α KO was detected. However, RAWM measures revealed a significant effect of microglial p38α suppression, albeit only in amyloidogenic mice, suggesting that abrogation of p38α signaling in microglia can have deleterious effects on amyloid-associated neuropathology. Neither genotype nor KO of p38α impacted NSR performance, amyloid burden, microglial morphology, or the pro-inflammatory cytokine profile. While it is surprising that p38 KO animals had worsened RAWM performance without concurrent changes in cytokine levels, it could be that microglial p38α signaling impacts spatial learning and memory via mechanisms that are independent of inflammatory processes. Future work will further investigate p38α signaling in other cell-types as well as across age and sex to better characterize the role of p38α in AD-associated neuroinflammation.

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Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Program #/Poster #: 273.13

Topic: B.09. Glial Mechanisms

Support: CONACYT 2019-000002-01NACF-08090

Title: Audiogenic stress differentially regulates proliferation/transformation of glial cells in the adult male rat´s brain.

Authors: *F. CRUZ MENDOZA[1], D. FERNÁNDEZ QUEZADA[1], Y. RUVALCABA DELGADILLO[1], S. LUQUÍN DE ANDA[1], F. JÁUREGUI HUERTA[2];
[1]Univ. De Guadalajara, Univ. De Guadalajara, Guadalajara, Mexico; [2]Univ. De Guadalajara, Univ. De Guadalajara, guadalajara, Mexico

Abstract: Environmental noise has been described as a stressor capable to activate the HPA axis and the stress responses. Stress and HPA axis instead regulates proliferation and the brain inflammatory responses. Glial cells are responsible for many tasks in the brain, including the homeostatic function. Astrocytes and microglial cells also regulate neuroinflammation through a glial reactivity process that can lead to diminished neurogenesis and impaired cognition. Here, we investigated if audiogenic stress is capable to affect the brain cell proliferation/survival and if glial cells respond with transformational changes associated to gliosis. To do so, we exposed adult male rats to a rat's audiogram fitted adaptation of some common annoying environmental sounds. Then, we applied immunohistochemistry in brain slices to identify changes in proliferation, survival and glial transformation of exposed subjects. Proliferation was measured by counting BrdU+ cells and glial transformation was determined by applying Sholl analysis in microglia (Iba1+ cells) and astrocytes (GFAP+ cells). Partial results shows a diminished cell survival rate and changes in glial ramification strongly dependent of the analyzed brain region. Then, we believe that audiogenic stress may also be considered a strong modulator of the brain proliferative and inflammatory responses.

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Poster

273. Microglial Mechanisms of CNS Injury and Disease

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Program #/Poster #: 273.14

Topic: B.09. Glial Mechanisms

Support: NIH R01 Grant MH123545

Title: Depletion of glucocorticoid receptors in prefrontal cortex pyramidal neurons mitigates stress-induced synaptic and behavioral deficits

Authors: *D. DADOSKY[1], H. ASRAT[1], L. VOLLMER[1], S. DAVIDSON[2], E. WOHLEB[1];
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Abstract: Neuroendocrine responses to chronic stress promote physiological and behavioral adaptations, in part, through glucocorticoid receptor (GR) signaling in various tissues. In particular, GR signaling has been implicated in stress-induced structural remodeling and synapse

loss on pyramidal neurons in the prefrontal cortex (PFC). This is significant because stress-induced synaptic loss in the PFC underlies behavioral deficits and working memory impairments. Our recent studies indicate that GR signaling engages neuron-microglia interactions and this contributes to synapse loss on neurons in the PFC. Despite this work, the cell type-specific role of GR signaling in the neurobiology of stress has not been studied. Thus, we aimed to determine how GR depletion in PFC pyramidal neurons influenced neurobiological and behavioral responses to chronic unpredictable stress (CUS). Transgenic mice with *loxP* sites flanking exon 3 of the *Nr3c1* gene (GR-flox) or wild-type conspecifics received bilateral infusion of AAV5-CaMKIIa-mCherry-Cre in the medial PFC to induce selective depletion of GR in pyramidal neurons. Immunohistology confirmed that GR-flox mice showed a significant reduction in GR levels in mCherry+ neurons compared to wild-type mice. Further studies showed that both wild-type and GR-flox mice had elevated plasma corticosterone levels and reduced weight gain after exposure to CUS, suggesting that GR depletion in PFC pyramidal neurons did not influence stress responses. In cognitive testing, we found that wild-type mice exposed to CUS had impaired discrimination in temporal object recognition task (TOR) and that this deficit was mitigated in GR-flox mice exposed to CUS. Other behavioral analyses such as nest building and sucrose consumption were evaluated, with no significant results found. Recent studies used fluorescence-activated cell sorting (FACS) and RT-PCR to examine neuron- and microglia-specific transcriptional changes mediated by GR depletion, such as *Redd1* and *Csf1*. Other studies are being carried out to evaluate changes in membrane excitability of mCherry+ PFC pyramidal neurons via patch clamp electrophysiology. Further immunohistology will be performed to quantify dendritic spine density and microglial morphology in the PFC following CUS. Altogether, our results provide strong evidence that GR depletion specifically in PFC pyramidal neurons attenuates neurobiological and behavioral consequences of CUS. This work provides fundamental insight into the cell type-specific molecular mechanisms that drive the neurobiology of stress and associated behavioral consequences.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Program #/Poster #: 274.01

Topic: B.09. Glial Mechanisms

Support: NIH UL1 TR001860
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Title: Bergmann glia exhibit structural and functional alterations in ataxia Canavan disease mice

Authors: *V. L. HULL[1,2], Y. WANG[1,2], F. GUO[1,2], L. BORODINSKY[1,2], D. PLEASURE[1,2]; [1]UC Davis, Sacramento, CA; [2]Shriners Hosp. for Children, Sacramento, CA

Abstract: Bergmann glia (BG), the radial astrocytes of the cerebellum, play pivotal roles in the structural and functional development of the cerebellar cortex and continue to support neuronal survival and synaptic function throughout adulthood. BG fibers guide the migration of the excitatory granule cells and intertwine with the complex dendritic arbors of Purkinje cells (PCs), neurons comprising the sole motor output of the cerebellum. During synaptogenesis, the previously smooth BG fibers generate lateral projections that enwrap the developing granule cell-PC synapses, forming microdomains which will provide ongoing synaptic support. Multiple animal models of BG ablation present with dysregulation of neuronal migration and loss of motor coordination, underscoring their vital role in these processes. PCs are particularly susceptible to damage in neurodegenerative diseases, namely the ataxias, and many studies have begun to explore BG alterations which accompany PC damage in ataxia. Our lab focuses on Canavan disease (CD), an inherited pediatric leukodystrophy resulting from inactivating mutations to the oligodendroglial enzyme aspartoacylase (ASPA). Without functional ASPA, responsible for cleaving the abundant brain metabolite N-acetyl-L-aspartate (NAA), patients with CD, as well as our murine model for the disease ("CD mice"), accumulate high levels of brain NAA which results in extensive spongiform brain degeneration, astroglial vacuolation and ataxia. We have shown that CD mice also present with PC dendritic simplification and spine loss. Here, we report that perturbations to BG morphology and physiology also occur in CD mice. We observe fragmented BG fibers, interrupted interactions between BG fibers and PC dendrites, as well as loss of Ca^{2+}-permeable AMPARs and altered Ca^{2+} dynamics in BG. In our therapeutic model, wherein we developed an antisense oligonucleotide targeting the knockdown of the NAA-synthesizing enzyme Nat8l, which lowers brain NAA and reverses ataxia and Purkinje cell damage in CD mice, we also observe repair of BG and increased expression of AMPARs. Given the intimate structural and functional relationship between BG and PCs, it is possible that BG damage may be a non-cell autonomous mechanism of PC degeneration in ataxias and other neurological conditions. We are working to elucidate the temporal relationship between damage occurring to each of these cell types during CD pathogenesis, as well as during repair in our therapeutic model. Taken together, these findings point to dysregulation of the PC-BG relationship as a driver for CD pathology and warrant further investigation for potential therapeutic targets for BG repair.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Sloan Research Fellowship in Neuroscience to Z.Q.
NIH R01NS118014 to Z.Q.

Title: Astrocytes modulate drug addiction by tonically releasing GABA through Swell1 channel

Authors: *J. YANG[1], Y. LIU[1], J.-N. CHEN[1], K. H. CHEN[1], J. M. BARABAN[2], Z. QIU[1,2]; [1]Dept. of Physiol., [2]The Solomon H Snyder Dept. of Neurosci., Johns Hopkins Univ., Baltimore, MD

Abstract: Drug-evoked synaptic plasticity in the mesolimbic system reshapes circuit function and drives addiction. Much work has focused on the potentiation of excitatory synaptic inputs onto ventral tegmental area (VTA) dopamine (DA) neurons. Disinhibition, the removal of an inhibitory brake on neuronal firing, also regulates circuit function. Recent evidence suggests that in response to drugs of abuse, inhibitory synaptic inputs can increase DA neuron activity by inhibiting local VTA GABA neurons. But how drug drives inhibitory transmission remains largely unknown. By releasing gliotransmitters, astrocytes actively regulate neuronal activity and synaptic transmission. Whether astrocytes play a role in drug-evoked inhibition of local VTA GABA neurons and disinhibition of DA neurons is unexplored. In this study, we found that chronic cocaine treatment in mice potentiates tonic GABA currents specifically in VTA GABA neurons but not in nucleus accumbens (NAc) projecting DA neurons, causing a firing decrease of VTA GABA neurons and firing increase of NAc-projecting DA neurons. Interestingly, cocaine-induced tonic GABA currents were largely mediated by GABA release from VTA astrocytes through GABA-permeable Volume-Regulated Anion Channel (VRAC). Deletion of Swell1, the only obligatory subunit of VRAC, in VTA astrocytes not only attenuated cocaine-evoked tonic GABA currents increase but also reversed cocaine-induced firing rate changes in both VTA GABA and NAc-projecting DA neurons. Importantly, cocaine-induced behaviors including locomotor sensitization and conditioned-place preference (CPP) were ameliorated in VTA astrocyte-specific Swell1 knockout mice. Furthermore, specific removal of the tonic inhibition onto VTA GABA neurons by deleting the δ subunit of extrasynaptic $GABA_A$ receptors using in vivo CRISPR-Cas9 genome editing also reduced the cocaine-induced changes in neuron firing and animal behaviors. Lastly, we found that chemogenetic activation of VTA astrocytes by expressing hM3Dq mimics cocaine-induced tonic GABA release and neuron firing changes and enhances cocaine addiction behavior. Together, our study indicates that enhanced tonic GABA inhibition is a novel mechanism for cocaine addiction and astrocytes play an important role in this process by releasing GABA through the Swell1 channel.

Disclosures: **J. Yang:** None. **Y. Liu:** None. **J. Chen:** None. **K.H. Chen:** None. **J.M. Baraban:** None. **Z. Qiu:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

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Topic: B.09. Glial Mechanisms

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FAPESP # 2018/05006-0
CNPq # 140027/2020-3

Title: Impact of TLR2 and TLR4 modulation on motoneuron survival and glial reactions after ventral root crush in mice

Authors: *L. CARTAROZZI, A. L. OLIVEIRA;
Structural and Functional Biol., Univ. of Campinas - Lab. of Nerve Regeneration, Campinas, Brazil

Abstract: Lesions to the peripheral and central nervous systems (PNS, and CNS, respectively) are followed by robust activation of innate immunity. Sciatic nerve crushing, for example, retrogradely induces, in the spinal cord, the upregulation of classically known immune molecules, such as the major histocompatibility complex class I (MHC-I) and Toll-like receptors (TLRs). Specifically, MHC-I, TLR2, and TLR4 stand out, as they exhibit pleiotropic roles in the CNS after peripheral lesions that are greatly related to synaptic plasticity. The detachment of presynaptic terminals in apposition to the axotomized motoneurons is a constitutive response to injury that is directly related to the glial cells which actively intermediate the process. Thus, it is hypothesized that a signaling system is established between axotomized motoneurons and glial cells, involving immune molecules such as MHC-I, TLR2, and TLR4. In this context, this study aimed to evaluate the influence of TLR2 and TLR4 modulation on the motoneuron survival and glial reactions in the spinal cord after unilateral ventral root crush (VRC). For that, 8 weeks old female mice from different strains were submitted to VRC: TLR2 *knockout*, TLR4 *knockout*, and C57BL/6J mice. C57BL/6J mice were further subdivided into a control group, Pam3CSK4 group (TLR2 agonist - 5 mg/kg; i.p.; 1 hour before surgery), and LPS group (TLR4 agonist - 1 mg/kg; i.p.; 1 hour before surgery). Seven days post-injury (dpi), the spinal cords were dissected out, cryoprotected, frozen, and sectioned for motoneuron counting and immunohistochemistry. Protocols concerning animal use and handling were approved by the Institutional Committee for Ethics in Animal Use (CEUA/IB/UNICAMP, Protocol number 4458-1). The analysis of motoneuron survival in Pam3CSK4 ($p = 0.016$), and LPS treated mice ($p = 0.003$), as well as in TLR4 KO mice ($p = 0.0003$) revealed an increased susceptibility to degeneration when compared to the control group. Additionally, microglial cells exhibited different patterns of activation, such as increased cell number in close contact with axotomized motoneurons. In LPS and Pam3CSK4 groups, a differential morphology of activated cells, such as hyper-ramified cells, corresponding to a higher activation degree, was more frequently found. Overall, the results described herein suggest that the higher susceptibility of motoneurons in the initial phase after a proximal root crush lesion is related to the modulation of TLR2 and TLR4 that initially act on the microglia.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Topic: B.09. Glial Mechanisms

Support: R01DA048815
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UCR Graduate Division

Title: Effect of Toxoplasma gondii infection on neuronal extracellular vesicles

Authors: *E. TABAIE[1], Z. GAO[2], K. BERGERSEN[3], W. ZHONG[2], E. WILSON[1];
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Abstract: Infection with the obligate intracellular parasite Toxoplasma gondii leads to Toxoplasmic encephalitis in immunocompromised patients. While T. gondii can infect any nucleated cell, spontaneous cyst formation is only found in neurons. Cysts remain lifelong in the host and as of now, there is no way to get rid of them. During infection, astrocytic glutamate regulation is disrupted and extracellular levels of glutamate in the central nervous system (CNS) reach non-homeostatic ranges. Astrocytes regulate CNS glutamate by adjusting uptake, release, and synthesis into glutamine. Through previous work in the lab, we have found that following T. gondii infection there is a decrease in astrocytic glutamate transporter, GLT-1, leading to a significant increase in the concentration of extracellular glutamate, which can lead to neurotoxicity and seizures. GLT-1 can be regulated by exosomes secreted by neurons. Exosomes are extracellular vesicles (EVs) containing proteins, lipids, DNA, mRNA, and miRNAs. EVs are derived from the fusion of multivesicular bodies with the plasma membrane and the extracellular release of the intraluminal vesicles. EVs function in intercellular communication, without the need for cellular contact. Exosomes are produced by any cell, including T. gondii. To test if Toxoplasma induces changes in EV production during neuronal infection, the number and concentration of EVs were measured from infected and uninfected primary murine neurons. To test if infected neuronal EVs result in changes to GLT-1 expression, purified EVs were incubated with primary murine astrocytes and host cell EV uptake was measured. Our preliminary results suggest that Toxoplasma infection changes EV production from neurons. To test if these EVs are taken up by astrocytes, EVs were labeled with PKH67, incubated with primary astrocytes, and confocal imaging was conducted. Results suggest that neuronal EVs localize with intracellular compartments of astrocytes. To determine possible functional changes induced by Toxoplasma-induced EV production, RNASeq analysis of astrocytes presented with EVs from uninfected or infected neurons will be presented. The long-term goals of this project will be to determine if GLT-1 expression is dysregulated during Toxoplasma infection via changes in EV content with potentially broad implications for regulation of the neuronal network during inflammation and infection.

Disclosures: E. Tabaie: None. **Z. Gao:** None. **K. Bergersen:** None. **W. Zhong:** None. **E. Wilson:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.05

Topic: B.09. Glial Mechanisms

Support: K08NS114170
2021 Martin-Reichenbach Endowment Junior Faculty Award
2021 True-Brown Fellowship

Title: Satellite microglia have a role in regulation of neuronal excitability and change in response to injury

Authors: R. O'BOYLE, H. MAIOLI, K. SYTSMA, *A. NOLAN;
Univ. of Washington, Seattle, WA

Abstract: Microglia, the primary mediators of innate immune activation in the brain, are increasingly recognized as key modulators of neuronal activity and excitability. There is growing evidence in many neurological diseases, including traumatic brain injury (TBI), that prolonged activation of the innate immune system can impede repair and promote disease, and it is not understood if or how microglia's impact on neuronal activity might contribute. One interesting microglial subtype that may be critical in the monitoring and feedback of neuronal excitability is the perineuronal satellite microglia. These microglia are juxtaposed adjacent to neurons with their soma and processes entwined around the neuronal cell body. To understand how these microglia modify neuronal excitability and change their interactions with neurons after injury, we utilized patch clamp recordings, immunohistochemistry, light microscopy and confocal imaging. We found an increase in the numbers of satellite microglia in the orbitofrontal cortex in both a murine model of TBI that is associated with network hyperexcitability and behavioral dysfunction with deficits in reversal learning several moths after TBI ($p=0.0095$, unpaired t-test), as well as human tissues from donors with a history of chronic TBI ($p=0.0177$, unpaired t-test) compared to sham and controls, respectively. In addition, preliminary analyses suggest increased interactions with an inhibitory neuronal subtype that we have found exhibits selective vulnerability with a decrease in excitability- the somatostatin-expressing (SOM+) subtype ($p=0.0051$ for neuron type, repeated measures, two-way ANOVA). Whole cell recordings in adult transgenic mice with GFP-labeled microglia (Tmem119-EGFP), utilized to record activity in neurons adjacent to and away from satellite microglia, also indicate that satellite microglia suppress neuronal excitability as measured by the action potential response to a series of depolarizing current steps ($p=0.0286$ for satellite microglial effect, repeated measures 2-way ANOVA), while preliminary data suggest this relationship may be altered at chronic time points after TBI. These findings support continued investigation of satellite microglial response and neuronal interaction after chronic injury.

Disclosures: R. O'Boyle: None. **H. Maioli:** None. **K. Sytsma:** None. **A. Nolan:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.06

Topic: B.09. Glial Mechanisms

Support: Ministry of Science and Technology, Taiwan. MOST 110-2320-B-A49A-504

Title: Fkbp51 mediates ischemic and excitotoxic brain injury involving regulation of astrogliosis

Authors: Y.-L. GAN[1], Y.-P. KANG[1], S.-H. LIN[2], C.-C. HUNG[1], *Y.-H. LEE[1];
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Abstract: FK506-binding protein 51 (FKBP51), encoded by *Fkbp5* gene, is a stress-inducible protein and serves as a multi-target cochaperone that regulates several cellular pathways, including glucocorticoid receptor, Akt and NF-κB signaling. However, the role of FKBP51 in neurotoxicity remained unclear. Here we used global knockout of *Fkbp5* (*Fkbp5*-KO) in mice to study the role of FKBP51 in glutamate excitotoxicity, a leading cause of brain damage in epilepsy and ischemic stroke. By using intracerebroventricular (icv) injection of excitotoxin kainic acid (KA), we found that *Fkbp5*-KO mice were more resistant than wild-type (WT) mice to the KA-induced seizure, neuronal loss and astrogliosis in hippocampus and cerebral cortex, as well as demyelination in corpus callosum and cingulum. In vitro study revealed that glia-neuron (GN) mix cultures and neuron-enriched cultures obtained from *Fkbp5*-KO mice were both more resilient to the NMDA-induced neurotoxicity compared to WT. NMDA treatment increased the phosphorylation of Akt and NF-κB subunit p65, and the effects were enhanced in neuron-enriched but blocked in GN mix cultures from *Fkbp5*-KO mice. We further established inducible astrocyte-specific *Fkbp5* conditional knockout mice to investigate the underlying mechanism of FKBP51-mediated astrogliosis after excitotoxic injury. We found that mice with astrocytic *Fkbp5* deletion had reduced KA-induced seizure activity and astrogliosis compared to WT mice. Together, these results suggest that FKBP51 mediates excitotoxic brain injury in both gray matter and white matter involving regulation of astrogliosis.

Disclosures: Y. Gan: None. **Y. Kang:** None. **S. Lin:** None. **C. Hung:** None. **Y. Lee:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.07

Topic: B.09. Glial Mechanisms

Support: NIH-NINDS Grant R01NS091617
Owens family foundation
Quantitative Neurobiology of Behavior Fellowship at University of Virginia

Title: Axon-schwann cell interaction in axonal injury

Authors: *E. STEPANOVA, K. GAMAGE, Y. YONG, A. SPANO, C. DEPPMANN;
Univ. of Virginia, Charlottesville, VA

Abstract: Axonal injury triggers a variety of signaling cascades culminating in axonal degeneration and fragmentation. Axons that are wrapped in myelin sheath, also undergo the process of demyelination after injury. In the peripheral nervous system demyelination occurs as a result of Schwann Cells (SCs) transdifferentiation, which is important for supporting axon degeneration, cleaning up debris and supporting axon regenerate later on. However, how precisely axons and SCs communicate to coordinate their reprogramming remains an open question.We have recently published that Death Receptor (DR6) mice do not go through the process of axonal degeneration, but have a substantial demyelination taking place (Gamage et al., 2017). Our unpublished work reveals that knocking out an additional gene, p75NTR, together with DR6 (n=8, 4 males and 4 females) not only preserves axons but also prevents injury induced demyelination 14 days post injury as revealed by light and electron microscopy. This suggests that p75NTR functionally interacts with DR6 to coordinate demyelination. Moreover, our in vitro work shows that addition of DR6 protein to in vitro DR6-/- myelinating neurons, actually induces demyelination (n=3). Given that DR6 is not expressed on SCs, but is expressed neuronally, our ongoing work investigates if axonal DR6 acts in trans on p75NTR on SCs to trigger demyelination in response to injury in vivo and in vitro.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.08

Topic: B.09. Glial Mechanisms

Title: Morphological characterization of neuro-immune interactions in response to peripheral conditioning

Authors: *J. S. JARA, E. R. HOLLIS;
Burke Neurolog. Inst., White Plains, NY

Abstract: Neuroinflammation is a critical regulator of axon regeneration in both the central and peripheral nervous systems. In the periphery, nerve injury elicits a robust regenerative program characterized by changes in gene expression and a significant macrophage response. Immune cells are highly plastic and undergo defined structural changes when activated. Within the dorsal root ganglia (DRGs), we have used high-resolution, three-dimensional imaging to characterize changes in macrophage morphology and neuro-immune interactions following sciatic nerve injury. Within intact sciatic-projecting L4 DRGs, macrophages largely exhibit an amoeboid morphology with a small population of elongated cells. Following sciatic nerve crush, we found that ipsilateral, activated macrophages expand into a more complex ramified morphology (Kalinsky et al., 2020). Here we show that these stellate-like macrophages closely interact with the large diameter, parvalbumin expressing, sensory neurons that exhibit robust regeneration-associated gene changes in response to peripheral injury. Three-dimensional image analysis shows that ramified macrophages enfold the somata of regenerating sensory neurons and exhibit extensive cell-cell contacts. Interestingly, these interactions appear to be dependent on sensory neuron identity as ramified macrophages do not interact with C-low threshold mechanoreceptors (tyrosine hydroxylase positive neurons). The selectivity of this interaction is not yet understood but is conserved across distinct methods of pro-regenerative peripheral conditioning. Our results characterize a novel neuro-immune interaction that correlates with conditioning-mediated regeneration. This selective cell-cell interaction may play a key role in neuroinflammation-mediated pro-regenerative signaling.

Disclosures: J.S. Jara: None. **E.R. Hollis:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

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Topic: B.09. Glial Mechanisms

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Kentucky Spinal Cord & Head Injury Research Trust training fund to Chen Lab
Haggerty Center for Brain Injury and Repair to Goldberg

Title: Genetic Stimulation of Astrocyte Reactivity Promotes Corticospinal Axon Sprouting

Authors: *M. CHEN[1], L. INGLE[2], E. PLAUTZ[2], X. KONG[2], R. TANG[2], N. GHOSH[2], M. ROMPREY[1], W. FENSKE[1], M. P. GOLDBERG[3];
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Abstract: Injury to the adult mammalian central nervous system triggers compensatory plasticity of spared axons - referred to as collateral axon sprouting - that can facilitate

neural recovery. The contribution of reactive astrocytes to axon sprouting remains elusive. Here, we investigated the role of axon degeneration-reactive astrocytes in the regulation of collateral axon sprouting that occurs in the mouse spinal cord after unilateral photothrombotic stroke of the primary motor cortex. We identified leucine zipper-bearing kinase (LZK) as a positive regulator of astrocyte reactivity to corticospinal axon degeneration. Remarkably, genetic stimulation of astrocyte reactivity, via LZK overexpression in adult astrocytes, enhanced corticospinal axon sprouting and resulted in a trend towards improved recovery of motor function. LZK promoted the production of astrocyte-derived ciliary neurotrophic factor (CNTF) that likely enhanced corticospinal axon sprouting in mice with astrocytic LZK overexpression after injury. Our demonstration that genetic stimulation of LZK-dependent astrocyte reactivity promotes corticospinal axon sprouting provides important insights to a beneficial role of reactive astrocytes in injury-induced axon growth, and highlights the therapeutic potential of targeting astrocytes for neural repair.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.10

Topic: B.09. Glial Mechanisms

Support: NIH NINDS 1R01NS115707

Title: In Vivo Two-Photon Imaging of Microglial and Neural Activity During One-Hour Daily Intracortical Microstimulation Following Electrode Implantation

Authors: *K. C. STIEGER, K. CHEN, S. WELLMAN, F. LI, J. GALLEGO, G. ZHANG, T. D. KOZAI;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Implantable recording and stimulating electrodes are valuable tools to gain insight into the function of different brain areas, restore impaired or lost sensation, and treat symptoms of neurological disorders. However, the long-term viability of these devices is challenged by gliosis and neurodegeneration around the electrode in part due to inflammatory reactivity of non-neuronal cells (e.g. microglia). Microglia are one of the first glial cells to respond to damage by extending their processes toward the site of injury and releasing pro-inflammatory cytokines and ultimately forming the first layer of glial encapsulation. Importantly, recent work has identified highly dynamic interactions between microglia and neurons that are linked with neural activity. Additionally, 2-20 Hz electrical stimulation for as short as 1 hour has been shown to promote anti-inflammatory phenotypes in microglia. In this work we hypothesize that brief daily 1-hour

intracortical microstimulation (ICMS) at 10 Hz following electrode implantation will modulate microglia-neuron interactions, and reduce microglial coverage of an implanted electrode. To test this hypothesis, we developed a mouse model that expresses GFP in microglia and the red calcium sensor, jRGECO1a, in pyramidal neurons. Next, we implanted a single-shank Michigan-style electrode into the visual cortex of these mice to quantify microglial process and soma dynamics and neuronal calcium activity under two-photon imaging during daily 1 hr ICMS. Microglia and neurons were imaged for 1 hour and 40 minutes (20 min pre-ICMS, 1h ICMS, and 20 minutes post-ICMS) daily for up to 3 days after electrode insertion. We quantified neuronal calcium activity and the volume of tissue surveilled by microglia during this time. The rate of microglia surveillance decreased during ICMS and remained lower than pre-ICMS for at least 20 minutes post-ICMS. Additionally, neural activity increased at the onset of ICMS, but tended to decrease throughout the 1h period. Interestingly, although the neural response to stimulation decreased from day 0 to day 3, the rate of microglia surveillance increased. These results indicate that ICMS may modulate microglial activity and therefore may influence the inflammatory reactivity in the days following electrode implantation.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.11

Topic: B.09. Glial Mechanisms

Support: CNPq #140027/2020-3
FAPESP #2018/05006-0

Title: Neuroprotection and glial reaction attenuation after mouse spinal root crush and treatment with NeuroHeal and NeuroBoost

Authors: *L. O. COSER, J. LOMBARDI, A. L. OLIVEIRA;
Structural and Functional Biol. - Lab. of Nerve Regeneration, Univ. of Campinas, Campinas, Brazil

Abstract: Spinal cord injury (SCI) generates local inflammation combined with neuronal and synaptic loss, which impairs important motor and sensory functions. Immunomodulatory and anti-inflammatory drugs have been studied in the SCI context, aiming at reducing inflammation and enhancing neuroprotective process after the lesion. Tempol (4-hydroxy-tempo) and dimethyl-fumarate have demonstrated significant results in terms of neuroprotection and reactive gliosis attenuation, and their combination has been named NeuroBoost (NB). Further, another drug combination called NeuroHeal (NH; acamprosate and ribavirin) demonstrated an important neuroprotective role for nerve regeneration and functional recovery after injury. Our study aimed

to compare the effectiveness of NB and NH following spinal root crush. For that, adult female C57BL/6J mice were subjected to lumbar ventral roots crush. Functional recovery was monitored with the Catwalk system (walking track test). Further, we analyzed the survival of the motoneurons, glial reactions, and the percentage of cell polarization. In terms of neuroprotection, our results demonstrated that NB significantly rescued spinal motoneurons by 14 days after injury (NB – 71%±7%; vehicle - 60% ± 8%; p=0.04). In contrast, animals treated with NH preserved motoneurons by seven days after injury (NH – 76% ±5%; vehicle - 62%±10%, p=0.04). We observed a functional recovery after twenty-one days post-lesion in animals treated with NB relative to the vehicle counterpart ($p<0.05$). However, NH-treated animals did not present significantly functional recovery after the treatment. Glial analysis revealed a decrease in astroglial reaction after treatment with both combinations at 7 and 28 days after treatment as compared to vehicle animals (7d - NB – 3.08±0.39; vehicle – 4.4±1.06, p=0.007, 28d - NB – 1.82±0.53; vehicle - 5.02±1.06, p=0.0003; 7d - NH – 3.04±0.83; vehicle – 4.4±1.06, p=0.031, 28d- NH – 2.27±0.45; vehicle – 5.02±1.06, p=0.0007). We also demonstrated a decrease in A1 and A2 astrocytes after the treatment with NB and NH. A reduction in microglial reaction was observed in NB-treated animals (NB – 3.43±1.43; vehicle – 5.91±1.91, p=0.04), with an increase in M2 profile after the treatment. Further, macrophage analysis demonstrated that NH-treated animals present an increase in M1 macrophage and a decrease in M2 profile after twenty-eight days of treatment. Both combinations show a decrease in Th1 lymphocytes after treatment. In summary, the present data suggest that the NB combination is neuroprotective and produces immunomodulation following root crush lesion, leading to an A2/M2 polarization earlier than the NH counterpart.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 274.12

Topic: B.09. Glial Mechanisms

Support: German Research Foundation Grant 270949263/GRK2162
The Multiple System Atrophy Coalition
Interdisciplinary Center for Clinical Research (IZKF) Erlangen, University Hospital Erlangen

Title: Pharmacological microglia depletion with PLX5622 affects motor cortical synaptic connectivity in a mouse model of multiple system atrophy

Authors: *C. GAUER[1], J. WINKLER[1], A. HOFFMANN[1,2];
[1]Dept. of Mol. Neurol., Univ. Hosp. Erlangen, Erlangen, Germany; [2]UK Dementia Res. Institute, Ctr. for Discovery Brain Sci., Univ. of Edinburgh, Edinburgh, United Kingdom

Abstract: Proper motor cortical circuit functions critically rely on precisely tuned synaptic excitation and inhibition executed by excitatory glutamatergic pyramidal neurons and gamma-aminobutyric acid (GABA)-releasing inhibitory interneurons. In particular, GABAergic interneurons play fundamental roles in refining and temporally sharpening the glutamatergic response of layer V pyramidal neurons. Microglia, the brain resident macrophages, are involved in shaping neuronal circuit function by surveilling neuronal microenvironment and pruning synapses. The small molecule inhibitor PLX5622, targeting the colony-stimulating factor 1 receptor (CSF1R), critical for microglia development and survival, provides a rapid and sustained depletion of microglia. In this study, we depleted microglia in a mouse model of multiple system atrophy (MBP29) administering PLX5622 for 12 weeks. While PLX5622 treatment protected MBP29 mice from premature death, it deteriorated their motor function. In order to understand the underlying pathomechanisms, we analysed the effect of microglia depletion on motor cortical GABAergic interneuron signalling in 4-months old MBP29 mice. Using an immunofluorescence approach we assessed the density of calretinin+ (CALR) and parvalbumin+ (PV) GABAergic interneuron subpopulations within the motor cortex (n=5/group, sex-matched). Neocortical GABAergic and glutamatergic synapses were characterized by quantifying pre- and postsynaptic protein levels using Western Blotting (n=4/group, sex-matched). MBP29 mice displayed an increased number of CALR+ GABAergic interneurons within the motor cortical layers II-V upon PLX5622 treatment while the PV+ GABAergic interneuron cell density remained unaltered. PLX5622 treatment did not restore decreased neocortical presynaptic protein levels (e.g. SNAP-25, synaptotagmin and synaptophysin). Furthermore, PLX5622 treatment increased glutamatergic synapse specific postsynaptic protein levels (e.g. PSD-95 and GluA2) within the neocortex of MBP29 mice. Collectively, these results imply that the synaptic balance of GABA and glutamate is shifted toward glutamate within the neocortex of both MBP29 mice and MBP29 mice treated with PLX5622. As CALR+ interneurons predominantly target neighbouring PV+ and calbindin+ GABAergic interneurons, the concomittant increase in CALR+ GABAergic interneuron cell density in MBP29 mice treated with PLX5622 may promote the motor cortical disinhibition of layer V pyramidal neurons. Consequently, an altered pyramidal neuron output might be linked to the deterioration of motor functions in MBP29 mice treated with PLX5622.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.13

Topic: B.09. Glial Mechanisms

Support: Blue Brain Project, a research center of the École Polytechnique Fédérale de Lausanne, from the Swiss government's ETH Board of the Swiss Federal Institutes of Technology
CRG grant from King Abdullah University of Science and Technology "KAUST-EPFL Alliance for Integrative Modeling of Brain Energy Metabolism" [OSR-2017-CRG6-3438]

Title: Using Information in a Biomolecular Network for Disease Analysis

Authors: *J. S. COGGAN[1], D. X. KELLER[2], H. MARKRAM[3], F. SCHUERMANN[4], P. J. MAGISTRETTI[5];
[1]Blue Brain Project, École polytechnique fédérale de lausanne, Lausanne, Switzerland; [2]Blue Brain Project, École polytechnique fédérale de Lausanne, Lausanne, Switzerland; [3]EPFL, Blue Brain Project, EPFL, Blue Brain Project, Lausanne, Switzerland; [4]EPFL - Blue Brain Project, EPFL - Blue Brain Project, Geneva, Switzerland; [5]King Abdullah Univ. of Sci. and Technol. (KAUST), Thuwal, Saudi Arabia

Abstract: With have demonstrated with a computational model of astrocytic energy metabolism the theoretical plausibility that this type of biomolecular network could code information about stimuli, in addition to its known functions in cellular energy and carbon balance. Results from simulations showed how a metabolic pathway can capture the intensity and duration profiles of a neuromodulator and respond in a dose-dependent manner, including with non-linear state changes that are similar to action potentials (Coggan JS et al., 2022). Such a mechanism can therefore translate information about external stimuli to production profiles of energy-carrying molecules such as lactate with better than realized precision. In a similar way, we now extend this concept to propose that the metabolic state-machines formed from these spatially discontiguous yet interdependent metabolic cascades of enzymatic reactions may quantitatively reflect or mediate the effects of disease-state changes. These networks might be able to codify a cell's internal state and allow them to report pathological signals or mutations. As a proof of concept, we employed the model to examine perturbations in a biomolecular network that reflect known clinical abnormalities in brain energy metabolism. This hypothesis might provide a new tool for analysis and prediction of disease state phenomena or control of desired product outcomes in synthetic biological systems. Citation: Coggan et al., (2022) J Theor Biol. doi: 7;540:111090 10.1016/j.jtbi.2022.111090.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 274.14

Topic: B.09. Glial Mechanisms

Support: NHMRC Project grant 1080197
NIH (R35 NS097283)

Title: Complexity of the primate astrocyte underpins diverse functions and responses to injury

Authors: *L. TEO[1], T. A. HOANG[1], A. G. BOGHDADI[1], T. CHOW[1], S. M. STRITTMATTER[2], N. SESTAN[3], J. A. BOURNE[4];
[1]Australian Regenerative Med. Inst., Monash Univ., Melbourne, Australia; [2]CNNR Program, Yale Univ., New Haven, CT; [3]Yale Univ. Sch. Med., New Haven, CT; [4]Section on Cell. and Cognitive Neurodevelopment, NIMH, Bethesda, MD

Abstract: Astrocytes play diverse roles in the CNS, ranging from homeostasis to active regulation of neural communication. However, less is known about the molecular signatures that drive this functional diversity across CNS evolution, beyond anatomical and spatial phenotypes. In addition, astrocytes also drastically alter their gene expression and function after injury, becoming reactive astrocytes (RAs). Traditionally considered to be functionally homogenous, recent advances are challenging this view. Here, we investigated the gene expression signatures of astrocytes and their correlation to functional specialization.
We performed snRNAseq on astrocytes from normal (n=6; 2-4YO) and injured (n=3) nonhuman primate (NHP; marmoset) neocortical grey matter (equal sex distribution). RAs nuclei from injured brains were collected 7d post-stroke from the proximal infarct. Data analysis focused on broad categorisation of astrocytes based on functional annotations, as well as interspecies comparison to open-source mouse and human astrocyte databases. The use of NHPs offer closer gene expression homology to humans than rodents, allowing direct comparison and translation of our findings to ensure the highest biological and clinical relevance.
In normal brains, astrocytes could be categorised into 3 functionally distinct clusters that partially align with the morphological (Protoplasmic, Interlaminar) and functional (Syt1+) categories of human astrocytes. Protoplasmic clusters were primarily associated with typical astrocyte metabolism. Syt1+ astrocytes, conserved from rodents to humans, were associated with bidirectional communication via glutamate synthesis and exocytosis. Primate-specific interlaminar astrocytes bore signatures distinct from their layer 1 counterparts in rodents, driving separate functions likely in response to a later evolutionary adaptation in more complex brains.
After focal stroke, RAs retained their pre-injury signatures. Protoplasmic RAs exhibited the most significant diversity, comprising seven distinct clusters forming “pairs” broadly associated with three functional categories: Increased astrocyte-specific metabolism, Response to inflammation, & Extrinsic apoptotic signalling. This was confirmed by functional connectivity between clusters and gene expression modules. These functions correlated to the NHP post-stroke timeline, wherein secondary neuronal apoptosis and peripheral immune infiltration peak occur.
These findings provide new insights into the functional adaptations of astrocytes in primates, their integrated roles in neuronal communication and their diverse response to injury.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Program #/Poster #: 274.15

Topic: B.09. Glial Mechanisms

Support: NIH grant M280001-NIH232

Title: Atypical astrocytes in the aging cortex are associated with areas of blood brain barrier leakage and disrupted glutamate homeostasis

Authors: *M. SOMMER[1], M. ARMBRUSTER[1], S. NASKAR[1], S. ROBEL[2], C. G. DULLA[1]; [1]Neurosci., Tufts Univ. Sch. of Med., Boston, MA; [2]Cell, Developmental, and Integrative Biol., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Proper astrocyte function ensures healthy and physiological brain activity, mediated in part by theexcitatory amino acid transporters, GLT1 and GLAST, that rapidly remove synaptic glutamate, and aninwardly rectifying K+ channel, Kir4.1, that mediates K+ influx from the extracellular space. Here wereport a novel astrocyte phenotype in the aging cortex in which astrocytes lack GLT1, GLAST and Kir4.1expression in a focal and age-dependent manner, but are not reactive (GFAP-negative). Previous workhas documented a similar astrocytic phenotype following mild traumatic brain injury and has referred tothese cells as *atypical astrocytes* (AtAs), but this is the first time AtAs have been reported in normalaging. Using immunohistochemical analysis of C57Bl/6 and EAAT2-tdT mice at various ages between p3and >1 year, we find that progressively more astrocytes lose the expression of GLT1, GLAST, Kir4.1, andother astrocytic proteins as mice age. Observed as early as P30, this loss of proteins in astrocytes is not agradual, global change but occurs cell-by-cell, where individual astrocytes lack immunolabeling butneighboring cells retain robust expression. Linear mixed modelling confirmed significant regional, sex,and age effects on AtA ratios, using data from ≥3 mice per age group and, when over P21, ≥3 mice ofeach sex. AtAs are most prevalent in the retrosplenial (RSC) and prefrontal cortices (PFC), occurring insignificantly lower numbers in the somatosensory cortex and rarely in the hippocampus. This phenotypeappears earlier and more abundantly in aging males than in female mice. AtAs express the astrocytemarker Sox9, and exhibit largely normal morphology, but do not express GFAP suggesting they are notthe result of astrogliosis. They are also negative for the neuronal marker NeuN, the oligodendrocytemarker MBP, and the OPC maker NG-2. Located near vasculature, AtAs are found in areas with normalage-related BBB disturbance, which was quantified using IV injection of fluorescently labeledcadaverine. Functional analysis using glutamate transporter current recording and iGluSnFR imagingsuggests that glutamate uptake is disrupted in areas where AtAs are abundant. Additionally, thisdisruption is regionally heterogeneous in the 1-year old RSC, indicative of sporadic loss of GLT1 andGLAST. A much higher membrane resistance is also observed in a subset of astrocytes in the RSC,consistent with the Kir4.1 loss observed in AtAs. Future studies aim to investigate the molecularunderpinnings of AtA generation and their functional consequences.

Disclosures: M. Sommer: None. **M. Armbruster:** None. **S. Naskar:** None. **S. Robel:** None. **C.G. Dulla:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.16

Topic: B.09. Glial Mechanisms

Support: NIH grant NS043277

Title: A tale of two metals: cell type selectivity of zinc and copper toxicity in cerebrocortical cultures

Authors: *J. R. GALE, K. A. HARTNETT, E. AIZENMAN;
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Abstract: Zinc, a redox-inert physiologically essential metal, has a well-established neurotoxic profile in many model systems, and well-characterized neuronal cell death-inducing signaling pathways. In contrast to neurons, astrocytes are highly resistant to zinc toxicity, likely as a result of robust zinc handling processes. Copper is another important essential metal, necessary for oxidative metabolism, antioxidant defense, and neurotransmitter synthesis. Copper, unlike zinc, readily undergoes redox cycling, making it inherently more reactive and potentially toxic. Indeed, a copper-mediated form of regulated cell death was recently described, appropriately termed “cuproptosis” (Tsvetkov et al., Science 375:1254; 2022). Cuproptosis is facilitated by the copper ionophore elesclomol. To our surprise, rat cortical neurons cultured in the presence of astrocytes were highly resistant to copper (0-3 μM) + elesclomol (0-1μM) (Cu/Es) treatment, showing virtually no lethality under all conditions tested. Nonetheless, high lactate dehydrogenase (LDH) levels were present in the culture medium following Cu/Es treatment, suggesting substantial tissue damage. Indeed, close inspection of the cultures following Cu/Es treatment revealed a highly disrupted astrocyte layer. Elimination of the neurons in the mixed cortical cultures with a 1 mM kainate overnight pre-treatment revealed that the Cu/Es-derived LDH signal largely arose from the injured astrocytes. Intriguingly, these experiments also suggested that neurons may partially protect astrocytes from Cu/Es induced toxicity. Using the same kainate paradigm, we confirmed the highly selective nature of zinc toxicity (0-30 μM; in the presence of 250 nM of the zinc ionophore pyrithione) towards neurons. Immunocytochemistry further validated the selective vulnerability of cultured astrocytes to Cu/Esc treatment. Glial fibrillary acid protein (GFAP) staining showed disruption of astrocyte architecture at Cu/Es levels as low as 2 μM Cu/30 nM Es. In contrast, microtubule associate protein 2 (MAP2) staining revealed normal neuronal morphology up to 2 μM Cu/200 nM Es, at which point dendritic beading was observed, likely as a result of excitotoxic damage due to astrocytic dysfunction. In fact, this beading could be rescued using the NMDA antagonist MK-801 (10 μM). Current studies aim to determine the mechanism of Cu/Es-induced astrocytic death, and to investigate the role of neuronal-glial crosstalk in this process. This work is likely

relevant to copper-induced neuronal dysfunction in Menkes and Wilson's diseases, as well as to age-related neurodegeneration, which has been linked to metal dyshomeostasis.

Disclosures: J.R. Gale: None. **K.A. Hartnett:** None. **E. Aizenman:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

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(YB) CAMH Womenmind post-doctoral fellowship
(YB) CAMH Discovery fund post-doctoral fellowship

Title: Chronic stress-induced anhedonia-like behavior can be mimicked by cortical astroglial ablation and reversed by increased astroglial activity

Authors: *S. A. CODELUPPI[1,2], M. XU[4], Y. BANSAL[1,2,3], A. LEPACK[4], S. WILBER[4], G. SANACORA[4], K. MISQUITTA[1,2], J. KNOCH[1], M. L. CHOW[1], E. SIBILLE[1,3,2], R. S. DUMAN[4], C. J. PITTENGER[4], M. BANASR[1,3,2,4];
[1]Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada; [2]Pharmacol. and Toxicology, [3]Dept. of Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [4]Dept. of Psychiatry, Yale Univ., New Haven, CT

Abstract: Astroglia loss and decreased expression of specific markers expressed by GFAP (glial fibrillary acidic protein)-astroglia have been found in the prefrontal cortex (PFC) of MDD patients and rodent chronic stress models. We examined the consequences of PFC GFAP-astroglia ablation on depressive-like behaviours and potential reversal of chronic stress-induced deficits by enhancing PFC GFAP-astroglia activity. GFAP-cre mice infused in the PFC with AAV5-DOI-CMV-DTR (diphtheria toxin (DT) receptor) were behaviourally assessed following i.p. injection with DT for anhedonia- and anxiety-like behaviors. We found that PFC astroglial ablation induced significant anhedonia- but not anxiety-like deficits. We also infused wild-type mice with an AAV5-GFAP-DREADD(designer receptor exclusively activated by designer drug)Gq in the PFC to activate GFAP-astrocytes upon clozapine-N-oxide administration. While PFC GFAP activation had no effects at baseline (no stress condition), it reversed only anhedonia-like deficits induced by chronic stress exposure. Current work focuses on validating increased astroglia activity using Ca2+ fiberphotometry and delta Fos staining. Our results demonstrate that cortical GFAP- astroglia loss is sufficient to induce anhedonia and that chronic stress-

induced anhedonia-like deficits can be reversed by increased GFAP-astroglia activity. This suggests a critical role of astroglia in the expression and the treatment of key symptoms of MDD.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Topic: B.09. Glial Mechanisms

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Title: Astrocytic GluN2C-containing NMDA receptors in the nucleus accumbens regulates cocaine-induced behavior and plasticity

Authors: *G. P. SHELKAR, P. J. GANDHI, J. LIU, S. M. DRAVID;
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Abstract: Abstract: Drugs of abuse, such as cocaine, lead to neural adaptations in the nucleus accumbens which underlie their addictive and reinforcing properties, and NMDA receptors play a critical role in these neuroadaptations. Recent studies have demonstrated the expression of NMDA receptors in non-neuronal cells including astrocytes. Here, we demonstrate the expression of GluN1/2A/2C NMDA receptor currents in astrocytes in the nucleus accumbens core. We found that selective ablation of the GluN1 subunit from astrocytes in the nucleus accumbens but not the medial prefrontal cortex enhanced extinction of cocaine preference memory. In contrast, astrocytic NMDA receptors did not affect cocaine conditioning or reinstatement. Cocaine conditioning upregulated GluN2C subunit expression, and GluN2C but not GluN2D subunit-containing NMDA receptor deletion facilitated extinction of cocaine memory. Furthermore, intra-accumbal administration of DQP-1105 (GluN2C/2D NMDA receptors antagonist) facilitated extinction of cocaine memory. Importantly, we found that several cocaine-induced neuroadaptations, including dendritic spine maturation and AMPA receptor subunit plasticity were under the control of astrocytic NMDA receptors. Impaired cocaine-mediated GluA1 subunit plasticity in GluN2C knockout was mediated by downregulation of neuronal pentraxin and astrocytic glypican 4/6. Together, these results identify a novel astrocytic NMDA receptor-mediated mechanism underlying the maintenance of cocaine-conditioned memory and neuroplasticity.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

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Program #/Poster #: 274.19

Topic: B.09. Glial Mechanisms

Title: Mu-opioid receptors in astrocytes are important for morphine withdrawal

Authors: *K. MURLANOVA, K. NOVOTOTSKAYA-VLASOVA, S. HUSEYNOV, O. PLETNIKOVA, M. J. MORALES, M. V. PLETNIKOV;
State Univ. of New York, Buffalo, Buffalo, NY

Abstract: Astrocytes have been implicated in responses to acute and chronic effects of opiates by changing their morphology and/or physiology. While astrocytes express mu-opioid receptors (MOR), the role of astrocyte MOR in mediating behavioral effects of morphine remains understudied. We evaluated the effects of astrocyte-restricted deletion of MORs in a series of behavioral tests, including morphine withdrawal, by selectively deleting in astrocytes the *Oprm1* gene that encodes MOR-1. To generate mice with conditional deletion of *Oprm1* (*Oprm1* cKO), *Aldh1l1-CreERT2* BAC transgenic mice were crossed with *Oprm1*$^{fl/fl}$, and conditional recombination in astrocytes was induced by tamoxifen treatment in one-month old mice. While no genotype-dependent changes were observed in locomotor activity, anxiety, novel object recognition, the acute analgesic effects of morphine or morphine-produced conditional place preference, *Oprm1* cKO mice exhibited significantly increased naloxone-precipitated aversion compared to controls. Brain astrocytes isolated from *Oprm1* cKO mice exhibited significantly elevated oxidative phosphorylation compared to control mice. These findings suggest that astrocyte MOR-1 could be involved in a homeostatic regulation of the endogenous opioid system via a functional link to oxidative phosphorylation in astrocyte mitochondria. We propose that astrocytes may provide new potential targets for ameliorating aversive states produced by opioid withdrawal.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Title: Astrocytic HIV-1 Nef expression and cocaine reduce glutamate transporters and increase cocaine-seeking behavior in rats

Authors: *J. PLA-TENORIO, B. VELAZQUEZ-PEREZ, M. CRUZ-RENTAS, G. RODRIGUEZ-TORRES, M. COLON-ROMERO, M. SEPULVEDA-ORENGO, R. J. NOEL; Ponce Hlth. Sci. Univ., Ponce, PR

Abstract: Cocaine use prevalence is higher in HIV-infected individuals and can contribute to the vulnerability and development of HAND. Both cocaine and HIV proteins contribute to neuronal damage during HAND progression through dysregulation of glutamate homeostasis in the brain. Part of the dysregulation is due to the reduction of astrocytic proteins such as glutamate transporter (GLT-1) and cystine/glutamate antiporter (xCT). When HIV replication is well-controlled with HIV treatment (cART), the neurotoxic HIV-1 Nef protein is more likely to cause pathology than other viral proteins. Nef is an early HIV viral protein expressed in approximately 1% of infected astrocytes. Even without measurable viral replication, astrocytic Nef expression could contribute to continued neuronal degeneration. In this study, we focus on the possible intersection of HIV-1 Nef neurotoxicity and cocaine consumption by examining the role of astrocyte Nef expression on glutamate homeostasis. We hypothesized that combined cocaine exposure and astrocytic Nef expression would exacerbate glutamate excitation inducing neurophysiological changes that strengthen synaptic transmission and reinforce cocaine-seeking behavior. To test this, we infused a lentiviral vector expressing Nef with a GFAP promoter into the nucleus accumbens (NAc), an important structure involved in the development of cocaine abuse, or a control vector into male and female Sprague Dawley (SD) rats (n=24). Five weeks after recovery, we injected the rats intraperitoneally with one dose of cocaine or saline and collected NAc tissues 24 hours later for molecular analysis. Protein bands of GLT-1 showed a significant decrease in glutamate transporter expression in **Nef**, **cocaine**, and **Nef + cocaine** groups compared to the **control** group ($p<0.05$). Expression of xCT only demonstrated a trend possibly decreasing in the **cocaine** group alone compared to the **control** group ($p=0.07$). RNA analysis of GLT-1 and xCT demonstrated no differences between the groups. Another group of male SD rats (n=12) was subjected to a behavioral paradigm of short-access cocaine self-administration and extinction. Preliminary data showed that rats with Nef expression in the NAc seem to have an increased cocaine-induced seeking behavior ($p=0.06$) compared to the control group. Currently, we are performing another group of rats for a cocaine-self administration experiment to have enough data for a reliable statistical analysis. In conclusion, our results suggest a convergence of HIV-1 Nef and cocaine in glutamate dysregulation and neurotoxicity that may explain the increase in cocaine seeking.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Topic: B.09. Glial Mechanisms

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BMBF (16GW0182 CONNEXIN, 01DN20001 CONNEX; to CS)

Title: A causal role of cytokine-mediated inhibition of astrocyte gap junction coupling in temporal lobe epilepsy

Authors: *P. BEDNER[1], T. DESHPANDE[1], J. MÜLLER[1], L. HENNING[1], M. SYLVESTER[2], V. GIESELMANN[2], C. STEINHÄUSER[1];
[1]Inst. of Cell. Neurosciences, [2]Inst. of Biochem. and Mol. Biol., Univ. of Bonn, Bonn, Germany

Abstract: Antiepileptic therapies mainly target neurons, but a high proportion of temporal lobe epilepsy (TLE) patients do not respond adequately to treatments and none of the available drugs cure the disorder. Consequentially, new therapeutic strategies and targets are urgently needed. We have previously shown that in hippocampal tissue from TLE patients with sclerosis (TLE-HS), surviving glia completely lack gap junction coupling. In a mouse model of TLE-HS, we further demonstrated that uncoupling and the resulting impairment of K^+ clearance temporally precede neuronal death and the onset of spontaneous seizures, suggesting a causal role in the development of epilepsy. Our recent Western blot and mass spectrometric analyses revealed that the early disruption of gap junctional coupling between astrocytes is not caused by reduced levels of gap junction proteins but by changes in the phosphorylation status of Cx43. Activation of astrocytic tumor necrosis factor receptor 1 (TNFR1) by the soluble form of the proinflammatory cytokine TNFα was found to mediate the posttranslational modification of the gap junction protein. Indeed, specific inhibition of sTNFα as well as genetic/pharmacological inactivation of TNFR1 protected astrocytes from seizure-induced uncoupling. Remarkably, inhibition of sTNFα also yielded significant antiepileptic and antiepileptogenic effects in our animal model. These effects were much less pronounced in transgenic mice lacking astrocyte gap junction proteins, demonstrating that astrocyte coupling is a major determinant of the antiepileptic/antiepileptogenic activity of sTNFα inhibition. Together, these data indicate that elevated levels of TNFα after an initial epileptogenic event trigger disruption of astrocytic gap junction coupling, which in turn plays a key role in the development and progression of TLE.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.22

Topic: B.09. Glial Mechanisms

Support: NINDS R01 NS113499
NINDS NS113499-S1
NINDS NS100706

Title: Uncovering the Role of Astrocytic Potassium Uptake in Glutamate Dynamics using the Controlled Cortical Impact Model of Traumatic Brain Injury

Authors: *J. GARCIA[1], M. ARMBRUSTER[3], S. QUINONES[1], S. NASKAR[2], C. G. DULLA[2]; [2]Neurosci., [1]Tufts Univ. Sch. of Med., Boston, MA; [3]Neurosci., Tufts Univ., Boston, MA

Abstract: Glutamate, the primary excitatory neurotransmitter in the central nervous system, is essential for normal brain function. In the healthy brain, once released from neurons, glutamate is transported into astrocytes by the excitatory amino acid transporters (EAATs) GLT-1 and GLAST. Previously, using iGluSnFR-based glutamate imaging and electrophysiology in the healthy adult mouse cortex, our lab has shown that glutamate uptake slowed up to threefold following bursts of neuronal activity. We suspect that this occurs when neuronal activity causes focal increases in extracellular potassium ($[K^+]_e$). This increase in $[K^+]_e$ drives local astrocyte depolarization, causing voltage-dependent inhibition of EAATs and prolonging extracellular glutamate transients. Dysregulation of both EAATs and astrocytic K+ uptake, mediated by the K^+ channel Kir4.1, can both contribute to disrupted glutamate dynamics, but the exact role these channels and transporters play is unknown. We hypothesize that EAATs, drive uptake while Kir4.1 shapes activity-dependent slowing of glutamate. In this study, we employed the controlled cortical impact (CCI) model of TBI in mice. It is well known that following TBI, astrocytes become “reactive” changing their morphology, protein expression, and function. Additionally, micro dialysis studies from individuals suffering from TBI reveal that the extracellular concentration of glutamate is elevated both before and during seizure onset. Three days after injury in our CCI model we found significantly elevated glutamate decay time constants and increased peak glutamate response in acute cortical slices. This effect was decreased 7 and 14 days after injury. Activity-dependent slowing of glutamate uptake was largely similar in the healthy and injured brain for all time points. Western blot, immunohistochemistry, and single-cell RNA sequencing data show significantly decreased levels of EAATs 3 days after TBI, while Kir4.1 expression remains unchanged after injury. GLT1 expression levels return to baseline 7 and 14 days after injury. These results support our model and demonstrate that TBI induces changes in glutamate uptake via decreased EAAT expression, but activity-dependent slowing of uptake is unchanged as Kir4.1 levels are not altered by TBI. Digging deeper into this mechanism, we will employ a floxed Kir4.1 mouse model and an astrocyte-specific Cre-virus to conditionally knockout Kir4.1. Using immunohistochemistry, iGluSnFR imaging, EEG, Genetically Encoded Voltage Indicators (GEVIs), and single-cell patch-clamp, we will further investigate the direct effect of potassium buffering on glutamate dynamics.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Topic: B.09. Glial Mechanisms

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Korea National Research Fund basic research program (NRF-2021R1F1A1063857)

Title: Deletion of PLCη1 in the lateral habenula astrocytes lead to reduced gliotransmission and depression-like behavior in mice.

Authors: *S. SONG, J. LEE, M. KANG, T. JUNG, B. KIM, J. KIM;
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Abstract: The lateral habenula (LHb) is an epithalamic brain structure that sends strong projections to the monoaminergic system of the midbrain. Growing body of evidence suggests that the imbalance of neural activity (hyperactivity/hypoactivity) in the LHb is responsible for several psychiatric disorders including depression, and this suggests that certain molecular and intercellular interactions between LHb cells including neuron and glia may be required to maintain its balanced activity. In this respect, we asked for the role of phospholipase Cη1 (PLCη1) in the lateral habenula astrocyte, in neural and behavioral modulations utilizing Cre-loxP system. We found that an astrocyte-specific deletion of PLCη1 reduced tonic glutamate current in the lateral habenula. Furthermore, the deletion impaired extrasynaptic long-term depression of the LHb neurons, which is dependent of the ambient glutamates outside of the perimeter of synapses. In addition, the immobile time during a forced swim test is significantly increased in the LHb astrocyte-specific PLCη1 KD mice, without changes in anxiety and cognitions. These findings suggest that the PLCη1 in LHb astrocytes maintains neural plasticity and balanced activities and keeps the organism from abnormal mood disorders and provide a novel therapeutic target for depression.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Topic: B.09. Glial Mechanisms

Support: R21DA052447

Title: Astrocyte-targeted CaMPARI2 as a novel tool to assess effects of cocaine on calcium signaling in nucleus accumbens astrocytes

Authors: *E. V. HARDER[1], A. TESTEN[2], R. MADANGOPAL[3], B. T. HOPE[4], K. J. REISSNER[1];
[1]Psychology and Neurosci., [2]Neurosci. Curriculum, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Neuronal Ensembles in Addiction Section, NIH, NIDA IRP, Baltimore, MD; [4]Behav Neurosci, NIH/NIDA, Baltimore, MD

Abstract: Intracellular calcium dynamics provide a primary means of signaling within astrocytes. The significance of astrocyte calcium signaling includes bidirectional communication with neurons and governance of neural function, which has been implicated in the regulation of complex behaviors. Reflecting the diverse roles for astrocyte calcium, there are distinct mechanisms and spatial domains of calcium within the cell body and peripheral processes. However, despite the substantial evidence for fundamental roles for astrocyte calcium in the cellular mechanisms of brain function and behavior, little is known about how drugs of abuse affect astrocyte calcium signaling (see Corkrum et al., 2020; O'Donovan et al., 2021; Wang et al., 2021). This is a particularly salient question, given the increasing evidence that astrocytes within the reward circuitry are chronically impaired both in structure and function following rodent drug self-administration (Testen et al., 2017, Kim et al., bioRxiv 2022.04.06.487393). Further, limitations of existing methods of calcium monitoring present significant hurdles toward assessment of astrocyte calcium elevations associated with behavior in deep brain structures in rat. Toward that challenge, we have developed two novel AAVs which express the photoconvertible, ratiometric calcium indicator CaMPARI2 (Moeyaert et al., 2018) under control of the astrocyte-specific GfaABC1D promoter, to allow irreversible marking of calcium-activated astrocytes. One variant is cytosolic and expressed primarily in the cell body, localized to primarily report somatic calcium elevations; in contrast, the membrane-associated Lck-fusion variant can report calcium elevations within the fine peripheral processes. Both variants demonstrate reliable, astrocyte-restricted expression in the nucleus accumbens. We further confirm and quantify photoconversion of astrocyte Lck-CaMPARI2 upon calcium stimulation in acute slices of nucleus accumbens in response to ATP, dopamine D1 receptor agonist SKF 38393, and direct application of cocaine. Ongoing studies are designed to characterize photoconversion of calcium-activated astrocytes *in vivo* in response to cocaine administration. Astro-targeted CaMPARI2 may be applied toward investigation of astrocyte calcium responses across a broad field of cells both in slice and in awake, behaving animals, imaged at high resolution, and with multiplexed assessment of gene and protein expression.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Program #/Poster #: 274.25

Topic: B.09. Glial Mechanisms

Support: 2RF1AG027297-11A1

Title: Calcium activity in astrocytes and astrocyte networks are altered in a hyperhomocysteinemia model of small cerebral vessel disease

Authors: *B. WEISS[1], C. GANT[1], R.-L. LIN[2], O. THIBAULT[2], D. M. WILCOCK[1], C. M. NORRIS[1], P. M. SOMPOL[1];
[1]Sanders Brown Ctr. on Aging, [2]Pharmacol., Univ. of Kentucky, Lexington, KY

Abstract: The hyperhomocysteinemia (HHcy) diet model of small cerebral vessel disease exhibits vascular inflammation, cerebral hypoperfusion, impaired neurovascular coupling, synapse dysfunction, and cognitive decline. Pathological changes in astrocyte structural features have also been noted, but functional astrocyte phenotypes have not been characterized. Here, we examined the Ca2+ transients from different morphological features of individual astrocytes and astrocyte networks in response to stimulation of the barrel cortex of fully awake mice pretreated with either a control or HHcy diet. Adult C57/BL6 mice were fed for 15 weeks with control chow or chow deficient in vitamins B6/B12 and enriched in methionine (HHcy diet). Mice were injected with AAV-Gfa104-lckGCaMP6f into barrel cortex, followed by two-photon imaging to assess GCaMP6f/Ca2+ transient activity. Baseline spontaneous activation of astrocytic Ca2+ networks was recorded, followed by air puff stimulation of contralateral whiskers (10Hz, 10 sec). Ca2+ transient amplitudes (Δf/f), rise/decay times, and number of transients, were measured for whole cell, and perivascular regions of interest, (ROI) and averaged across the field of view (FOV). The temporal relationship between perivascular Ca2+ transients and dilatory responses in immediately adjacent microvessels was also investigated. Though spontaneous Ca2+ transients were comparable in amplitude, rise, and decay across diet conditions, the number of transients per ROI was reduced in HHcy diet mice. Whisker stimulation evoked a significant and synchronous Ca2+ elevation among ROIs in the FOV of both diet groups. However, the number of active ROIs and the functional connectivity between ROIs were significantly reduced in HHcy vs. control diet mice. Despite these network deficits, evoked Ca2+ transient amplitudes in individual ROIs were significantly larger with faster activation/decay kinetics in HHcy mice. Initiation of Ca2+ transients at perivascular ROIs following whisker stimulation also occurred significantly earlier, relative to peak changes in vascular tone, in HHcy mice vs. control diet mice. The results show that diet induced vascular pathology is associated with complex changes in astrocytic Ca2+ signaling that may trigger and/or maintain reactive astrocyte phenotypes. Interactions between vascular smooth muscle cells and astrocytes may also contribute to deleterious effects on cerebral blood flow and neuronal activity, leading to overall neural dysfunction and cognitive impairment.

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Poster

274. Astroglia: Neuron Interactions in Disease

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Program #/Poster #: 274.26

Topic: B.09. Glial Mechanisms

Support: RF1 AG064859

Title: Early loss of astrocyte p38alpha MAPK has sexually dimorphic impacts on measures of neuronal excitability and cytokines in aged mice

Authors: *J. C. GANT, M. J. COLEMAN, V. A. DAVIS, C. M. NORRIS, L. J. VAN ELDIK, D. J. BRAUN;
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Abstract: Background: The p38α MAPK (p38) signaling pathway is ubiquitously expressed in the brain where it plays central roles in neuroinflammation, metabolism, synaptic development and communication, and modulation of blood brain barrier integrity. Its activity in astrocytes has largely been described as immunomodulatory; however, it has also recently been reported to contribute to long term depression and associated behaviors. Astrocytes are also known to be important to healthy brain aging and neuroprotection, but the specific involvement of astrocytic p38 signaling is unclear. We therefore used conditional KO mice to determine how the loss of p38 in astrocytes early in adulthood (3 months) affects synaptic function and cytokine expression phenotypes in later life (20+ months).
Method: Mice were bred homozygous for floxed p38, heterozygous for a Rosa reporter, and with or without tamoxifen-inducible Cre recombinase under control of the astrocyte aldh1l1 promoter. When animals were about 3 months of age they were placed on tamoxifen diet (400 ppm) for 4 weeks, after which they were returned to standard chow and allowed to age until 20-24 months prior to undergoing electrophysiological endpoints or collection of brain tissue for biochemical analyses. Electrically evoked field excitatory post synaptic potentials (fEPSPs) were recorded in hippocampal CA1 stratum radiatum in acutely prepared brain slices and cytokines from the whole hippocampus were measured from a subset of mice that did not undergo electrophysiological recordings.
Results: Males and females showed baseline differences in measures of I/O, fiber volley amplitude, early vs. late potentiation, and paired pulse facilitation. Additionally, knockout of astrocyte p38 generally had opposite effects on these parameters in males and females. Interestingly, the pattern of hippocampal IL-1β cytokine levels mirrored these differences.
Conclusions: These data indicate that there may be qualitative differences in male versus female brain aging that are differentially impacted by astrocyte p38 signaling. The implications of these findings are currently being explored to determine the underlying mechanisms for these sexually dimorphic effects and further determine what functional differences may exist for hippocampal-dependent behaviors.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.27

Topic: B.09. Glial Mechanisms

Support: NIHRF1 AG027297

Title: Overexpression of astrocytic glutamate transporters alleviates hyperexcitability in mouse model of Alzheimer's Disease

Authors: *J. GOLLIHUE, J. C. GANT, S. KRANER, C. NORRIS;
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Abstract: Astrocytic glutamate transporters shape synaptic transmission and prevent neuronal excitotoxicity by quickly and efficiently clearing glutamate from the synaptic cleft. In mouse models and human cases of Alzheimer's Disease (AD), the astrocyte-specific glutamate transporter (Glt1 in rodents and EAAT2 in humans) is decreased. Our lab, and others, have hypothesized that reduced astrocytic glutamate uptake leads to hyperexcitability and synapse loss in AD. To further test this hypothesis, we used an AAV equipped with a GFAP promoter to over-express Glt1 in hippocampal astrocytes of 5XFAD mice that show characteristic signs of astrocyte reactivity, Glt1 loss, hyperexcitability, and synapse dysfunction. Compared to a control AAV vector (AAV-Gfa2-Luciferase), AAV-Gfa2-Glt1 significantly increased the expression of glutamate transporters in hippocampal tissue. Next, we performed electrophysiological field recordings to examine the role of astrocyte-specific glutamate transporters in hyperexcitability and synaptic function. Animals received injections of AAV-Gfa2-Luc in one hippocampus, and AAV-Gfa2-Glt1 in the contralateral hippocampus. Field recordings were performed to assess excitatory postsynaptic potentials in the hippocampal CA1 stratum radiatum elicited by stimulation of CA3 axons. We then compared within-animal differences for measures of neuronal hyperexcitability, synaptic strength, and long-term synaptic potentiation. Results indicate that there is more hyperexcitability in control tissues (i.e. treated with AAV-Gfa2-luc) compared to tissues treated with AAV-Gfa2-Glt1, as evident by decreased population spike thresholds. The results confirm that loss of glutamate transport is key "loss-of-function" phenotype associated with reactive astrocytes in AD. Experiments to assess the impact of Glt-1 overexpression on synaptic function and plasticity are ongoing, as are measures of brain metabolism and cognitive function.

Disclosures: J. Gollihue: None. **J.C. Gant:** None. **S. Kraner:** None. **C. Norris:** None.

Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.28

Topic: A.03. Stem Cells and Reprogramming

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

Title: Human in vitro model to investigate the effect of inflammation on the glial cells in multiple sclerosis

Authors: I. TUJULA[1], T. HYVÄRINEN[1], J. LOTILA[1], H. JÄNTTI[4], S. OHTONEN[4], L. SUKKI[2], K. TORNBERG[2], D. VOULGARIS[5,6,7], T. MALM[4], A. HERLAND[5,6,7], P. KALLIO[2], S. NARKILAHTI[3], ***S. HAGMAN**[1];
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Abstract: Multiple sclerosis (MS) is a chronic inflammatory disease with the complex pathogenesis driven by inflammation and axonal degeneration. Although multiple treatment options are available for the inflammatory disease subtype of MS, finding an effective treatment option for progressive disease form has been largely unsuccessful. Recent data suggest that the CNS-resident cell types, astrocytes and microglia, are the key players in the MS disease pathogenesis and disease progression. Understanding their functions in the complex neuroinflammatory axis need to be further investigated. Bidirectional communication of glial cells may lead to enhanced immune responses, disturbed metabolism, loss of trophic support and axonal injury.Here we took advantage of human induced pluripotent stem cell (hiPSC) -derived microglia and astrocytes. We induced a reactive phenotype in astrocytes with tumor necrosis factor (TNF)-α and interleukin (IL)-1β and in microglia with lipopolysaccharide (LPS) and interferon (IFN)-γ, and characterized their inflammatory phenotypes. Finally, we used novel microfluidics chips to reveal their bidirectional communication in the pathogenic processes of MS.Cytokine-stimulated hiPSC-derived astrocytes showed a typical change in morphology that is a known hallmark of astrogliosis. In addition, astrocytes showed increased gene and protein expression of inflammatory mediators. Moreover, hiPSC-derived microglia expressed cell type specific markers, and showed typical microglial functions, such as phagocytosis and intracellular calcium transients. Stimulation of microglia activated inflammatory signaling pathways and induced secretion of inflammatory mediators. Lastly, we engineered a novel microfluidic chip for studying the bidirectional communication of hiPSC-derived astrocytes and microglia in controlled, compartmentalized culture environment. Co-cultures were successful and microglial migration through the microtunnels was demonstrated and both cell types responded treatments

selectively. In conclusion, our human in vitro MS model is promising and enables interrogation of intercellular communication between astrocytes and microglia in inflammatory environment.

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Poster

274. Astroglia: Neuron Interactions in Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 274.29

Topic: B.09. Glial Mechanisms

Support: CIHR Grant - PJT165852
CAMH - Discovery Fund

Title: Astroglial and neuronal calcium dynamics in response to stress in rodents

Authors: *Y. BANSAL[1], S. A.-M. CODELUPPI[1], J. KNOCH[1], J. MUIR[2], R. C. BAGOT[3], E. SIBILLE[4], M. BANASR[1];
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Abstract: Astroglial and neuronal calcium dynamics in response to stress in rodents
Bansal Y[1] **,** Codeluppi S[1,2], Knoch J[1,2], Muir J[4], Bagot RC[4,5], Sibille E[1,2,3], Banasr M[1,2,3]
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Astrocytes are ubiquitous glial cells in central nervous system that regulate synaptic transmission and neurotransmitter recycling through bidirectional interaction with neurons. Specifically, astrocytes respond to altered neuronal activity through changes in intracellular Ca^{2+} concentrations and release gliotransmitters, which in turn act on neurons. Emerging research identify a critical role of astroglial dysfunctions in the major depressive disorder- and stress-related synaptic deficits. Thus, investigating changes in astroglial and concomitant neuronal Ca^{2+} transients in response to stress may help elucidate the astroglial involvement in depressive-like symptoms. In this study, neuronal and astrocytic Ca^{2+} transients were recorded using fiberphotometry in mice. Astroglial and neuronal specific adeno-associated viruses were infused in the prefrontal cortex to record Ca^{2+} transients using fiber optic cannula. We first investigated the changes in astroglial and neuronal Ca^{2+} mobilization baseline activity and reactivity to acute stress (tail pinch and immobilization stress). Further we also examined the effects of PFC

specific astroglial activation and CaMKII specific PFC neuronal inhibition and activation using designer receptors exclusively activated by designer drugs approach on neuronal and astroglial Ca^{2+} transient respectively. Current studies are focusing on examining chronic stress effects on neuronal and astrocytic Ca^{2+} transients. This work will allow identification of cell activity alterations in response to stress and will shed light on the dynamic relationship between the astrocytic and/or neuronal dysfunctions associated with stress and depression.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.01

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ)
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)
Alzheimer’s Association
Serrapilheira Institute
D'Or Institute for Research and Education

Title: Suppression of lipoxin A4 associates with cognitive deficits in aging and Alzheimer's disease

Authors: *M. V. LOURENCO[1], F. A. PAMPLONA[2], G. VITÓRIA[2], F. K. SUDO[2], F. C. RIBEIRO[1], A. R. ISAAC[1], C. A. MORAES[3], P. F. LEDUR[2], K. KARMIRIAN[2], Í. M. ORNELAS[2], L. M. LEO[3], B. PAULSEN[4], G. COUTINHO[2], C. DRUMMOND[2], N. ASSUNÇÃO[2], B. VANDERBORGHT[2], C. CANETTI[1], H. C. CASTRO-FARIA-NETO[3], P. MATTOS[2], S. T. FERREIRA[1], S. K. REHEN[2], F. A. BOZZA[3], F. TOVAR-MOLL[2];
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Abstract: Age increases the risk for cognitive impairment and is the single major risk factor for Alzheimer's disease (AD), the most prevalent form of dementia in the elderly. The pathophysiological processes triggered by aging that render the brain vulnerable to dementia involve, at least in part, changes in inflammatory mediators. Here we show that lipoxin A4 (LXA4), a lipid mediator of inflammation resolution known to stimulate endocannabinoid signaling in the brain, is reduced in the aging central nervous system. We demonstrate that genetic suppression of 5-lipoxygenase (5-LOX), the enzyme mediating LXA4 synthesis, promotes learning impairment in mice. Conversely, administration of exogenous LXA4 attenuated cytokine production and memory loss induced by inflammation in mice. We further show that cerebrospinal fluid LXA4 is reduced in patients with dementia and positively associates with memory performance, dementia stage, brain-derived neurotrophic factor (BDNF), and AD-linked amyloid-beta. Our findings suggest that reduced LXA4 levels may lead to vulnerability to age-related cognitive disorders and that promoting LXA4 signaling may comprise an effective strategy to prevent early cognitive decline in AD.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1 AG074566
NIH Grant U54 AG054345
NIH Grant R01 GM057391
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NIH R03 AG063250

Title: Plcg2 variants elicit differential microglial responses and disease pathology in Alzheimer's disease

Authors: *S. J. BISSEL, A. P. TSAI, P. B.-C. LIN, C. DONG, E. MESSENGER, M. MOUTINHO, G. XU, Y. LIU, A. OBLAK, K. NHO, B. T. LAMB, G. E. LANDRETH; Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Recent studies have highlighted that many genetic risk variants for Alzheimer's disease (AD) are predominately expressed in microglia and are associated with innate immune

responses. Among these risk genes is phospholipase C gamma 2 (PLCG2), a key signaling hub protein that regulates immune effector function. Notably, coding missense variants in *PLCG2* are linked to altered AD risk. The hyperfunctional P522R variant of *PLCG2* confers protection against AD, and we have identified a novel variant encoding the less active M28L variant, which is associated with elevated AD risk. However, the contribution of *PLCG2* variants on AD pathogenesis is unknown. Gene expression analysis of RNA-Seq data from human brains linked expression of *PLCG2* variants to inflammation-related pathways. To systematically explore the effect of *PLCG2* variants in AD, we created mice harboring the *PLCG2* variants and crossed them with 5xFAD amyloidogenic mice. Primary murine microglia isolated from variant mice showed differential uptake capacity of fluorescently labeled amyloid-beta peptide. The less active M28L risk variant disrupted protein interactions between PLCG2 and upstream signaling elements, diminished microglial response to plaques, suppressed cytokine concentrations, downregulated disease-associated microglial gene expression, and exacerbated plaque deposition. Conversely, the protective, hypermorphic P522R variant altered microglial disease-associated populations, stimulated microglial response to plaques with altered cytokine levels, decreased plaque deposition, and ameliorated the impairment of synaptic plasticity and Y-maze alternation. Collectively, our study provides evidence that the M28L variant had accelerated and exacerbated disease-related pathology, while the P522R variant appeared to mitigate disease severity and progression. Overall, our findings suggest that *PLCG2* plays an important role in AD pathophysiology.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: CNPq/Brazil 406436/2016-9
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Title: Oxytocin attenuates microglial activation and restores social and non-social memory in the APP/PS1 mouse model of Alzheimer’s disease

Authors: *M. SELLES[1], J. T. FORTUNA[3], Y. P. DE FARIA[3], B. MONTEIRO LONGO[4], R. C. FROEMKE[2], M. V. CHAO[5], S. T. FERREIRA[6];
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Abstract: Alzheimer's disease (AD) is the main cause of dementia in the elderly and is characterized by memory loss, social withdrawal and neurodegeneration, eventually leading to death.
Brain inflammation has emerged as a key pathogenic mechanism in AD. We hypothesized that oxytocin, a pro-social hypothalamic neuropeptide with anti-inflammatory properties, might be implicated in the pathophysiology of AD, and that the oxytocin system could be a therapeutic target in AD. To test this hypothesis, we first studied the effect of Aβ oligomers on oxytocin production *in vitro* using hypothalamic slices and *in vivo* in wild-type mice. Next, we measured oxytocin production in the APP/PS1 transgenic mouse model of AD. We evaluated oxytocin production by quantification of hypothalamic oxytocin mRNA levels by qPCR, and found decreased oxytocin levels in the three models, compared to controls (Aβ *in vitro*: p= 0.01, Aβ *in vivo*: p<0.01, APP/PS1 mice: p=0.04, n= 3-9 mice per group). We then developed and validated a protocol to rescue brain oxytocin levels via intranasal delivery of exogenous oxytocin (p=0.03, n=10-12 mice per group), and tested the therapeutic potential of chronic intranasal oxytocin in aged APP/PS1. At a cellular level, we found that treatment with oxytocin reduced microglial activation, evaluated as Iba1 immunoreactivity by immunohistochemistry (p<0.01, n=7-9 mice per group). Behavioral tests revealed that while social and non-social memory was impaired in APP/PS1 mice, oxytocin treatment rescued both social and non-social memory in the 5-trial social task (p=0.01) and novel object recognition task (p=0.04), respectively (n=11-15 mice per group). Our findings point to oxytocin as a potential therapeutic target to reduce brain inflammation and correct memory deficits in AD.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.04

Title: WITHDRAWN

Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIAAA Grant AA024829
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NICHD Grant P50HD103573

Title: Alcohol abuse during adolescence promotes Alzheimer's disease pathology in adulthood by persistent enhancement of proinflammatory signaling.

Authors: A. BARNETT[1], E. DAVID[2], A. ROHLMAN[2], V. D. NIKOLOVA[2], S. S. MOY[5], R. P. VETRENO[3], ***L. COLEMAN**[4];
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Abstract: Epidemiologic studies find an association between heavy alcohol consumption and the incidence of Alzheimer's Disease (AD), with consumption during young adulthood possibly having a greater impact (Mukamal et al 2003, Langballe et al 2015). Therefore, we investigated if adolescent alcohol abuse promotes progression of AD pathology with aging. Triple transgenic-AD mice (3xTg-AD, APP^{Swe}, tau^{P301}, $Psen1^{tm1Mpm}$) underwent either adolescent intermittent ethanol (AIE, 5g/kg/day, i.g., **2 days on 2 days off) or water gavage from P25-55. Mice were then left without treatment and assessed at P200, an early time point when intraneuronal Aβ and tau accumulation is beginning in this model. In female 3xTg-AD mice,** AIE increased levels of the early pathologic feature of intraneuronal $A\beta_{1\text{-}42}$ in entorhinal cortex (ENT), subiculum, and amygdala by P200. AIE also increased levels of phosphorylated tau-Thr181, a marker for disease progression in humans. Adolescent ethanol also persistently reduced memory retention, locomotor activity (open-field and NORT habituation), and increased anxiety-like behavior (thigmotaxis) in 3xTg mice. Along with increases in early AD behavioral and protein pathology, AIE persistently increased expression of multiple proinflammatory genes in the hippocampus, including IL-1β (7.5-fold), MCP-1 (6.7-fold), IL-6 (9-fold), and IFNα (10-fold). Strikingly, the expression of proinflammatory genes was strongly correlated with the levels of $A\beta_{1\text{-}42}$ and p-tau-181 (R^2=0.86 to 0.99). Therefore, we investigated if blocking proinflammatory signaling with minocycline during AIE could prevent enhancement of AIE pathology. Minocycline on AIE treatment days (30mg/kg) blocked ethanol-induced increases in $A\beta_{1\text{-}42}$ in amygdala and p-tau-181 in hippocampus and prevented AIE-induced thigmotaxis and memory loss. To ascertain the translational relevance of these findings we measured levels of Aβ1-42 and p-tau Thr181 and Ser214 in human subjects diagnosed with AUD who began drinking during adolescence (19.2±1.3 years old onset, average age of death 48.5±3.0 years old). Similar to findings in 3xTg-AD mice, subjects with AUD had increased levels of p-tau214 and p-tau181 in the frontal cortex (vm-PFC/BA25, 50% and 30% increases

respectively) and the hippocampus (27% and 20% increases). $A\beta_{1-42}$ was also increased in vm-PFC by 31%. Thus, heavy alcohol use promotes AD molecular and behavioral pathology in mice and humans, with induction of proinflammatory signaling playing a key role. (Funded by NIAAA)

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

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Program #/Poster #: 275.06

Topic: C.02. Alzheimer's Disease and Other Dementias

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NIH Grant R01AG053588

Title: Type I IFN response-dependent microglia MEF2C deregulation and neuroinflammation in Alzheimer's Disease

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Abstract: Alzheimer's disease (AD) is a chronic neurodegenerative disorder with multifactorial etiology. The role of microglia in the pathogenesis of AD has been increasingly recognized in recent years; however, the detailed mechanisms shaping microglial phenotypes in AD-relevant pathological settings remain largely unresolved. Myocyte-specific enhancer factor 2C (Mef2C) is a transcription factor with versatile functions. In addition to its critical functions in muscular cells and lymphocytes, Mef2C is emerging as a pivotal player in the maintenance of microglial homeostasis. Patients with Mef2C loss-of-function demonstrate brain abnormalities and cognitive deficits, and Mef2C polymorphism has been linked with increased susceptibility to sporadic AD. Moreover, recent studies have attributed aging-related microglial changes to type I interferon (IFN-I)-associated Mef2C deregulation. However, the functional status of microglial Mef2C and its contribution to microglial activation in AD has never been comprehensively investigated. In this study, we have found that suppressed Mef2C nuclear translocation was an early and prominent microglial phenotype in a mouse model of brain amyloidosis (5×FAD mice), which exacerbated with age. Echoing the early Mef2C deregulation and its association with microglial activation, transcriptional data showed elicited IFN-I response in microglia from

young 5×FAD mice. Amyloid beta 42 (Aβ42) in its oligomeric forms promoted Mef2C deregulation in microglia on acute organotypic brain slices with augmented microglial activation and synapse elimination via microglial phagocytosis. Importantly, these oligomeric Aβ42-mediated microglial changes were substantially attenuated by blocking IFN-I signaling. The simplest interpretation of the results is that Mef2C, concurring with activated IFN-I signaling, constitutes early microglial changes in AD-related conditions. In addition to the potential contribution of Mef2C deregulation to the development of microglial phenotypes in AD, Mef2C suppression in microglia may serve as a potential mechanistic pathway linking brain aging and AD.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

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Program #/Poster #: 275.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG018440-16A1

Title: In vivo reduction of α-synuclein improves neuro-pathological, neuro-inflammatory and behavioral phenotypes in a mouse model of Alzheimer's Disease

Authors: *A. LEITAO[1], B. SPENCER[1], C. WU[2], M. MANTE[1], A. CONROY[1], J. ALMARAJ[1], J. B. FLORIO[3], E. MASLIAH[4], R. RISSMAN[1];
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Abstract: *In vivo* reduction of α-synuclein improves neuro-pathological, neuro-inflammatory and behavioral phenotypes in a mouse model of Alzheimer's Disease
Andre D. G. Leitao[1], Brian Spencer[1], Chengbiao Wu[1], Abigail Conroy[2], Jessica Amalraj[1], Michael Mante[1], Jazmin Florio, Eliezer Masliah[3], Robert A. Rissman[1]
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the pathological accumulation of beta amyloid (Aβ), Tau and α-synuclein (α-syn). The role of α-syn has been extensively studied in Parkinson's disease, but less so in AD. Recent studies have shown that α-syn may also play a role in AD and its downregulation could be protective against the toxic effects of Aβ accumulation. We hypothesized that selectively knocking down α-syn improves the neuropathological, biochemical, and cognitive findings in AD.Amyloid precursor protein transgenic (APP-Tg) mice were used to model AD and knockdown of α-syn was

performed by RNA interference. We have selectively reduced α-syn levels by stereotaxic bilateral injection of either LV-shRNA α-syn or LV-shRNA-luc (control) into the hippocampus of mouse lines with different disease paradigms: non-transgenic (non-Tg), APP-Tg, α-syn-Tg. Water maze and open field testing were used to measure cognitive deficits. Biochemistry and immunohistochemistry were performed on each hemibrain.We have successfully utilized this strategy to down-regulate α-syn as shown by lower levels of α-syn through biochemistry and immunohistochemistry of hemibrains of mice treated with LV-si-α-syn. Importantly, this correlates with a significant reduction in the number of Aβ plaques and tau, suggesting a link between Aβ, tau and α-syn in pathology. Furthermore, we found that down-regulation α-syn results in amelioration of neuropathological phenotypes of microglial activation (Iba1) and astrocytosis (GFAP). Morris water maze and open field testing indicate that α-syn reduction restores context dependent memory and ameliorates hyperactivity phenotypes in mouse models of AD.Overall, this study provides evidence that α-syn downregulation improves some of the neuropathological and cognitive deficits and could be of therapeutic value in AD.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.08

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: BrightFocus Foundation grant A20201166F
Eli Lilly-Stark Neuroscience Research grant EPAR1536
NIH grant RF1AG068400

Title: Hexokinase 2 antagonism fine-tune microglial response in Alzheimer's disease

Authors: *J. F. CODOCEDO, C. MERA-REINA, S. PUNTAMBEKAR, B. T. CASALI, P. B.-C. LIN, P. MARTINEZ, C. A. LASAGNA-REEVES, G. E. LANDRETH;
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Abstract: Hexokinase 2 antagonism fine-tune microglial response in Alzheimer’s Disease
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Microgliosis and neuroinflammation are hallmarks of Alzheimer’s disease (AD). Pro-inflammatory microglia meet their increased energy demand by reprogramming metabolism, specifically, switching to favor glycolysis over oxidative phosphorylation, suggesting that

modulations of microglial metabolism might be a new therapeutic strategy for treating AD. However, the metabolic determinants of such changes have not yet been identified. In the brains of 5xFAD mice and postmortem brains of AD patients, we found a significant increase in the levels of Hexokinase 2 (HK2), an enzyme which acts as the first and rate limiting step in glycolysis. We hypothesize that microglial HK2 expression plays a key role in the neuroinflammatory response of microglia. Accordingly, we propose that the antagonism of HK2 could reduce disease progression by blocking its transition to a neurodegenerative phenotype.We generated mice that have a conditional deletion of HK2 gene in microglial cells by crossing 5xFAD;CX3CR1-Cre^{ERT2} mice with mice harboring a floxed HK2 allele. Microglial deletion of HK2 was induced with tamoxifen at two months. Three months later, we assessed HK2 cortical levels, as well as several AD hallmarks. Similarly, we evaluated the acute effect of its pharmacological inhibition with Lonidamine. To ascertain the biological process affected by HK2 inhibition in AD, we performed transcriptomic analysis of the cortical samples with NanoString. We determined that the increase in HK2 occurs selectively within activated microglia associated with amyloid plaques. Here we show that the pharmacological inhibition of HK2 acts to modulate specific features of the microglial response, fine-tuning the resulting phenotype that displayed reduced microgliosis but sufficient barrier formation, increase uptake of Aβ and reduced expression of neurotoxic mediators. *In vivo* this translates in the preservation of synaptic proteins and improved cognitive performance. Similar results were obtained in HK2-haploinsufficient microglia in 5xFAD mice. These findings demonstrate that the upregulation of HK2 in microglia contributes to their chronic pro-inflammatory activation, and its antagonism elicits beneficial effects in a murine model of AD.

Disclosures: **J.F. Codocedo:** None. **C. Mera-Reina:** None. **S. Puntambekar:** None. **B.T. Casali:** None. **P.B. Lin:** None. **P. Martinez:** None. **C.A. Lasagna-Reeves:** None. **G.E. Landreth:** None.

Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.09

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH 1R56AG069192-01 award

Title: Subclinical Expression of Herpes Simplex Virus-1 proteins as a Driver for Alzheimer's Disease Pathologies

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Abstract: DNA and RNA of Herpes Simplex Virus 1 (HSV-1) were found in the brains and serological samples of Alzheimer’s disease (AD) patients. Such molecular presence of HSV-1 in

AD patients is especially intriguing as HSV-1 virions are rarely detected in AD brains. To follow the molecular footsteps detected, we imaged viral proteins in postmortem human AD brains at superior resolution using expansion microscopy, a tissue manipulation method that physically expands the samples by a factor of 4.5x, allowing a 40 nm imaging resolution, and immunolabeled herpetic proteins, AD pathologies and cell markers. Protein levels previously undetectable with standard methods, i.e., immediate-early herpetic protein were found across large brain areas, while late proteins of HSV-1 were not detected. Importantly, we found that HSV-1 immediate early proteins strongly co-localized with AD pathologies. Consequently, we hypothesized that expression of HSV-1 proteins during latency may be linked to AD pathology. We are now characterizing the HSV-1 proteome in AD brains by imaging key latent and non-latent proteins in expanded AD brain slices and examining their colocalization with AD pathologies across brain areas and disease stages. Finally, we are inducing HSV-1 latency in human brain organoids and imaging the relationships between viral proteins and the formation of AD pathologies via expansion microscopy. Pathogens may be triggers of immune responses driving AD; this study would shed light on one common pathogen, HSV-1, while serving as a framework to unveiling molecular causation between infectious agents and AD hallmarks.

Disclosures: **V. Hyde:** None. **V. Pablo:** None. **L. D'Aiuto:** None. **O. Shemesh:** None.

Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.10

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: P01AG062817

Title: The Ketogenic Diet Reduces NeuroInflammation and increases Synaptogenesis and also rescues LTP in PSAPP mice

Authors: J. DI LUCENTE[1], A. TOMILOV[5], L.-W. JIN[7], I. MAEZAWA[8], J. RAMSEY[2], C. MONTGOMERY[3], J. RUTKOWSKY[2], J. SHORE[6], K. KIM[6], ***G. CORTOPASSI**[4];
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Abstract: The Ketogenic diet (KD) has been shown to increase longevity and increase cognitive function in aging mice (PMID: 28877457, 28877458). We tested whether KD may oppose AD in three ways, in a long-term 6 mo. KD of PSAPP Alzheimer’s mice *in vivo*, a short-term KD of 6 wks of aged C57B mice *in vivo*, and a 24hr study of human IPSC microglia driven to inflammation by A-beta *in vitro*. PSAPP mice were fed for 6 months an isocaloric carbohydrate standard chow diet or ketogenic diet. Significant increases in blood ketone Beta-hydroxy

butyrate (BHB) was observed. Long-Term Potentiation (LTP) thought to be the molecular substrate of memory was significantly deficient in PSAPP mice on the carbohydrate standard chow diet, but was rescued in mice on KD PSAPP mice to within wildtype levels. In these KD PSAPP hippocampi--a significant increase in p-Creb and p-Erk pathways and pro-BDNF was observed, these pathways are thought to support synaptic plasticity. To investigate shorter-term KD effects, 11 month C57 mice were fed KD or CD for 6 weeks, hippocampus extracted for nucleic acids and RNAseq performed. The KD mediated significantly stimulated hippocampal synaptic plasticity genes (Icam5, Hsf1, Gale, Wars), reduced complement-related genes (Cast, She, Vcam1, Serping1), and reduced microglial inflammatory genes known to be triggered by A-beta amyloid: SPP1, Smoc1, TSPO, cyclooxygenase 1. As BHB is thought to mediate the KD's benefits, we tested the consequences of 24h BHB administration on human IPSC microglia *in vitro*. BHB significantly reduced the A-beta-mediated rise in inflammatory markers IL-1b, IL-6, TNF-alpha and NOS2. In summary, 6 months of KD suppresses the PSAPP-mediated loss of LTP observed on the carbohydrate diet and stimulates synaptic genes *in vivo*. 6 weeks of KD changes lipid metabolism genes in the hippocampus, inhibits microglial inflammation genes, and stimulates synaptic genes *in vivo*. And 24 hr of BHB admistration strongly suppresses A-beta mediated microglial inflammation *in vitro*. These data support a hypothesis in which the KD and its mediator BHB suppress the A-beta dependent microglial hyperinflammation which may result in 'overpruning' of synapses and defective short term memory consolidation.

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Poster

275. Neuroinflammation and Immune Responses in Alzheimer's *In Vivo* Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 275.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R21AG073710
R56AG068089
Cure Alzheimer's Fund

Title: Siglec family members are associated with specific subsets of microglia in a disease stage-specific manner in AD

Authors: *T. LI[1], K. TU[1], A. GONZALEZ-GIL[2], R. L. SCHNAAR[2];
[1]Pathology, [2]Pharmacol., The Johns Hopkins Univ., Baltimore, MD

Abstract: Microglia are genetically implicated in the susceptibility to the progression of Alzheimer's disease (AD), which has stimulated enhanced focus on these cells and their regulatory pathways as therapeutic targets. Among several microglial genes linked to AD

susceptibility via genome-wide association studies (GWAS) is CD33, also known as sialic acid-binding immunoglobulin-like lectin 3 (Siglec-3). Siglec-3 is likely immune inhibitory, as it has been postulated that an excess of microglial inhibitory signals by Siglecs results in reduced phagocytosis and exacerbated progression of proteinopathy in AD. Understanding of Siglec regulation of microglia in AD would not only shed light on the mechanism of AD development, but also provide new therapeutic targets for AD. Siglecs are a family of 14 transmembrane proteins in humans. Most Siglecs are expressed on overlapping subsets of immune cells and most Siglecs are immune inhibitory, suggesting that Siglec-3 is only the tip of the iceberg for the roles of Siglecs and Siglec ligands in the regulation of microglia. We hypothesize that microglia activity is modulated by specific Siglec inhibitory pathways at different stages of AD pathogenesis. Identification of the specific Siglec pathways during AD development could open new potential avenues for mechanism-based therapy. Here, we found that *Siglec* family members are associated with specific subsets of microglia that are activated in a disease stage-specific manner in both mouse models of AD and AD patients. For example, Siglec-F is upregulated in response to Aβ pathology and is selectively expressed in Aβ-associated DAMs, while Siglec-G/10 is upregulated in white-matter-associated microglia subtypes in late-stage AD. These findings are consistent with a model that *Siglec* family members are associated with specific subsets of microglia in a disease stage-specific manner in AD, and have important implications for the identification of novel molecular targets and therapeutic strategies for the treatment of AD.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 276.01

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant AG068286

Title: Acetyl-coa carboxylase regulates lipid droplet formation in senescent microglia

Authors: M. COOK[1], S. G. DIXON[1], S. K. MISHRA[2], ***G. Y. WANG**[2];
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Abstract: The accumulation of senescent cells in aged tissues has been implicated in a variety of age-related diseases, including cancer and neurodegenerative disorders. Recent studies have demonstrated a link between age-associated increase of senescent glial cells in the brain and the pathogenesis of Alzheimer’s disease (AD). However, there is a lack of *in vitro* cellular models of senescent human microglia, which significantly limits our approaches to study AD pathogenesis. Here, we report that ionizing radiation (IR) dose-dependently induces premature senescence in

HMC3 human microglial cells. Senescent microglial cells displayed the senescence-associated secretory phenotype (SASP), as evidenced by the production of a high level of neuroinflammatory cytokine interleukin-6 (IL-6). Phosphorylated p53 and p21 expression levels were substantially increased in IR-induced senescent microglia compared to control proliferating cells, suggesting a role of the p53/p21 pathway in IR-induced senescence of microglial cells. Interestingly, we found that senescent microglia accumulate higher levels of lipid droplets (LD) than proliferating cells. An analysis of human brain specimens using supper-resolution confocal microscopy confirmed the accumulation of LDs in senescent microglia in the hippocampus of AD patients compared with age-matched control subjects. Blocking of acetyl-CoA carboxylase (ACC), a key enzyme for lipid *de novo* synthesis, by TOFA markedly inhibits LD formation in senescent microglial cells. Taken together, these results suggest that ACC may play a critical role in regulating LD formation in senescent microglia.

Disclosures: M. Cook: None. **S.G. Dixon:** None. **S.K. Mishra:** None. **G.Y. Wang:** None.

Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 276.02

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH-1R01AG065836-01

Title: Disruption of Perineuronal nets in mouse models of Alzheimer Disease

Authors: *L. CHAUNSALI[1], B. P. TEWARI[2], C. PRIM[1], H. SONTHEIMER[1];
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Abstract: Alzheimer Disease (AD) is the most common form of dementia characterized by progressive loss of memory and cognition. Extracellular deposition of amyloid plaque and intracellular accumulation of tau tangles are the key pathological features of AD brains however, treatment strategies targeting Aβ and tau have not resulted in a satisfactory outcome. Recent studies provide evidence of changes in the extracellular matrix structures called perineuronal net (PNNs) in the AD mouse models and human AD brains. PNNs are primarily composed of chondroitin sulfate proteoglycans, tenascins, link proteins, and hyaluronan, and are known to stabilize synapses and regulate neuronal plasticity, thereby implicated in memory and cognition. PNNs are found in several brain regions including the cerebral cortex and hippocampus and surround the majority of parvalbumin-expressing (PV+) GABAergic inhibitory neurons and a small population of excitatory neurons. To establish the causal role of PNN disruption in memory and cognitive decline, we first defined a timeline of PNN disruption in J20 and 5XFAD models. Our immunohistochemical staining and confocal imaging results suggest that PNN disruption precedes memory and cognitive decline in AD mouse models. To answer whether PNN disruption is due to altered synthesis of PNN constituents or PNN remodeling agents, we

performed qPCR, western blotting, and enzyme activity assay. Our data suggest that PNN disruption is mainly due to an increased expression and functional activity of matrix metalloproteinase 3. The upregulation was found at an early age in the hippocampus and at a later age in the cortex thereby following the spatiotemporal course of PNN disruption and Aβ accumulation. Overall, our findings suggest that PNN disruption progresses with amyloid plaque deposition in AD mouse models and precedes memory and cognitive decline. Preventing PNN disruption by inhibition of ECM remodeling agents may have a positive outcome in AD-associated memory deficits.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

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Program #/Poster #: 276.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: P30 AG028383
R00 AG044445
R21AG066865

Title: High-volume microglia staining in the presence of disease pathology

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Abstract: Despite the variations in clinical manifestation, many of the age-related neurodegenerative diseases share similar pathological features, such as neurofibrillary tangles (NFT) comprised of abnormally phosphorylated tau protein and extracellular plaques containing amyloid-beta (Aβ) proteins. Microglial changes are also believed to be associated with neurodegenerative disease as well. Recent studies suggest that loss of neuronal support from microglia acts as a point of inflection leading to disease pathology, while others speculate that it is a toxic gain of function that can exacerbate disease progression-whether through the secretion of toxic cytokines or by aiding in the formation of pathology. Despite the continued innovation of techniques such as scRNA-Seq, histological examination of stained tissue remains the most robust and reliable method to visualize pathology topographically in FFPE archived brains. Using various combinations of antibodies in tandem allows for visualization of potential sub-populations of microglia. Traditionally, immunofluorescence has been one of the most widely used methods to visualize multiple proteins of interest within a cell, although, this method is often limited in its efficacy and the number of targets that can be detected. However, using a recently developed method of multiplex staining, we are able to visualize the spatial and morphological changes taking place in microglia with more than 10 different makers in the presence of disease-associated pathology. We are therefore curious to see what changes can be

observed in regards to the loss of homeostatic microglial markers that directly associate with disease pathology as opposed to cells not in direct proximity to pathology. Using multiplex staining in FFPE samples of human mid-temporal gyrus and 8 well-characterized and commercially available antibodies, we investigated changes in immunoreactivity and microglial morphology at various distances from plaque and tangle pathology associated with Alzheimer's disease. Our working hypothesis is that loss of homeostatic microglial markers and changes in microglial morphology are related to their proximity to pathology. Understanding the changes occurring in microglia and their spatial association to disease pathology may allow for a better insight into the role of microglia in disease.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

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Topic: C.02. Alzheimer's Disease and Other Dementias

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VA Merit Award 101 BX004161-01
NIH R01 ES028104
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Title: Ozone dysregulates astrocyte-associated proteins in the amyloid plaque microenvironment

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Abstract: Urban air pollution exposure, including ozone (O_3), has been associated with increased Alzheimer Disease (AD) risk and there is recent support that urban air pollution exposure may increase amyloid plaque pathology. However, the underlying cellular mechanisms driving this effect are poorly understood. Astrocytes surround amyloid plaques and have been implicated as regulators of amyloid pathology. At present, how inhaled pollutants, particularly O_3, may affect the astrocytic response is largely unknown. To begin to explore this, 10-11 week old male 5xFAD mice were exposed to filtered air (FA) or 1 ppm O_3 for 13 weeks (4 hours/day and 3 days/week). Analysis of ThioS-stained plaques demonstrated that O_3 increases amyloid plaque load. The total GFAP positive area and cell number were increased in the cortex of 5xFAD mice exposed to 1ppm O_3, supporting an increase in the number of cortical astrocytes. The Nanostring GeoMx Digital Spatial Profiling (DSP) platform was used to assess multiplex

protein expression co-localized with GFAP positive astrocytes, that were either in physical contact with amyloid plaques (plaque-associated astrocytes) or plaque distant in the cortex. DSP analysis revealed that the expression of 16 proteins were shared regardless of plaque association phenotype or O_3 exposure. When comparing plaque-associated astrocytes to plaque distant astrocytes, 9 proteins were changed in only FA exposed mice, such as Neprilysin and Ki-67, supporting that a baseline change in plaque-associated astrocyte proteins occurs, regardless of O_3 exposure. However, only Myelin Basic Protein (MBP), CSF1R and Clec7a protein expression was dysregulated in O_3 exposed mice, suggesting that O_3 exposure impedes the protein expression changed with astrocyte plaque association. However, in direct comparison of only plaque-associated astrocytes between FA and O_3 groups, 12 proteins, including Vimentin, GPNMB, Clec7a, MBP were upregulated with O_3, further supporting that O_3 regulates a unique signature of protein changes around plaques-associated astrocytes and suggesting potential changes in astrocyte interactions with microglia and myelin in the peri-plaque space. Taken together, these findings support that the impact of O_3 on astrocytes is dependent on the plaque microenvironment and may provide much needed insight into how urban air pollution affects amyloid pathology.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Program #/Poster #: 276.05

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH
UH Foundation

Title: Amyloid-β Induces morphological and cellular changes in cells of the oligodendroglia lineage linked to myelin disruption

Authors: *D. D. DELGADO, R. A. NICHOLS;
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Abstract: Loss of white matter integrity is a feature of natural aging and Alzheimer’s disease (AD). Notably, the spread of AD pathology reflects the pattern of myelination during normal development but in reverse (neuropathologic retrogenesis), where later myelinated brain regions develop AD pathology before early myelinated regions. Classical neuropathologic changes in AD (i.e. accumulation of soluble oligomeric amyloid-β) are correlated with progressive neuronal death, but may also induce changes in the cells of the oligodendroglia lineage and damage myelin.This project sought to elucidate the cellular responses of the oligodendroglia cells to amyloid-β (Aβ) and the subsequent impact on white matter structure. Using neonatal C57BL/6J

mice, we were able to establish a variety of primary glial cultures: oligodendrocyte precursor cells (OPC), oligodendrocytes, and oligodendrocyte-neuron co-cultures. We assessed the effects of Aβ on OPC's self-replicative ability and survival using EdU and TUNEL detection assays. Preliminary results indicate that Aβ may have a time-dependent effect on OPC replication, survival, as well as differentiation. Using immunocytochemistry, we found that a small population of OPCs exposed to 1μM Aβ for a prolonged period of time (5 days) seemingly switch glial fates to type II astrocytes indicated by the loss of neuron glial 2 proteoglycan (NG2) and A2B5 expression concomitant with the induction of expression of the glial fibrillary acid protein (GFAP). We further investigated the effects Aβ may have on the myelinating role of oligodendrocytes by creating an *in vitro* myelination assay utilizing an oligodendrocyte-neuron co-culture. Neurons were visualized via expression of BacMam-GFP, while axons and myelin were immunostained for neurofilament H (NF-H) and myelin basic protein (MBP), respectively. To corroborate the data from the *in vitro* primary culture models, *ex vivo* organotypic brain slices were used to further assess changes to the oligodendroglia population and the integrity of white matter upon exposure to Aβ.Our preliminary results showcasing the effects of Aβ on cells of the oligodendroglia lineage and CNS white matter suggest that impaired myelin maintenance leading to demyelination may be more prominent in AD than previously thought. Hence, characterizing changes in white matter alterations due to AD-related pathogenesis beyond the normal aging process is key to furthering our understanding of AD pathology.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: conacyt national postgraduate scholarship 958097

Title: Effects of high glucose concentration on morphometabolic functions and generation of hyperphosphorylated Tau in the C6 astrocyte model

Authors: *K. A. HERNÁNDEZ CONTRERAS, M. E. HERNÁNDEZ-AGUILAR, D. HERRERA-COVARRUBIAS, F. ROJAS-DURÁN, G. E. ARANDA-ABREU;
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Abstract: Diabetes mellitus (DM) is a risk factor for the development of Alzheimer's disease (AD), with alterations in glucose metabolism and neuroinflammation being highlighted as part of this link. These functions are regulated by astrocytes in brain tissue. Morphological and functional modifications of activated astrocytes occur analogously in glioma derived C6 cells. In this study, we aimed to evaluate the effect of high glucose concentration on morphometabolic functions and generation of hyperphosphorylated Tau (pTau) in the C6 astrocyte model. We

seeded 25000cells/cm^2 (for morphological assessments) and 40000 cells/cm^2 in DMEM medium with a glucose concentration of 6mM (control) or 35mM (high glucose/HG) and incubated them under standard conditions for 72 hours. The trypan blue exclusion viability assay was performed. We evaluated the "incidence of spindle morphology", "soma/cell index" and "cell area" by taking phase contrast photomicrographs at 20x and using image J software to analyze them. We assessed cellular glucose uptake by measuring the residual glucose concentration (RGC) in the culture medium in two parallel assays, we exposed both groups to a glucose concentration of 6 mM (assay A) and 35mM (assay B) for 24 hours each assay. Mitochondrial activity, intracellular reactive oxygen species (ROS) generation and Tau protein (Tau) and pTau generation were assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay and dichloro-dihydro-fluorescein diacetate assay and ELISA assay respectively. Each assay was performed in triplicate. The results were analyzed with JASP software. Similar viability (100%) and proliferation ($p=0.063$) for both groups, lower incidence of spindle morphology in the HG group ($p<0.001$), similar soma/cell index ($p=0.1577$), higher mean cell area in the HG group ($p=0.011$), a similar RGC for both groups ($p=0.1391$) in assay A and a lower RGC in the HG group ($p=0.0240$) in assay B, in addition we observed a higher mitochondrial activity in the HG group ($p<0.001$), a higher intracellular ROS concentration in the HG group ($p=0.0289$), a similar Tau concentration in both groups ($p=0.220$) and a higher concentration of pTau in the HG group ($p=0.020$). Our findings suggest that exposure of astrocyte-like cells to HG during a subacute lapse leads to a partial modification of cell morphology linked to hypertrophy and a regulated increase in cellular uptake and mitochondrial glucose metabolism associated with an increase in intracellular ROS generation that we suggest is associated with alterations in Tau processing leading to an increase in pTau generation.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Support: NIA grant U54 AG054345
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Title: The role of INPP5D in tau pathology

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Abstract: Background Alzheimer's disease (AD) is the most common form of dementia characterized by extracellular deposits of beta-amyloid and neurofibrillary tangles composed of hyperphosphorylated Tau (pTau). Recently, large-scale genomic studies have highlighted microglial genes as significant risk factors for developing Late-onset AD (LOAD). Inositol polyphosphate-5-phosphatase-D (*INPP5D*) is associated with AD through regulation of the innate immune system and is thought to act as a break on microglia activation. We previously reported that elevated *INPP5D* expression is positively correlated with amyloid burden. However, the relationship of *INPP5D* in tau pathology remains unclear. In this study, we hypothesize that *INPP5D* inhibition will increases microglial function and alleviate tau pathology. **Methods** To assess the role of *INPP5D* in tau pathology, we performed fluorescence-resonance energy transfer (FRET)-based tau seeding assay on human LOAD brain-samples. Furthermore, we crossed *Inpp5d*-deficient (*Inpp5d*$^{+/-}$) mice with tau mice (PS19). Transcriptomic analysis was completed using the NanoString nCounter assay to determine the genetic profiles modulated by *Inpp5d* deficiency. Fluorescently labeled mutant Tau was utilized to measure tau internalization in primary microglia. **Results** Increased tau-seeding is positively correlated with *INPP5D* expression in LOAD-subjects. Increased *Inpp5d*expression in PS19 mice is positively correlated with pTau (S202/T205) levels. We observed an up-regulated immune- and cell migration-related genes in PS19:*Inpp5d*$^{+/-}$mice. *Inpp5d*-deficient primary microglia showed increased tau internalization compared to wild-type microglia. **Conclusion** We confirmed that elevated *INPP5D* expression in human LOAD subjects is recapitulated in PS19 mice, and this is positively correlated with tau pathology. Alterations in immune- and cell-migration-related pathways in PS19:*Inpp5d*$^{+/-}$mice suggest that *Inpp5d* modulates the disease-progression through these pathways. Furthermore, increased tau internalization due to reduced *Inpp5d* expression in microglia may attenuate tau pathology. These results suggest that therapeutic interventions aimed at tauopathies by inhibition of *INPP5D* may be beneficial.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 276.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association Grant AARGD-22-928829
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Title: Deep spatial phenotyping of microglia in post-mortem Alzheimer's Disease brain tissue

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Abstract: Recent high-plex single-cell transcriptomic analyses have shown remarkable heterogeneity of microglia across neurodegenerative disease conditions and progression stages. While single-cell RNA sequencing (scRNA-seq) has been instrumental in revealing microglia subpopulations, it lacks spatial information for each cell and does not provide enough insight into potential roles of inflammatory microglia in disease progression. The scarcity of fresh human brain tissue imposes an additional bottleneck for RNA-seq studies, whilst paraffin-embedded tissues are widely available. To this end, the PhenoCycler spatial biology platform permits deep single-cell phenotypic characterization of microglial cells in situ and in formalin-fixed paraffin-embedded tissues. The PhenoCycler experiment is highly-multiplexed, affords morphological acuity as well as an unprecedented ability to comprehensively phenotype diverse cell types, including immune cells in the cerebral vasculature and other residents of the brain parenchyma.
Using the PhenoCycler technology, we characterized microglial subpopulations and their spatial biology in relationship to pathogenic features associated with Alzheimer's Disease (AD). We imaged 8 post-mortem tissue samples with over 25 markers and performed custom segmentation based on nuclei or specified neuronal and/or glial markers. Our nuclei-based segmentation identified over 500,000 single cells, while our targeted membrane-based segmentation captured over 100,000 microglial cells. Via unsupervised clustering, we next identified multiple different microglial subtypes with unique expression patterns of 10 inflammatory and pro-inflammatory biomarkers. Our results show the existence of different microglial cell populations, whose presence varies between AD and control brains. With the added segmentation of Aβ plaques, we were able to identify specific microglial subpopulations that express blood-derived macrophage markers (CD163, CD11c) in parenchymal ramified cells close to Aβ plaques. We also observed that increased microglial/macrophage activation markers were correlated with the distance of the cell to the plaques.
This study is the first to comprehensively map the spatial biology of inflammatory microglia in post-mortem human brain tissues. Our observations will enhance our understanding of the complex roles that are played by microglial during the onset and progression of Alzheimer's disease.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant T32 NS007433-21
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Title: Role of Na/H exchanger isoform 1 in reactive astrocytes in human AD brains

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Abstract: Alzheimer's Disease (AD) pathogenesis includes remarkedly early development of astrogliopathology in the brain. Elevation of astrocytic marker protein, glial fibrillary acidic protein (GFAP), in plasma and cerebrospinal fluid (CSF) has been suggested as one of early biomarkers for AD diagnosis. Previous findings in our lab have shown that Na+/H+ exchanger isoform 1 (NHE1) expression and activation in reactive astrocytes contribute to neuroinflammation and cognitive function deficits in murine models of ischemic stroke and vascular stenosis. The underlying mechanisms involve coordinated NHE1-mediated H+ efflux in support of NADPH oxidase (NOX) signaling in production of reactive oxygen species (ROS) and cytokines. However, whether NHE1 protein plays a role in AD-associated reactive astrogliosis, and pathogenesis remains unknown. Using histochemistry and immunostaining assays in control and AD patient post-mortem brain tissues with and without other comorbidities, we first observed significantly increased expression of NHE1 protein in reactive astrocytes of AD brains, compared to controls. Elevation of NHE1 protein was detected in GFAP+ reactive astrocytes and ~70-80 % of GFAP+ reactive astrocytes expressed NHE1 protein in cortical, hippocampal and amygdala regions of the AD brains. In quantification of reactive astrocytes association with Amyloid-Beta (Aβ) in different AD brain regions, we found that reactive astrocytes are increased in brain regions with higher Aβ load, a well-known hallmark of AD. We are currently examining the relationship of NHE1 expression in GFAP+ reactive astrocytes and parenchymal Aβ plaque pathology. These findings will reveal whether NHE1 protein in reactive astrocytes plays a role in homeostatic dysfunction of astrocytes, neuroinflammation and AD pathogenesis.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Minciencias, the National Government for the management of the National System of Science, Technology, and Innovation (SNCTI) in Colombia, Project 111577757128

Title: Gliovascular alterations in frontal cortex characterize Alzheimer's disease and APOE3-Christchurch homozygote resistance

Authors: J. HENAO-RESTREPO[1,2], ***P. VALDERRAMA-CARMONA**[1,2], N. OROZCO-SANTA[1,2], C. LÓPEZ-MURILLO[1,2], J. GÓMEZ[3], J. GUTIÉRREZ-VARGAS[1,4], R. MORAGA[5], J. TOLEDO[5], J. L. LITTAU[6], S. HÄRTEL[5], J. ARBOLEDA-VELASQUEZ[7], D. SEPULVEDA-FALLA[8], F. LOPERA[3], G. CARDONA-GÓMEZ[2], A. VILLEGAS[3], R. POSADA-DUQUE[1,2]; [1]Inst. de Biología, Facultad de Ciencias Exactas y Naturales, [2]Área de Neurobiología Celular y Molecular, Grupo de Neurociencias de Antioquia, [3]Grupo de Neurociencias de Antioquia, Facultad de Medicina, SIU, Univ. de Antioquia, Medellin, Colombia; [4]Hlth. Sci. Fac., Remington Univ. Corp., Medellin, Colombia; [5]Anat. and Developmental Biology, Lab. of Scientific Image Analysis SCIAN-Lab, Univ. de Chile, Santiago, Chile; [6]Inst. of Neuropathology, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; [7]Massachusetts Eye and Ear Inst., Harvard Med. Sch., Boston, MA; [8]Mol. Neuropathology of Alzheimer's Disease, Inst. of Neuropathology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Astrocytes become reactive, promoting protection and tissue repair. However, astroglial reactivity is typical of brain pathologies, including Alzheimer's disease (AD). Considering the heterogeneity of the reactive response, the role of astrocytes in the course of different types of AD has been underestimated in humans. Interestingly, Colombia has the largest human group known to have familial AD (FAD). This group carries the autosomal dominant and fully penetrant mutation E280A in PSEN1, which causes early-onset AD. Recently, our group identified an E280A carrier who did not develop FAD. The individual was homozygous for the Christchurch mutation in APOE3 (APOEch). Since astrocytes are critical to brain health and disease, in this study, we characterized astrocyte reactivity and structural integrity of the gliovascular unit (GVU) that could underlie the pathogenesis or protection of AD in the postmortem human frontal cortex of SAD, FAD and APOEch. Through histological and 3D analyses, we show that astrocytes and the GVU are differentially altered in SAD and FAD. Outstandingly, APOEch displayed the mildest effects despite presenting the highest burden of amyloid β (Aβ). Specifically, we demonstrate that, while astrodegeneration is prominent in SAD, and FAD is characterized by reactive-like glia, astrocytes in APOEch resemble those of controls. We revealed that glutamate-regulatory astrocytic proteins are altered in the three conditions but to a lesser extent in APOEch. Although we showed modification of the gliovascular configuration and disturbance of endothelial-related proteins in AD, only SAD exhibited structural deterioration of the vessels. In contrast, none of these gliovascular changes were detected in APOEch. This study provides new insights into the potential relevance of astrocytes

and the GVU in the pathogenesis of and protection against AD, and it controverts the leading role of Aβ as a determinant of this disease.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 276.11

Title: WITHDRAWN

Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 276.12

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant 1RF1AG057247

Title: Understanding microglial function and morphology using a mouse model deficient in Alzheimer's disease associated PLCG2 gene

Authors: *H. STALEY, J. BOLES, O. URIARTE HUARTE, N. NEIGHBARGER, C. COLE, T. E. GOLDE, P. CHAKRABARTY, M. G. TANSEY;
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Abstract: Alzheimer’s disease (AD) is a neurodegenerative disease that can cause severe cognitive impairments including memory loss, changes in personality, and trouble speaking. Pathologically, it is characterized by the presence of beta-amyloid protein plaques in the brain, and tau tangles within neurons. In addition, many AD cases have been shown to exhibit significant neuroinflammation. What is poorly understood is whether this inflammation is causative of neurodegeneration, or just a physiological response to other dysfunctional processes. A big player implicated in the propagation of this neuroinflammation is an innate immune cell-type found in the brain called microglia, which not only release neuroinflammatory markers in response to various stimuli, but also perform phagocytosis. Recent studies have suggested that genetic mutations in microglia can cause significant changes in the progression and outcome of

AD. One particular example of this is a genetic mutation that changes a proline residue to an arginine in phospholipase C gamma 2 (PLCG2 P522R), which has been linked to slower rates of cognitive decline and reduced $pTau_{181}$ and tTau levels in cerebrospinal fluid. Understanding the mechanism behind this protective action could be important for determining future therapeutic targets, of which there are currently very few for the treatment of AD. To assess changes in microglial function caused by alterations in the PLCG2 gene NanoString Single Molecule Counting was used to compare inflammatory pathway signaling in PLCG2 knock out and PLCG2 wild type mice. In addition, microglial morphology and proliferation changes were assessed through immunohistochemical staining with Iba-1, and quantified using NearCYTE with regional specificity. Results indicated that alterations in the PLCG2 gene can cause changes to inflammatory pathway signaling, suggesting that PLCG2 may play a significant role in the propagation of inflammation by microglia. In addition, loss of PLCG2 caused a reduction in microglial proliferation and changes in cellular morphology. Studies to investigate how P522R affects microglia phenotypes are underway. Completion of these studies will shed light on the importance of PLCG2 in the regulation of essential microglial functions, and provide evidence to support a prominent role for PLCG2 in the neuroimmune cross-talk underlying age-related neurodegenerative diseases.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Program #/Poster #: 276.13

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH/NIA grant AG056114

Title: Alzheimer’S-associated plcg2 variant p522r ameliorates multiple disease-related phenotypes in an amyloid ad mouse model

Authors: X. GU[1], ***C. LEE**[1], P. LANGFELDER[1], A. J. DE LA ROCHA[2], Z. PAMONAG[1], L. RAMANATHAN[1], W. GE[3], A. BHADURI[3], X. YANG[1];
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Abstract: A rare P522R polymorphism in phospholipase C-γ2 (Plcg2) has been reported to associate with reduced risk against Alzheimer’s disease (AD). Plcg2 is highly expressed in microglia in the brain and is a key downstream component of TREM2 signaling. Hypermorphic mutations in PLCG2 in humans lead to inflammation and autoimmune disorders, suggesting a key role for this enzyme in the regulation of myeloid cell function. To study how the P522R variant elicits protective effects against AD, we developed a Plcg2-P522R knock-in (KI) mouse model using CRISPR/Cas9 gene editing. We found that Plcg2-P522R reduced multiple disease-

related phenotypes in the 5xFAD mice, including but not limited to amyloid deposition and dystrophic neurites. Importantly, the microglia derived Plcg2-P522R mice exhibited enhanced phagocytosis fibrillary Aβ. Our ongoing analysis of bulk cortical tissue and single-nuclei transcriptomic studies of 5xFAD/Plcg2-P522R mouse cohort will help to discern the molecular impact of Plcg2-P522R mutation in the disease-associated microglial gene signatures as well as similarity or differences from those modified by TREM2 loss-of-function or gene-dosage increase. Together, our study used a novel Plcg2-P522R knockin mouse model to reveal the preclinical benefit of this AD-protective variant in a mouse model of amyloid AD deposition, and hence provide further credence and readouts to pursue Plcg2 in AD therapy.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

Location: SDCC Halls B-H

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Program #/Poster #: 276.14

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Exploring autophagy activation as a means to improve microglial lipid metabolism in neurodegeneration

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Abstract: Alzheimer Disease (AD) affects approximately 6 million people in the United States and currently has no disease modifying therapies. AD is defined by the accumulation of amyloid β (Aβ) plaques and tau tangles in the brain and it is known that alterations in the activities of glia play important roles in shaping the disease course. Much of the polygenic risk for AD is derived from variants in genes expressed by microglia, specifically those involved in endolysosomal and lipid processing pathways, and microglia containing lipid droplets (LD-MG) have been observed in post-mortem human AD brains. As previous interventions targeting Aβ have not yet been successful in clinical trials, the concept of modulating microglial lipid metabolism is a novel and exciting avenue being explored in preclinical studies, however we need to know more about how lipid metabolism governs microglial functional states. In mouse models of amyloidosis, microglia transition from a homeostatic to a disease-associated transcriptional state that may represent a plaque-compacting phenotype. While microglial activity may be beneficial in the early amyloid phase of AD, pharmacological or genetic inhibition of microglia has been shown to be protective in mouse models of tauopathy. Given recent evidence suggesting that the LD-MG observed in aged mice have pro-inflammatory, hypo-phagocytic phenotypes, we hypothesize that the LD-MG in AD will have similar alterations and could thus be partially

responsible for microglial contributions to neurodegeneration. To begin evaluating the importance of microglial lipid accumulation on inflammatory phenotypes and disease progression, we have partnered with the lab of Dr. Jeffery Kelly at the Scripps Institute to screen a library of autophagy activating compounds that putatively function via mTOR-independent mechanisms. An initial compound screen using the immortalized BV2 mouse microglial cell line identified 10 compounds that promoted the clearance of at least 50% of accumulated neutral lipids. Subsequent validation experiments confirmed that many of these compounds are also effective at clearing neutral lipid deposits induced by myelin debris in mouse primary microglia. Ongoing studies are aimed at identifying the molecular targets of these novel compounds, the arms of lipid metabolism they engage, and the effects of lipid clearance on microglial inflammatory phenotypes in AD-relevant models. Our studies will both identify molecular targets amenable for promoting lipid flux in myeloid cells and evaluate the potential of modulating microglial lipid metabolism for the treatment of diseases marked by aberrant lipid metabolism like AD.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant RF1NS093652
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Title: Complement C3aR regulates microglial metabolism and response to amyloid beta via Hif-1 signaling

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Abstract: Alzheimer’s disease (AD) is characterized by deposition of amyloid beta (Aβ) derived from the amyloid precursor protein (APP), accumulation of neurofibrillary tangles containing hyper-phosphorylated tau protein and neuroinflammation driven by over activation of microglia and astrocytes. The complement pathway is a critical regulator of innate immunity and neuroinflammation. Microglia, a major contributor to neuroinflammation and a crucial player in AD pathogenesis, is the major cell type expressing complement C3a receptor (C3aR) in the central nervous system. Modulating microglial response through C3aR could be a potential

approach to target disease progression. Using a C3aR Td-tomato genetic reporter line, we identified two subpopulations of microglia, C3aR+ and C3aR-. Microglia expressing C3aR were significantly enriched surrounding amyloid-β plaques in an APP knock-in (APP-KI) mouse model of AD. Transcriptomic analysis of C3aR-positive microglia identified significant up regulation of hypoxia-inducible factor (HIF-1) signaling in APP-KI mice compared to wild-type controls. Using primary microglial cultures, we found that cells lacking C3aR have lower HIF-1α expression and higher ATP levels. These are associated with improved receptor recycling and phagocytic capabilities and higher resistance to hypoxia mimetic agent exposure. These results implicate that C3aR negatively regulates microglial metabolism through the HIF-1 signaling pathway. To validate these effects in vivo, we genetically knocked out C3ar1 in APP-KI mice and found that C3aR ablation improved microglial phagocytic and clustering abilities, reduced plaque load, and improved memory and learning. Taken together, our data indicates that complement C3aR regulates microglial metabolism and its response to Aβ pathology via HIF-1 signaling and that C3aR inhibition may be therapeutically beneficial against AD.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Title: Characterization of an Alzheimer’s disease-associated risk variant in progranulin indicates hyperactivation of microglia around β-amyloid plaques

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Abstract: Progranulin encoded by *GRN* is a lysosome-resident protein, which plays an important role in neurodegeneration. In the brain, progranulin is predominantly expressed in microglia, but also in neurons. Recently, it was shown that *GRN* rs5848 variant significantly associates with a higher risk of having Alzheimer's disease (AD). Here, we have utilized β-amyloid-positive brain biopsy samples and monocyte-derived microglial like (MDMi) cells obtained from idiopathic normal pressure hydrocephalus (iNPH) patients to study the effects of the *GRN* rs5848 variant on microglial and β-amyloid pathology as well as functions of MDMi cells upon myelin and lipopolysaccharide (LPS) treatments. MDMi cells were established from the peripheral blood monocytes of iNPH patients. For quantitative immunohistochemical (IHC) analyses, brain biopsy samples from the frontal cortex of shunt-operated iNPH patients were used. The quantitative IHC analysis showed significant clustering of the microglia around the β-amyloid plaques in iNPH patients, who were homozygous for the *GRN* rs5848 variant. Interestingly, the total β-amyloid plaque area was significantly reduced in iNPH patients with the homozygous *GRN* rs5848 variant as compared to iNPH patients without the risk variant. Importantly, MDMi cells with homozygous *GRN* rs5848 variant background showed an increased inflammatory cytokine response upon LPS treatment. Finally, bulk RNA sequencing data obtained from myelin-treated *GRN* rs5848 variant-carrying MDMi cells revealed differentially expressed genes that were enriched to the pathways relevant for MHCI- and MHCII-related antigen presentation, and interferon α, β and γ responses. These results suggest that hyperactivated microglia encompassing the *GRN* rs5848 variant background migrate to the sites of β-amyloid deposition to restrict plaque growth. Consistent with this idea, both RNA sequencing and inflammatory cytokine secretion point to an increased proinflammatory response in the cultured MDMi cells owing to the AD-associated *GRN* rs5848 risk variant.

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Poster

276. Alzheimer's Disease and Related Dementias: Glia

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Title: Effect of a fiber-enriched diet on neuroinflammation and cognition in Alzheimer's disease

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Abstract: Introduction: Alzheimer's disease (AD) is an age-related dementia. Recent studies have suggested cognitive improvements due to dietary soluble fiber intake. Fiber decreases astrogliosis (neuroinflammation), improving cognition through microbial products, like butyrate. However, there is no sufficient data about the potential action mechanism of soluble fiber on astrocytes activation. **Methods**: Four months male wild type (WT) and APP/PS1 transgenic (Tg) mice were fed for 2 months with control diet (AIN-93), AIN-93 + 5% fructans (soluble fiber, 5%-F), or 5%-F + an antibiotic cocktail (ampicillin [1 g/l], neomycin [1 g/l], metronidazole [1 g/l], and vancomycin [0.5 g/l], diluted in drinking water [5%-F+Abx]). Spatial, working, and recognition memory were assessed by water maze (WM), T-maze (TM), and novel object recognition (NOR) paradigm respectively. Anxiety was evaluated by elevated plus maze (EPM). Brain was extracted and astrogliosis was assessed by immunofluorescence (IF) against glial fibrillary acidic protein (GFAP). Butyrate was quantified in feces by chromatography. **Results**: Soluble fiber-enriched diet decreased anxiety and improved working, space, and recognition memory scores compared to Tg AIN-93 fed animals [ANOVA, $p<0.05$]. Interestingly, Tg 5%-F fed animals showed an increased concentration of fecal butyrate [ANOVA, $p<0.0001$], and a decreased neuroinflammatory process [ANOVA, $p<0.001$] in comparison to Tg AIN-93 fed animals. The effects of fiber disappear with the use of antibiotics. **Conclusion**: We demonstrated that ingestion of soluble fiber (i.e. fructans) effectively increases the concentration of butyrate, reduces neuroinflammation, and restores cognitive scores to control values in an AD mice model. This work was financed by Consejo Nacional de Ciencia y Tecnología, CONACyT-163235 INFR-2011-01 to J.G.-M. and Consejo Nacional de Ciencia y Tecnología CONACYT A1S-42600 to C.P.-C.

Disclosures: D. Cuervo-Zanatta: None. **J. García-Mena:** None. **C. Perez-Cruz:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG051266
NIH Grant AG059695
NIH Grant AG065651
Bright Focus Foundation Grant A2015275S
Alzheimer's Research UK research fellowship

Title: Deposition of Aβ4-x proteoforms in Alzheimer's plaques and cerebrovascular deposits is further promoted by their enhanced brain retention properties

Authors: *J. GHISO[1], E. CABRERA[1], T. LASHLEY[2], A. ROSTAGNO[1];
[1]New York Univ. Grossman Sch. of Med., New York, NY; [2]Univ. Col. of London, London, United Kingdom

Abstract: The molecular heterogeneity of Alzheimer's Aβ deposits is significantly more complex than originally anticipated, extending well beyond the classic Aβ1-40/Aβ1-42 dichotomy and being substantially expanded by the presence of multiple post-translational modifications that exponentially increase the proteome diversity. Although N-terminal truncations at Glu 3 and Glu 11 and their subsequent cyclation to pyroglutamate are perhaps the modifications most extensively studied, many others have been reported, among them peptides starting at Ala 2, Phe 4, Arg 5, and Leu 17 of Aβ. Species beginning at Phe 4 and bearing an intact C-terminus are especially relevant. This truncation favors the formation of a poorly soluble, β-sheet-rich, and aggregation-prone peptides with high amyloidogenic propensity, requiring formic acid for extraction from brain deposits. Indeed, Aβ4-42 proteoforms have been reported as a major component of amyloid plaque cores and the limited studies available seem to indicate their relative abundance in patients with AD, Down's syndrome, and vascular dementia. To investigate the brain clearance characteristics of Aβ4-x species in comparison with full-length Aβ40 and Aβ42, synthetic homologous were biochemically and biophysically characterized, radioiodinated at Tyr 10 and utilized in *in vivo* brain clearance experiments in 5-6-week-old C57Bl/6 mice. Brain efflux of monomeric forms of all Aβ species tested was fast, with no significant differences in brain retention within the 60 min duration of the experiment, whereas retention of oligomeric preparations was consistently higher than that of their monomeric counterparts. Remarkably, retention of Aβ4-x truncated species was significantly greater than that of the full-length peptides, highlighting the importance of truncated species with high oligomerization capacity as central pathogenic elements in AD. An in-house-generated anti-Aβ4-x monoclonal 18H6 - specific for Aβ species starting at position 4 and blind for the full-length Aβ40 and Aβ42 - used in double and triple immunohistochemical analysis in combination with thioflavin S and antibodies specific for Aβ40 and Aβ42 C-termini. These studies confirmed the presence of Aβ4-x in thioflavin positive parenchymal plaques, and cerebrovascular deposits, in agreement with their amyloidogenic properties and poor clearance characteristics. Since the process of multimerization is concentration dependent, failing to remove these oligomeric forms of Aβ from the brain in likely to further exacerbate the formation of fibrillar deposits, self-perpetuating the amyloidogenic loop and worsen amyloid-mediated pathogenic pathways

Disclosures: J. Ghiso: None. **E. Cabrera:** None. **T. Lashley:** None. **A. Rostagno:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant 1RF1AG077570
NIH grant 1R01NS096151
NIH grant R21AG067445

Title: Chronic sleep deprivation increases CSF PDGFRb level

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Abstract: Pericytes, a primary component of the neurovascular unit (NVU), play a crucial role in keeping brain homeostasis through several mechanisms including regulating cerebral blood flow (CBF). Since the CBF volume fluctuations are modulated by sleep, we hypothesize that pericytes might be also directly modulated by sleep and sleep deprivation (SD) may cause damage to pericytes. Platelet-derived growth factor receptor beta (PDGFRb) is mainly expressed in brain pericytes and plays a critical role in pericyte functions. Shedding of soluble PDGFRb (sPDGFRb) into CSF is a sensitive marker of pericyte damage. sPDGFRb was measured by sandwich ELISA in mice CSF underwent either acute SD (ASD, 4h SD between ZT0-ZT4) or chronic SD (CSD, 4h/day, ZT0-ZT4, for 10 consecutive days). To test if sleep enrichment will protect pericytes, sPDGFRb was measured in narcoleptic APP/PS1 mice and age-matched non-narcoleptic APP/PS1 mice. The results showed that one-time ASD did not significantly change CSF sPDGFRb level (2806±522 pg/ml in the control group versus 2229±374 pg/ml in the ASD group), while CSD increased the CSF sPDGFRb level significantly (7055±1717 pg/ml in the CSD group). However, 2-week recovery after CSD returned the sPDGFRb level back to normal level (2707±592 pg/ml). Compared to the non-narcoleptic APP/PS1 mice (5701±1103 pg/ml), age-matched narcoleptic APP/PA1 mice have a significantly reduced sPDGFRb level (3201±591 pg/ml). These results demonstrated that chronic prolonged waking can cause pericyte damages which are reversible after recovery. Narcolepsy reduces the pericyte damage in Alzheimer's disease (AD) mice model. More studies are needed to further test whether the protective effects of sleep on AZ are or fully, or partially mediated by the protection of brain pericytes.

Disclosures: P. Li: None. **M. Liu:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Cerebrovascular MCT1 is unchanged in Alzheimer's Disease, whereas GLUT1 is decrease

Authors: C. TREMBLAY[1], M. LECLERC[1,2], P. BOURASSA[1,2], D. A. BENNETT[3], F. CALON[1,2];
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Abstract: The brain is a highly demanding organ, involving glucose and ketones as main sources of energy. GLUT1 and MCT1 respectively transport glucose and ketones across the blood-brain barrier. While reduced glucose uptake by the brain is one on the earliest signs of Alzheimer's disease, changes in the uptake of ketone bodies have not been evidenced yet. Here, to probe for changes in GLUT1 and MCT1, we performed Western immunoblotting in microvessel extracts from the parietal cortex of 60 participants of the Religious Orders study. Participants clinically diagnosed with AD had lower cerebrovascular levels of GLUT1, whereas MCT1 remained unchanged. GLUT1 reduction was associated with lower cognitive scores for global cognition, episodic and semantic memories, and perceptual speed. No such association were found for MCT1. Cerebrovascular levels of both transporters were not significantly associated with age of death, Aβ or tau pathologies. These results suggest that, while a deficit in GLUT1 located on the blood-brain barrier may underlie the failed transport of glucose to the brain in AD, such a deficit was not observed for MCT1. These results support the use of ketones bodies as an alternative energy source for the aging brain.

Disclosures: C. Tremblay: None. **M. Leclerc:** None. **P. Bourassa:** None. **D.A. Bennett:** None. **F. Calon:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA R01 AG060238
NIA R01 AG062251
NIA RF1 AG033570
AHA 636269

Title: Depletion of endothelial specific Caveolin-1 induces brain insulin impairments in type 2 diabetes: Implications for Alzheimer's disease

Authors: *A. SHETTI[1], A. RAMAKRISHNAN[1], L. ROMANOVA[5], W. LI[2], K. VO[1], I. VOLETI[1], I. RATNAYAKE[3], R. MINSHALL[4], S. COLOGNA[2], O. LAZAROV[1];
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Abstract: Type 2 diabetes Mellitus (T2DM) is a major risk factor for the development of Late onset Alzheimer's disease (LOAD). Impairments in Insulin signaling, and vascular dysfunction are thought to be involved in the development of LOAD in T2DM, but a potential link connecting these pathologies is currently unknown. Here we show that the levels of endothelial enriched protein caveolin-1 (Cav-1) is depleted in the brain microvessels of db/db ($Lepr^{db}$) diabetic mice. This reduction is correlated with reduced insulin receptors expression and altered

insulin signaling in brain microvessels as well as parenchymal cells of db/db mice. Furthermore, impairments in insulin signaling are observed in the brains of endothelial- specific Cav-1 knock out mice, suggesting a role of endothelial Cav-1 in regulating brain microvascular and parenchymal insulin signaling. By utilizing brain endothelial cell cultures, we show that loss of Cav-1 leads to reduced insulin uptake and signaling. Importantly, we show that Cav-1 interacts with insulin receptor-β and its loss destabilizes insulin receptor localization within the plasma membrane lipid rafts. Interestingly, restoration of Cav-1 expression in endothelial cells using Adeno Associated Virus (AAV) expressing Cav-1, rescues impairments in insulin receptors. Overall, our study shows that Cav-1 is essential for the regulation of insulin signaling in the cerebrovasculature and that loss of Cav-1 can compromise insulin metabolism, leading to brain pathology. This study unravels a novel mechanism that may underlie T2DM- induced Alzheimer's disease.

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Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: JPMJMS2024

Title: Effect of carbon monoxide on pericytes and oligodendrocytes in chronic cerebral hypoperfusion

Authors: *K. YASUDA, T. MAKI, R. TAKAHASHI;
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Abstract: [Background] Subcortical ischemic vascular dementia (SIVD) is the most common subtype of vascular dementia. Pericytes in neurovascular unit are a key effector for the regulation of blood-brain-barrier integrity (BBB) and cerebral blood flow. Decreased pericyte coverage with BBB disruption is reported to precede white matter lesions with oligodendrocyte (OL) loss after chronic cerebral hypoperfusion in a mouse model of SIVD. Previous reports have also shown that ischemic pericytes suppress OL differentiation, while normal pericytes support OLs. Carbon monoxide (CO) at low concentration has multiple beneficial effects for the neurovascular unit impairment, such as the trauma-induced loss of pericytes and neuronal cells. However, whether carbon monoxide treatment can rescue the damaged pericytes and oligodendrocytes in SIVD remains to be investigated. [Aim] The purpose of present study is to examine the therapeutic effects of CO on disrupted pericytes and oligodendrocytes in a mouse model of SIVD. [Methods] We used low concentrations of CO-releasing molecule (CORM)-3 for *in vitro*

and *in vivo* experiments. For *in vitro* experiments, we examined the effects of CORM-3 on mouse pericytes under ischemic conditions. WST/LDH assay and western blot analysis (WB) were performed to assess the viability and phenotype of pericytes, respectively. For *in vivo* experiments, we used a mouse model of prolonged cerebral hypoperfusion and white matter ischemic lesions generated by bilateral common carotid arteries stenosis (BCAS). CORM-3 was administered retro-orbitally to the BCAS-operated mice on day1 after surgery. WB and immunohistochemistry were performed on day28 after BCAS. [Results] *In vitro* studies demonstrated that CORM-3 rescued the ischemia-induced cytotoxicity of pericytes in a dose-dependent manner, as evaluated by WST/LDH assay. WB analysis revealed that CORM-3 suppressed the ischemia-induced increase in the expressions of Hif1-α, BMP4 and MMP-9 in pericytes. WB analysis of *in vivo* studies revealed that CORM-3 treatment significantly ameliorated the decreased expression levels of myelin basic protein (OL marker) in the BCAS-operated mice. As in the *in vitro* studies, CORM-3 treatment attenuated the expression levels of Hif1-α, BMP4 and MMP-9 in the BCAS-operated mice. [Conclusions] The present study indicated that CORM-3 rescued the loss and dysfunction of pericytes and oligodendrocytes under ischemic conditions. Carbon monoxide treatment may provide a therapeutic approach for SIVD by mitigating pericyte and OL damages.

Disclosures: K. Yasuda: None. **T. Maki:** None. **R. Takahashi:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.06

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Fibrillar tau elicits metabolic reprogramming in endothelial cells and cerebrovascular dysfunction

Authors: *R. A. GUZMAN-HERNANDEZ[1], S. FOSSATI[2];
[1]Neural Sci., Temple Univ., Philadelphia, PA; [2]Neural Sci., Temple Univ., Phiadelphia, PA

Abstract: Alzheimer’s Disease, the most common form of dementia, is characterized by the accumulation of amyloid-beta plaques and neurofibrillary tangles formed by hyperphosphorylated tau in the brain, leading to neuronal loss and cognitive decline. The neurovascular unit, a complex system of several cell types surrounding brain vessels, is extremely important for blood flow regulation, clearance of amyloid and tau, and proper brain function. Tau aggregates are mostly produced by neurons and glial cells and can reach endothelial cells at the vessel walls through tau spreading at astrocytic end-feet and perivascular spaces. Therefore, tau aggregates may exacerbate cerebrovascular dysfunction, causing blood brain barrier (BBB) permeability, endothelial cell stress and death. However, the mechanisms responsible for the effects of tau on endothelial cells and the impact of tau aggregates on mitochondrial microvascular dysfunction remain to be understood. Mitochondrial dysfunction is

a key pathological feature in cell stress and death pathways; for instance, mitochondria participate in the apoptotic pathway by releasing Cytochrome C and ROS, and ATP production is necessary for the cell to undergo programmed cell death. However, the effects of tau on endothelial mitochondrial processes and eventual dysfunction, as well as the contribution of tauopathy to vascular dysfunction, remain to be elucidated. We hypothesize that aggregated tau fibrils cause mitochondrial dysfunction and alterations in bioenergetics in endothelial cells, resulting in cell death and BBB permeability. We challenged immortalized human brain microvascular endothelial cells (D3) with 5nM, 10nM and 25nM aggregated 1N4R tau. These cells exhibited a reduction in trans-endothelial electrical resistance, measured through the ECIS Zθ system as an *in vitro* model of the BBB. After 72 hours treatment, 25nM tau induced a significant increase in apoptotic cell death, as well as secondary necrosis, yet no significant changes in cell death were observed at 24 or 48 hours. Extracellular flux analysis (Agilent Seahorse) of D3 cells demonstrated bioenergetic alterations starting at 24 hours of treatment. Aggregated tau (25nM) caused an increase in glycolysis at 24 hours, which reverted at 48 and 72 hours, where mitochondrial ATP production increased. These results suggest that a metabolic reprogramming caused by aggregated tau in endothelial cells may contribute to mitochondrial deregulation, possibly leading to EC inflammatory activation, apoptosis, and BBB dysfunction. This study will help providing insights into the molecular processes surrounding neurodegeneration in the neurovascular unit.

Disclosures: R.A. Guzman-Hernandez: None. **S. Fossati:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.07

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: CONACYT, UJAT-CB-2015-257849

Title: Metformin modifies the expression of LRP-1 and RAGE transporters in the hippocampus of rats with metabolic syndrome.

Authors: *F. HERNANDEZ-LANDERO[1], E. MARTINEZ-ABUNDIS[1], E. DE LA CRUZ HERNANDEZ[2], N. GOMEZ-CRISOSTOMO[3];
[1]Univ. Juarez Autonoma De Tabasco, Comalcalco, Mexico; [2]UNIVERSIDAD JUAREZ AUTONOMA DE TABASCO, COMALCALCO, Mexico; [3]UNIVERSIDAD JUAREZ AUTONOMA DE TABASCO (DIVISION A, Comalcalco, Mexico

Abstract: Metabolic syndrome (MS) is the main cause of hyperglycemia, promoting damage to microvascular structures that modify the blood-brain barrier (BBB) permeability and function. MS is associated with either an increase in the accumulation of amyloid (Aβ) in patients with cognitive impairment and alterations in the expression of RAGE and LRP-1 transporters located

in the BBB. Receptor for advanced glycation end products (RAGE) leads Aβ efflux from the brain, whereas low-density lipoprotein receptor-related protein-1 (LRP-1) transports Aβ in the opposite direction. Hyperglycemia can alter the expression of these receptors in BBB; furthermore, reversing this condition may return the receptors to normal levels. Metformin has protective vascular and neuronal effects, preventing oxidative stress and inflammation and improving vascular perfusion. Clinical reports demonstrate a slow down of neurodegeneration and improved patients' cognitive abilities after metformin consumption. This work aims to evaluate the effect of metformin on RAGE and LRP-1 levels in a murine model of MS. Weanling male Wistar rats were fed with hypercaloric diets (high sucrose, HSD or high fat, HFD) for 12 months to develop MS. Subsequently, metformin was administered by gastric cannula for five weeks at 100mg/kg/day. At the end of treatment, body weight, blood pressure, and spatial memory were determined. The animals were sacrificed, and body fat percentage, glucose, and triglyceride levels were measured. The hippocampus was dissected to quantify RAGE and LRP-1 levels by western blot. The results show that after 12 months of consumption of HSD or HFD, the rats developed MS (obesity, hyperglycemia, and hypertension). After treatment with metformin, the body weight and blood glucose levels decreased. As a result of 12 months of HSD and HFD consumption, RAGE levels increased, whereas LRP-1 decreased; interestingly, metformin administration reverses these changes in the HSD group. Finally, the treatment with metformin improved spatial memory. In conclusion, metformin reversed the metabolic alterations and the changes in RAGE and LRP-1 levels in MS rats. These changes could explain the improvement in spatial memory by favoring the clearance of Aβ in the CNS.

Disclosures: F. Hernandez-landero: None. **E. Martinez-Abundis:** None. **E. De la cruz hernandez:** None. **N. Gomez-Crisostomo:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.08

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Open Philanthropy

Title: Dysregulation of the gut microbiome contributes to compromised blood-brain barrier integrity in ApoE4 transgenic mice

Authors: *M. ZHANG, M. T. HUUSKONEN, A. P. SAGARE, A. CHAKHOYAN, K. KISLER, A. R. NELSON, Y. WANG, B. V. ZLOKOVIC;
Zilkha Neurogenetic Inst., Keck Sch. of Med. of USC, Los Angeles, CA

Abstract: Dysregulation of the gut microbiome contributes to compromised blood-brain barrier integrity in *ApoE4 transgenic* mice.
Zhang M, Huuskonen MT, Sagare AP, Chakhoyan A, Kisler K, Nelson A, Wang Y, Zlokovic

BV
[1]Zilkha Neurogenetic Institute, Department of Physiology and Neuroscience, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA
Recent studies suggest that brain vascular dysfunction contributes to cognitive impairment and Alzheimer's disease (AD). *APOE4* is a major genetic risk factor for AD. *APOE4* exacerbates blood-brain barrier (BBB) breakdown and degeneration of brain vascular pericytes resulting in dysregulation of cerebral blood flow and increased amyloid deposition, thereby affecting neuronal function. In addition to genetic risk factors, emerging evidence suggests that the gut microbiome is closely related to aging-associated neurodegenerative diseases including AD. However, it remains unclear whether the gut microbiome impacts BBB integrity. We investigated the difference in gut bacterial taxa in *ApoE3* and *ApoE4* transgenic mice and its contribution to the BBB permeability by treating animals with a cocktail of antibiotics amoxicillin-clavulanic acid through drinking water for two weeks. We found that the antibiotic-induced alteration in the gut microbiome restored BBB integrity in *ApoE4* mice as analyzed by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and accumulation of pericapillary fibrinogen deposits. Further analysis using shotgun metagenomics of fecal pellets from before and after antibiotic treatment and analyses of inflammatory cytokines in serum revealed disrupted balance between 3 major families including Bacteroidaceae from two dominant phyla Bacteroidetes and Firmicutes that may contribute to the compromised BBB integrity through elevated levels of pro-inflammatory cytokine TNF-alpha in blood in *ApoE4* transgenic mice. These preliminary findings suggest that differences in gut microbiome between *ApoE3* and *ApoE4* transgenic mice may influence BBB permeability and that targeting TNF-alpha might reduce proinflammatory activity of previously shown BBB-degrading cyclophilin A-matrix metalloproteinase-9 pathway in pericytes which may help restore *APOE4*-mediated BBB disruption in mice.
This work is supported by the Open Philanthropy grant to B.V.Z.
Theme: Theme C: Neurodegenerative Disorders and InjuryCategory: C.02. Alzheimer’s disease and other dementiasSection: C.02.e. Vasculature, BBB, and AD

Disclosures: **M. Zhang:** None. **M.T. Huuskonen:** None. **A.P. Sagare:** None. **A. Chakhoyan:** None. **K. Kisler:** None. **A.R. Nelson:** None. **Y. Wang:** None. **B.V. Zlokovic:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.09

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH P20GM121307
Malcolm Feist Postdoctoral Fellowship (CCDS)

Title: Neurogranin regulates blood-brain barrier integrity and amyloid-β clearance

Authors: J. PARK[1], S. ALEXANDER[2], K. STOKES[2], *H. NAM[1];
[1]Pharmacology, Toxicology, and Neurosci., [2]Mol. Cell. Physiol., LSU Hlth. Sci. Center-Shreveport, Shreveport, LA

Abstract: Blood-brain barrier (BBB) disruption promotes amyloid-β (Aβ) accumulation in the neurovasculature that contributes to the pathophysiology of Alzheimer's disease (AD). Clinical researchers discovered a reduction of neurogranin (Ng) expression in postmortem brains of AD patients. Although Ng is predominantly expressed in the neuron, we previously reported that Ng is also expressed in the endothelial cells that contribute to the endothelial activation process. Thus, we hypothesize that Ng is expressed in human cortical microvessel endothelial cells (hCMEC/D3) and that depletion of Ng impacts BBB breakdown by inducing endothelial activation. To test this hypothesis, we generated Ng knockdown in hCMEC/D3 and analyzed Ng-mediated molecular signaling and cellular permeability change. In this study, we found that Ng is expressed in the hCMEC/D3 and that Ng knockdown decreased AKT and mTOR activity and increased VCAM-1 expression indicating endothelial dysfunction. Then, we measured how the lack of Ng alters physiological responses in the BBB. Ng knockdown cells show a decreased transendothelial electrical resistance (TEER) response, indicating that lack of Ng disrupts BBB integrity. Moreover, we observed increased Aβ permeability in Ng knockdown cells compared to mock control cells. Overall, we are the first study to explore how Ng expression in the BBB plays an essential role in neurovasculature and BBB integrity. Our future in vivo study will support the role of Ng in BBB integrity and the Aβ clearance mechanism related to AD. Our study on the Ng mechanism will provide a better understanding of the vascular hypothesis of AD pathophysiology.

Disclosures: J. Park: None. **S. Alexander:** None. **K. Stokes:** None. **H. Nam:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Do APOE genotype and amyloid-beta interact in the emergence of AD pathology?

Authors: *S. ANDERLE, K. SHAW, J. HENDERSON, H. TREWHITT, L. MCMULLAN, C. N. HALL;
Univ. of Sussex, Brighton, United Kingdom

Abstract: Neuronal function is possible thanks to nutrients and oxygen delivered via the blood flow in a process called neurovascular coupling. However, changes in the brain's energy supply may predispose to Alzheimer's disease (AD) as cardiovascular risk factors increase AD risk, decreases in cerebral blood flow are observed before beta amyloid accumulation and cognitive decline, and beta amyloid accumulation may be promoted by hypoxia. The APOE4 gene is a risk

factor for AD and a cardiovascular risk factor, suggesting it may promote AD via effects on the vasculature. To date no experiments have been able to test whether APOE4-mediated vascular deficits really do promote Aβ formation, one of the hallmarks of AD, mostly due to a lack of appropriate models and experimental designs to study the effects of ApoE and Aβ. To test the interacting effect of APOE and Aβ on neurovascular function, we created a mouse model containing human APOE3 or APOE4 genes and APPSwe/Ind under the control of a tet-Off promoter. Aβ production is suppressed when doxycycline (dox) is administered in chow and can be triggered by removing doxycycline from the diet. We performed a cranial window surgery over V1 or CA1 to allow us to visualise neurons and vasculature in the mouse brain using two-photon microscopy and record haemodynamic measures using Laser Doppler Flowmetry and haemoglobin spectrometry. *In vivo* recordings were done prior to Aβ production, then at 4-weeks and 12+-weeks after dox diet removal. To see if Aβ accumulated around blood vessels, we injected the mice with Methoxy-X04 prior to each imaging session. We performed Barnes Maze, Novel Object Recognition and Y-maze tests at baseline, 6-8w off dox and 15-17w off dox to test for cognitive differences. Mice were perfused with FITC-gelatine at Baseline, 6w off dox and 17w off dox. We performed immunohistochemistry for Aβ, hypoxia and inflammation. Net haemodynamic measures at baseline showed that over time there is a gradual decrease in the average levels of blood oxygen saturation (SO2). We also found that the percentage of responsive vessels decreases over time, indicating that the lower SO2 levels might be a consequence of the reduced ability of vessels to respond to neuronal activation. On the Barnes maze test, APOE3 and APOE4 mice showed similar performance at baseline, suggesting the absence of a genotype effect on cognition. However, at 6-8 weeks off dox APOE4 mice showed longer latency times than APOE3 mice, suggesting impaired cognition due to Aβ accumulation in the APOE4 mice. Data collection and analysis is still ongoing.

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Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.11

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Prmt4-mediated preconditioning through adenosine in alzheimer’s disease

Authors: *M. S. B. UDO[1], V. TESIC[1], C. T. CITADIN[2], C. H. ACOSTA[2], G. A. CLEMONS[2], W. C. CARR[1], C. Y. C. WU[1], R. H. C. LEE[1], H. LIN[1];
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Abstract: Alzheimer’s disease (AD) is one of the most common and progressive neurodegenerative disorders in the US covering about 70% of all cases of dementia. Women are more likely to develop a rapid progression of AD than men when predictive factors such as

enhanced stroke severity are considered. Derangements in cerebral blood flow have been recently observed alongside tau and β-amyloid accumulation in AD physiopathology. Currently, there is no effective treatment available to prevent, attenuate, or reverse the disease, they only treat the symptoms; therefore, innovative therapies are needed in the field. Our approach for this study was derived from lessons learned from ischemic preconditioning. Therefore, our research led to the investigation of using adenosine (a known preconditioning agent) and its' associated (A2AR) pathways as a preconditioning agent to prevent age-related AD development/progression. Adenosine is an endogenous mediator involved in cerebral vascular tone regulation, a promoter of neurovascular coupling to enhance overall brain perfusion and neuronal activity. Attenuation of neurovascular coupling is associated with the progression of AD with patients having an increased expression of A2AR under low oxygen tension. We believe adenosine control involves the protein arginine methyltransferase 4 (PRMT4), part of a novel enzyme family that can play a role in neurovascular function; and is specifically involved in the methylation of arginine residues, which is a post-translational modification involved in mRNA splicing, DNA repair, signal transduction, protein interaction, and transport. We have evidence to suggest that adenosine (and its' associated pathways)and PRMT4 are both enhanced in the 3xTg-AD mice being related to neurovascular coupling and brain hypoperfusion in AD pathology. These results suggest that adenosine may be used as a preconditioning agent against AD development and/or progression, controlled by PRMT4.

Disclosures: M.S.B. Udo: None. **V. Tesic:** None. **C.T. Citadin:** None. **C.H. Acosta:** None. **G.A. Clemons:** None. **W.C. Carr:** None. **C.Y.C. Wu:** None. **R.H.C. Lee:** None. **H. Lin:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Canadian Institutes of Health and Research (CIHR) no: 10021444

Title: Brain levels of Angiotensin-Converting Enzyme 2 (ACE2) are associated with a neuropathological diagnosis of AD and with cognitive decline.

Authors: *L. REVERET[1,2], M. LECLERC[1,2], V. ÉMOND[1], A. LOISELLE[1], P. BOURASSA[1,2], C. TREMBLAY[1], D. A. BENNETT[4], S. S. HÉBERT[1,3], F. CALON[1,2]; [1]Ctr. de recherche Univ. Laval, Ctr. de recherche Univ. Laval, Québec, QC, Canada; [2]Pharm., [3]Med., Univ. Laval, Quebec, QC, Canada; [4]Rush Univ. Med. Ctr., Rush Univ. Med. Ctr., Chicago, IL

Abstract: The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a major cause of death, particularly in the elderly. The geriatric population in which cognitive

decline due to Alzheimer's disease (AD) is frequent was disproportionately affected by the pandemic. In addition, central nervous system (CNS) manifestations have been reported in a significant subset of SARS-CoV-2 infected patients. Since the principal entry receptor utilized by SARS-COV-2 is Angiotensin-Converting Enzyme 2 (ACE2), we examined whether *postmortem* ACE2 protein and mRNA levels were altered in parietal cortex samples from two different AD cohorts, totalling 142 cases. Both immunoblot and RT-qPCR analysis revealed higher concentrations of ACE2 protein and mRNA in persons with a neuropathological diagnosis of AD, compared to age-matched controls. Brain levels of ACE2 were inversely correlated with *antemortem* cognitive scores. We found that ACE2 protein was highly enriched in microvessels of mice compared to brain parenchyma, but not in humans. These observations were confirmed with immunostaining in both species. Levels of ACE2 in the soluble fractions were negatively correlated with blood-brain barrier markers and positively with β-amyloid pathology. Our data suggest that higher levels of ACE2 in the brain might contribute to AD pathology and to the increased risk of CNS SARS-CoV-2 infection in cognitively impaired individuals.

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Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: 1R21AG067613-01

Title: Vascular and Water Content Alterations in the Novel Human Amyloid-Beta Knock-In (hABKI) Mouse Model

Authors: *R. PAD, A. JULLIENNE, B. NOARBE, A. OBENAUS;
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Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disease that targets brain regions such as the hippocampus and cerebral cortex essential for cognitive and behavioral functions. In the late-onset or sporadic form of AD, aggregation of sticky and misfolded cerebral amyloid-beta (AB) plaques can induce an inflammatory response and cause neuronal degeneration, potentially deposit in blood vessels, reduce blood flow, and lead to a modified vasculature. While most prevalent AD mouse models are designed to recapitulate familial AD (such as 5xFAD), few studies have focused on sporadic AD which accounts for 95% of AD cases. In this study, we used genetically altered human AB knock-in (hABKI) mouse model that mimics sporadic AD at ages 4, 12, and 18 months. We performed high-resolution T2-weighted magnetic resonance imaging (MRI) to assess brain water content. We then undertook

morphological alterations of cortical vessels across sex and age in hABKI compared to wildtype (WT) mice. We hypothesized a progressive age-related reduction in whole-brain water content that would be accompanied by decrements in vascular density and complexity in the hABKI mice. Our study reports that both hABKI and WT mice exhibited a progressive decline in brain water content with advancing age, especially in male mice. In hABKI mice, a global decrease in vessel density and complexity was observed with age that was predominantly evident in female mice. These studies in relevant mouse models of AD can inform the physiological alterations that are progressive in human AD.

Disclosures: R. Pad: None. **A. Jullienne:** None. **B. Noarbe:** None. **A. Obenaus:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

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Program #/Poster #: 277.14

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH 1F31NS116926
NIH R01NS126091
EVMS Ryan Translational Research Fund F20-20

Title: Distinct pericyte subsets differentially contribute to amyloid beta loads in Alzheimer’s Disease

Authors: *D. G. BOHANNON, D. LONG, L. L. WELLMAN, W.-K. KIM;
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Abstract: Alzheimer’s disease (AD) is the most common form of dementia and is pathologically characterized by β-amyloid (Aβ) plaque formation and vascular dysfunction. Recent studies have shown that the blood-brain barrier (BBB) and brain vascular cells, pericytes (PCs) in particular - may play a direct role in both Aβ buildup and changes in neurovascular function. Our group identified a traditional smooth muscle actin (SMA) negative subset of PCs, type-1 PC (PC1), and a disease-associated, SMA positive PC subset, type-2 PC (PC2) and sought to determine whether they differentially interact with Aβ in AD. Using brain tissues from human AD patients and cognitively normal aged subjects, human brain microvascular pericyte cultures, and wild-type mice injected intracerebroventricularly with either Aβ40 or Aβ42, we investigated the potential roles for PC1 and PC2 in Aβ regulation and AD. Our initial findings demonstrated that patents with a high percent of total pericytes which are PC2 (%PC2) are associated with less BBB-breakdown, less dense Aβ42 plaques, less PC death, and higher tissue Aβ40:Aβ42 ratios. In an attempt to identify the mechanism by which PC2 may reduce Aβ-related AD pathologies, we sought to identify the independent role of PC1 and PC2 on Aβ40 and Aβ42 in vitro and in an in vivo mouse model. Our findings suggest that PC1 and PC2 employ different mechanisms to regulate Aβ levels and alter the phenotype and progression of disease.

Collectively, our findings shed light on some of the discrepancies in the field concerning the role of pericytes in AD. By differentiating PCs into functionally distinct subtypes, we have been able to demonstrate clear differences between PC1 and PC2 in Aβ uptake and processing, which would likely convolute the data when combined into a single population. Further elucidating the mechanisms and triggers that activate Aβ clearance by PCs in a subset-specific manner may prove useful in reducing the build-up of excessive Aβ and toxic plaque formation without producing undesirable immune cell activation in the CNS of AD patients.

Disclosures: D.G. Bohannon: None. **D. Long:** None. **L.L. Wellman:** None. **W. Kim:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.15

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: Canadian Institutes of Health Research (CIHR) Grant PJT 168927]

Title: Direct observation of a reduced response of cerebrovascular insulin receptor in the 3xTg-AD mouse model of Alzheimer’s disease

Authors: *M. LECLERC[1,2], C. SUGÈRE[1], P. BOURASSA[1,2], C. TREMBLAY[1], D. A. BENNETT[3], F. CALON[1,2];
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Abstract: Alzheimer's disease (AD) is an age-related disorder, sharing risk factors with metabolic diseases, such as type 2 diabetes. Recent evidence indicates that the AD brain displays a lower response to insulin. However, cellular localization and mechanisms involved remain poorly understood. Most insulin secreted by the pancreas must first interact with the blood-brain barrier (BBB) before having an impact on brain function. The insulin receptor (INSR) is formed by 2 extracellular α chains, which exist under 2 isoforms, a short one (INSRα-A) and a long one (INSRα-B), combined with 2 intracellular β-chains (INSRβ) containing auto-phosphorylation sites. We first showed that INSR is concentrated at the level of the cerebral vasculature in both human and mouse, whereas INSRα-B was absent from the parenchymal fractions. We then observed lower cerebrovascular levels of isoform INSRα-B compared with individuals with AD, leading to a shift toward a higher INSRα-A/B ratio, consistent with cerebrovascular insulin resistance. In agreement with human data, lower INSRα-B levels were also observed in the 3xTg-AD mouse model of AD neuropathology, but only at an old age. In order to directly investigate cerebrovascular INSR activation, insulin was delivered into the carotid to interact with the BBB using a technique called *in situ* cerebral perfusion (ISCP), followed by an extraction of brain microvessels. We first confirmed the method in young C57Bl6 with insulin or

saline perfusion, or a competitive INSR antagonist (S961) or a phosphorylation inhibitor (AG1024). We found that an intracarotid perfusion of insulin induced the phosphorylation of INSRβ, restricted to microvessels. No increase in the phosphorylation of INSRβ was detected in the parenchyma. We next applied the BBB INSR activation protocol in 16-month-old 3xTg-AD mice. We confirmed that these 3xTg-AD mice also exhibited lower levels of vascular INSRα-B, while the INSR precursor (pro-INSR) and total INSRβ levels remained unchanged. A shift toward a higher INSRα-A/INSRα-B ratio was detected in 3xTg-AD mice, consistent with brain insulin resistance. More importantly, intracarotid insulin perfusion failed to phosphorylate vascular INSRβ in 3xTg-AD mice, suggesting that AD neuropathology induces insulin resistance at the BBB. Overall, the present data in postmortem AD brains and in an animal model of AD neuropathology indicate that defects in the insulin receptor located at the BBB strongly contribute to brain insulin resistance in AD.

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Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

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Program #/Poster #: 277.16

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH/NINDS 1R01NS096225-01A1
AHA 22PRE903112
AHA 19TPA34850047
Louisiana State University Research Council
Joanna G. Magale Foundation

Title: Prmt4-mediated notch1 methylation induces bbb dysfunction and cbf derangement in alzheimer's disease

Authors: *G. CLEMONS[1], C. H. ACOSTA[1], M. S. B. UDO[2], V. TESIC[2], C. T. CITADIN[1], C. Y.-C. WU[2], R. H.-C. LEE[2], H. LIN[2];
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Abstract: Background: Alzheimer’s disease (AD) is a devastating pathology, which contributes massively to the long-term care burden in the United States. Women are more likely to develop dementia than men and tend to have a more rapid disease progression. While AD-related dementia is associated with tau and amyloid beta proteinopathies, derangements in cerebral blood flow (CBF) have also been observed in humans and mice. Additionally, in the aged AD brain cerebral vascular dysfunction has been shown to augment blood brain barrier (BBB) dysfunction. These changes may be due to the loss of junctional proteins and a subsequent ionic imbalance which contribute to neurovascular uncoupling and cognitive decline. Soluble levels of

NOTCH1 is lower in human AD patients may be a factor in the BBB decline seen in AD. Reduced NOTCH1 is linked to blood brain barrier permeability (more leakage) resulting in brain damage. The intersection of the neuronal and vascular NOTCH1 functions make it a promising and target in AD and cerebrovascular pathology. PRMT4 has been shown to be a specific methylator of the intracellular domain of NOTCH1, methylation of this domain leads to its degradation. As stated previously, lower NOTCH1 is associated with BBB compromise. PRMT4 may be an upstream regulator of the NOTCH1 signaling cascade, and this axis can be manipulated through both pharmacological and AAV approaches to improve BBB stability in AD. **Aim: The aims of this study are to 1) determine how protein arginine methyltransferase (PRMT) enzyme PRMT4 influences NOTCH1 expression in the context of AD cerebrovascular pathology, and 2) to determine how regulation of PRMT4 may rescue CBF in AD to improve functional outcomes. Method:** Real-time qPCR and ProteinSimple capillary-phoresis were used to quantify mRNA and levels. Magnetic bead precipitation was used to purify NOTCH1 from protein lysates. Neurovascular coupling was measured using two-photon laser scanning microscopy in tandem with manual whisker stimulation. Novel texture discrimination task as well as novel object recognition and modified T-maze were used to assess cognitive and behavioral outcomes. **Results/Conclusion:** Our preliminary data suggest that 3xTg female mice have 1) higher levels of PRMT4 protein and decreased NOTCH1 intracellular domain expression in the hippocampus, 2) enhanced type-1 PRMT methylation of NOTCH1, 3) reduced junctional proteins in the brain, 4) compromised neurovascular coupling, and 5) poor functional outcomes.

Disclosures: G. Clemons: None. **C.H. Acosta:** None. **M.S.B. Udo:** None. **V. Tesic:** None. **C.T. Citadin:** None. **C.Y. Wu:** None. **R.H. Lee:** None. **H. Lin:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.17

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIA Grant R01 G21042975

Title: A novel CNS pericyte marker for studying blood-brain barrier in AD pathogenesis

Authors: X. GUO, ***S. XIA**, T. GE, Z. ZHAO;
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Abstract: Background: Although there is emerging evidence of a relevant relationship between pericyte loss and blood-brain barrier (BBB) breakdown, the role of pericytes in neurodegenerative disorders still requires in-depth investigation. So far, no genetic marker has been identified for a very accurate classification of pericytes in central nervous system (CNS), especially BBB, which become a major hurdle for genetic manipulations and linage tracing of

pericytes in CNS disorders, including Alzheimer's disease (AD) and dementia.**Method**: We compiled multiple mouse transcriptomic datasets and performed secondary analysis with the Seurat Package to identify new pericyte markers. Next, we generated an *Atp13a5-2A-CreERT2-IRES-tdTomato* knock-in mouse model for studying CNS pericytes. In addition, we examined *Atp13a5*-postive pericyte loss in the 5xFAD transgenic AD model with immunostaining using ATP13A5 polyclonal antibodies. **Result**: We identified that *Atp13a5* is specific to brain pericytes than other current markers. *Atp13a5* is also developmentally regulated, and its appearance coincides with BBB establishment around embryonic day E15. Profiles of the tdTomato reporter in this new model are on a par with the bioinformatic results, as they are only found in the CNS and colocalized exclusively with $CD13^+$ pericyte profiles. Interestingly, tdTomato profiles are completely absent in brain regions known to be outside of the BBB, which is completely different from the $CD13^+$ pericyte profiles in those regions particularly in the highly vascularized pituitary gland, suggesting that *Atp13a5* is also a marker to differentiate the BBB pericytes from the non-BBB pericytes in mice. As *Atp13a5*-expressing pericytes were present in cortex and hippocampus in wild type mice, we found a dramatically loss in 5XFAD mouse brain.**Conclusion**: Therefore, we believe that the identification of *Atp13a5* as a new specific CNS pericyte marker and the *Atp13a5-2A-CreERT2-IRES-tdTomato* knock-in model will advance our studies in blood-brain barrier and vascular contributions to AD and other neurodegenerative disorders. Also, our findings in 5XFAD mice may have implications for the pathogenesis of AD that are associated with neurovascular dysfunction and pericyte loss.

Disclosures: X. Guo: None. **S. Xia:** None. **T. Ge:** None. **Z. Zhao:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: TCRD-108-32
TCRD-109-45

Title: Elevated PDGFR-β under vascular injury can effectively predict shunt efficiency in patients with normal pressure hydrocephalus

Authors: *H. Y. HUANG[1], P. H. TSENG[2], S. T. TSAI[2];
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Abstract: CSF protein analysis has been recommended for idiopathic normal pressure hydrocephalus (iNPH) diagnosis. However, iNPH patients with other neurodegeneration diseases, such as Alzheimer's disease and cerebral small vessel disease, exhibit an equivocal distribution of cerebrospinal fluid (CSF) protein profiles, leading to different shunt responses.

Several studies reveal not all iNPH patients with an Alzheimer's disease (AD) pathology of CSF protein distribution show poor or non-response to shunt. The white matter damages often exist in iNPH patients, indicating that small vessel injury and blood-brain barrier breakdown are risks for the disease. The possibility of progressive morbidity of vascular injury leading to irreversible damages may be one of the reasons for the inefficiency of shunt. Thus, combining dementia and vascular risk protein markers in CSF (Aβ42, t-Tau, p-Tau, PDGFR-β, NFL, and Trem2) for iNPH prognosis is necessary. We prospectively studied 53 iNPH patients undergoing shunt surgery and followed up for one year. Here, the correlation between CSF protein levels and cognitive impairment in iNPH patients was determined, but only Aβ42, p-Tau, and PDGFR-β levels were simultaneously associated with ventricular dilation. In iNPH patients with cerebral small vessel disease, we found that PDGFR-β levels were significantly elevated and prognostic prediction using PDGFR-β levels is evident (r=0.4303, p=0.0282). Using receiver operating characteristic curve (ROC) analysis, calculating the area under the ROC (AUC) for preoperative Aβ42 and PDGFR-β levels provided a more reliable predictive score for postoperative cognitive improvement (AUC=0.7177, Aβ42; AUC=0.8107, PDGFR-β). Cutoff values for Aβ42 and PDGFR-β levels determined by ROC analysis were used to discriminately compare improvements in prognostic symptoms. In the cohort with high Aβ42 levels (>450 pg/ml), significant cognitive improvement after shunt was observed in iNPH patients who had high preoperative levels of PDGFR-β (>480 pg/ml). These results suggest that preoperative PDGFR-β levels in CSF may provide predictable shunt outcomes in patients undergoing shunt intervention. In iNPH patients, the impact of vascular injury appears to be more critical than AD risk factors, leading to differential therapeutic effects on cognitive function.

Disclosures: H.Y. Huang: None. **P.H. Tseng:** None. **S.T. Tsai:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

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Program #/Poster #: 277.19

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 3P2012130701A1S1
NIH Grant HL149264-01A1

Title: Excess acid labile sulfide is detrimental to general cognitive function

Authors: *T. H. REEKES[1], C. R. LEDBETTER[2], K. Y. STOKES[3], J. S. ALEXANDER[3], S. PARDUE[4], C. G. KEVIL[4], E. A. DISBROW[5];
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Abstract: The pathogenesis of Alzheimer's disease (AD) includes factors such as age and deposition of neurodegenerative β-amyloid and phospho-tau. There is growing evidence that

vascular stress is also a major contributor. We reported that elevated plasma sulfides correlated with both vascular and cognitive impairment in AD. Furthermore, sulfides mediated the relationship between measures of memory and cerebral small vessel disease (cSVD). Here, we tested the hypothesis that domain specific cognitive dysfunction and structural brain changes are associated with plasma sulfide dysregulation in AD.
We collected neuropsychological data, plasma sulfide levels and cSVD lesion data from 115 participants (48 AD). Our neuropsychological battery consisted of measures of global cognitive function (Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog)) and 6 core domains: 1) attention and working memory (Digit Span, WAIS-IV); 2) executive function (Verbal Fluency and Color Word Interference, D-KEFS); 3) language (Verbal Fluency, (D-KEFS)); 4) episodic memory (Logical Memory, WMS-IV); 5) visuospatial function (Benton Judgement of Line Orientation); and 6) processing speed (Symbol Digit Modalities Test (SDMT)). We measured three sulfide pools, protective antioxidant free sulfides, as well as *acid-labile* (e.g., iron-sulfur clusters) and *bound* (per- and polysulfides) sulfides. Finally, we measured cSVD burden using fluid-attenuated inversion recovery (FLAIR) MRI.
Plasma acid labile sulfide was significantly increased in AD, and in all subjects was associated with poorer performance across tests: ADAS-Cog ($R^2 = 0.144$, $p < 0.0001$); Digit Span ($R^2 = 0.260$, $p < 0.0001$); Verbal Fluency switch and switch accuracy ($R^2 = 0.082$, $p = 0.008$; $R^2 = 0.059$, $p = 0.024$) and Color Word inhibition and inhibition/switch ($R^2 = 0.219$, $p = 0.003$; $R^2 = 0.256$, $p = 0.001$); Verbal Fluency semantic fluency ($R^2 = 0.106$, $p = 0.002$); Logical Memory I ($R^2 = 0.307$, $p < 0.001$) and Logical Memory II, ($R^2 = 0.201$, $p = 0.002$); and the SDMT ($R^2 = 0.127$, $p < 0.001$). Increased acid labile sulfide was also correlated with FLAIR lesion volume ($R^2 = 0.110$, $p = 0.028$).
Elevated acid labile sulfide is associated with reduced cognitive function across multiple domains. How these plasma and brain changes are linked is unclear. The acid labile sulfur pool includes iron sulfur clusters. Increased iron load is associated with cognitive decline, aggregation of β-amyloid and phospho-tau, brain volume changes and severity of cSVD. Whether elevated plasma acid labile sulfides reflect iron deposition in the brain remains to be determined.

Disclosures: **T.H. Reekes:** None. **C.R. Ledbetter:** None. **K.Y. Stokes:** None. **J.S. Alexander:** None. **S. Pardue:** None. **C.G. Kevil:** None. **E.A. Disbrow:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.20

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Ressler Family Foundation

Title: Gliovascular signaling precedes blood-brain barrier disruption and neuronal loss in a mouse model of tauopathy

Authors: *Y. KOMURO[1], E. MILIOTOU[1], O. MINAEVA[2], J. MONCASTER[2], N. HUA[2], S. CARMICHAEL[1], L. GOLDSTEIN[2], J. D. HINMAN[1];
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Abstract: Cerebrovascular abnormalities have long been known to cause vascular dementia and are increasingly thought to contribute to the pathogenesis of Alzheimer's disease and related tauopathies. However, the contribution of tau-mediated pathways to vascular dysfunction is unknown. Using the P301S mouse model of tauopathy, we employed dynamic contrast enhanced MRI (DCE-MRI) to demonstrate widespread blood-brain barrier (BBB) dysfunction in late stage tauopathy. Quantitative spatial analysis of BBB breakdown in the frontal cortex and hippocampus of P301S transgenic mice was confirmed post-mortem using Gd-MIMS to detect residual gadolinium in cerebral tissue. To identify molecular pathways regulating intercellular signaling between neurons and gliovascular cells that are pathologically altered in advance of BBB disruption, we developed a cell-type specific viral approach for transcriptional profiling using RiboTAG to identify novel pathways within the multicellular environment of the neurovascular unit (NVU) in tauopathy. Viral constructs using cell-type specific promoters (hSynapsin, GFAP, or PDGFRβ) to drive expression of antigen-tagged ribosomes (TRAP) were used to study variance in spatial and temporal gene expression patterns in NVU cell types in P301S transgenic mice prior to widespread BBB disruption. Coupling vTRAP with endothelial MACS-Seq in P301S transgenic mice, we generated multiple cell-type specific transcriptomic databases of the neurovascular unit across several pathologically-relevant time points that precede the BBB disruption seen by DCE-MRI. Gene ontology analysis indicates that gliovascular cells express multiple molecular programs driving neuronal differentiation, neurite outgrowth, and synaptogenesis in early pathological stages. This specific and temporally regulated coordination of pathways in pericytes and astrocytes to support neurons injured by tau pathology may lessen with age and increased stress, leading to a loss of homeostatic maintenance and cascading neuronal injury. This suggests that there may be a paradigm for gliovascular prevention and rescue of neurodegeneration and implicates novel perivascular molecular pathways in the early pathogenesis of Alzheimer's disease and related tauopathies.

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Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG049952

Title: Psgl-1 as a potential therapeutic target in improving cerebral blood flow in app/ps1 mouse models of alzheimer's disease

Authors: *D. A. RIVERA[1], A. BUNCE[1], O. BRACKO[2], N. NISHIMURA[1], C. B. SCHAFFER[1];
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Abstract: In addition to the presence of amyloid plaques and neurofibrillary tau tangles normally associated with Alzheimer's disease, mouse models and human patients also exhibit a decrease in cerebral blood flow of ~30% which is seen prior to plaque deposition and tangle formation. Previous work in our lab has identified neutrophils arresting within capillaries, causing stalled blood flow in that capillary segment, as a potential cause of the blood flow deficit. Application of antibodies against the Ly6G neutrophil surface marker, which plays a role in neutrophil adhesion, led to a decrease in capillary stalling rates, an increase in cerebral blood flow, and an improvement in memory of APP/PS1 mouse models to wild-type levels. However, the Ly6G surface marker does not have a true mouse-to-human conserved analog and therefore requires identification of alternative conserved receptors within the neutrophil adhesion cascade. Antibodies against multiple parts of the cascade, such as p-selectin, PSGL-1, and $\alpha_4\beta_1$ integrin, were screened using multi-exposure laser speckle imaging to measure cerebral blood flow continuously up to 3 hours and chronically up to a week post administration to identify potential targets for which cerebral blood flow improved in APP/PS1 mice. We found that treatment with PSGL-1 (4mg/kg IP), a leukocyte receptor for both p-selectin and e-selectin, increased vascular blood flow by a median value of 26% (IQR = 21-51%) and overall perfusion by a median value of 56% (IQR = 46-65%) within 2 hours (n=7, linear mixed model, p=0.0001). To verify the effect seen with laser speckle imaging, we used multiphoton imaging with acute treatments of the antibody (4 mg/kg, IP) to measure the incidence of capillary stalling and arteriole flow speeds before and after treatment. In addition, we tested whether this improvement in blood flow was enough to improve object recognition and spatial memory as seen with antibody treatment against Ly6G.

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Poster

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Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG049952

Title: Vascular oxidative stress contributes to neutrophil arrest in brain capillaries and decreased cerebral blood flow in an Alzheimer's disease mouse model

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Abstract: Cerebral blood flow (CBF) is decreased by 30% in both patients and animal models of Alzheimer's disease (AD). Previously, we found that the arrest of neutrophils in the capillary leading to about 2% of cortical capillary stalls is responsible for the CBF deficit in the AD mouse model overexpressing the human transgenes APP KM670/671NL and PSEN1 L166P (APP/PS1). However, the upstream mechanisms leading to the arrest of neutrophils and capillary stalls remain largely unexplored. Here, we investigated whether vascular oxidative stress produced by the NADPH oxidase NOX2 leads to increased neutrophil arrest and capillary stalls in APP/PS1 mice. First, we treated 10-11 month-old APP/PS1 mice, using the peptide inhibitor gp91-ds-tat (10 mg/kg i.p. for two weeks). We found that neocortical ROS production was decreased by 55% ($p < 0.05$; n=6 per group; one-way ANOVA) assessed with hydroethidine staining. With *in-vivo* two photon imaging, we also observed that the fraction of capillaries with stalled blood flow was decreased by 67% ($p < 0.05$; n=3-5; Kruskal-Wallis test), CBF was increased by 29% ($p < 0.05$, n=6 vessels per mice, 3-5 mice; one-way ANOVA), and performance on short-term memory tasks was improved with NOX2 inhibition in APP/PS1 mice, as compared to control treatment ($p < 0.05$; n=9-10; one-way ANOVA). Interestingly, Aβ levels and amyloid plaques, measured with ELISA and Methoxy X04 staining respectively, were comparable between gp91ds-tat and control peptide-treated APP/PS1 mice. NOX2 inhibition was also associated with a decrease in microgliosis, as shown by a 40% decrease in the area of IBA1 staining ($p < 0.05$; n=7; one-way ANOVA), in the cortex and a decrease in VCAM-1 expression around penetrating arterioles and capillaries, as shown by *in vivo* immunolabelling. Inhibiting the DNA repair enzyme poly (ADP-ribose) polymerase (PARP) with 3-aminobenzamide (10 mg/kg, i.p.), as well as promoting decomposition of peroxynitrite with FeTPPS (10 mg/kg, i.p.) was also associated with an increase in CBF ($p < 0.05$; n=5; Kruskal-Wallis test), further implicating ROS pathways in capillary stalling. Finally, RNA sequencing and gene set enrichment analysis (GSEA) of cerebral microvessels revealed pathways involved in inflammation, cell adhesion, neutrophil chemotaxis and migration, among others, to be upregulated in APP/PS1 and then downregulated with NOX2 inhibition, as compared to control treatment (q-value < 0.01). This study implicates the NOX2 pathway as a molecular mechanism contributing to capillary stalling and CBF reduction in a mouse model of AD and could represent a molecular pathway with therapeutic potential for AD.

Disclosures: **N. Ruiz:** None. **O. Bracko:** None. **M. Swallow:** None. **A. Omurzakov:** None. **B. Njiru:** None. **M. Ali:** None. **K. Falkenhain:** None. **H. Chang:** None. **S. Dash:** None. **H. Uchida:** None. **T. Sanchez:** None. **C. Iadecola:** None. **L. Park:** None. **N. Nishimura:** None. **C. Schaffer:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.23

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R21AG066001
NSF GRFP
NIH AG057622

Title: Simultaneous 2-photon calcium imaging of cortical neurons to investigate links between cerebral blood flow deficits and excitation/inhibition balance

Authors: *R. ZIRKEL, M. ISAACSON, K. YANG, M. LAMONT, N. NISHIMURA, C. B. SCHAFFER;
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Abstract: Alzheimer's disease (AD) is characterized by progressive, irreversible neurodegeneration, leading to memory loss and cognitive decline. In mouse models of AD, it has been shown that global decreases in cerebral blood flow (CBF) are brought on by the plugging of capillaries by arrested neutrophils, and that the administration of the neutrophil-specific mouse Ly-6G antibody (aLy6G) reduces these capillary stalls in minutes and improves cognitive function within hours. This suggests that at least some aspects of neural activity impairments are reversible, but the mechanism of this recovery - and what specific aspects of neural activity are recovered - is not yet known. One potential link between CBF and neural activity is the excitation/inhibition (E/I) balance, as some inhibitory neurons may be more sensitive to energy deficiencies associated with CBF decrease than excitatory neurons. We developed a method using *in vivo* 2-photon calcium imaging to distinguish excitatory and inhibitory neurons and simultaneously record neural activity of both. We use this labeling to determine how E/I balance is affected by CBF deficits and recovery with aLy6G treatment (4 mg/kg) in the APP/PS1 mouse model of AD. We transfected inhibitory and excitatory cell types in layer 2/3 of the primary visual cortex (V1) of mice using mDlx-mRuby2 (10^12 vg/mL) and CaMKII-EYFP (10^11 vg/mL) AAV1s, respectively, while also co-labeling all neurons with a calcium indicator using the Syn-GCaMP6s AAV9 (10^12 vg/mL). These dilutions enabled calcium imaging and cell-type identification in the same tissue, along with imaging of methoxy-X04 to detect amyloid plaques and Texas-red-dextran in the vasculature to detect blocked capillaries. Using 780, 920, and 1030 nm laser excitation to differentially excite this set of fluorophores and dichroic filters to separate the emission spectra into different color imaging channels, we identified dozens of cell bodies in each imaging plane as excitatory or inhibitory through spectral unmixing. Additionally, using an automated imaging and registration procedure with the ScanImage microscope control software, we could consistently re-locate neurons from a previous imaging session without manually searching the tissue. Using these methods, we demonstrate imaging of the same excitatory and inhibitory neurons at multiple timepoints to compare neural activity (both spontaneous and visual stimulus-evoked) before and after aLy6G treatment. Using these tools, we can study how excitatory and inhibitory cell activity is affected by CBF deficits in AD mice.

Disclosures: R. Zirkel: None. **M. Isaacson:** None. **K. Yang:** None. **M. Lamont:** None. **N. Nishimura:** None. **C.B. Schaffer:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: CIHR Postdoctoral Fellowship

Title: Impact of hypertension in the apolipoprotein e4 targeted replacement mouse on cerebrovascular pathology and spatial working memory

Authors: *L. J. TRIGIANI, N. E. CHERNAVSKY, R. KIM, A. A. MISTRY, D. A. RIVERA, K. E. MCGARTY, N. H. ALLAN-RAHILL, M. LAMONT, N. NISHIMURA, C. B. SCHAFFER;
Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Cerebrovascular alterations have been observed based on apolipoprotein E (ApoE) status whereby carriers of the ε4 allele have elevated cerebral blood flow (CBF) in early adulthood but experience a faster CBF and cognitive decline later in life relative to non-carriers. More severe cognitive decline is observed in hypertensive individuals, an association that is strongest in ε4 carriers. Here we investigated the impact of these strong and prevalent contributors to late-onset dementia and how they interact to alter cerebrovascular function.
To compare effect of genotype, we used male and female mice with a targeted replacement of human ε4 or ε3 allele (6-8 months, n=9-15/group). Mice were assessed twice: at baseline, and following two weeks of angiotensin II (AngII, 500ng/kg/min via subcutaneous minipumps). A spontaneous alternation Y-maze was used to assess spatial working memory, mice were imaged using laser speckle contrast to measure tissue perfusion, and z-stacks of capillary beds and line scans of penetrating arterioles were acquired using two-photon microscopy with a 70kDa dextran-conjugated dye, Hoechst, and rhodamine 6G injected retro-orbitally to assess capillary stalling and blood flow speeds. Mice were perfused following the last imaging session.
At baseline, ε3 and ε4 mice had similar levels of spatial memory function, but after two weeks of AngII, ε4 mice showed an impairment (p<0.01). Following AngII, ε4 mice showed a significant decrease in volumetric blood flow in arterioles (β=6.89, p<0.05), and capillary speed (p<0.05, n=6-10 vessels/mouse), along with an increased incidence of non-flowing capillary segments (p<0.05) not observed in ε3 mice; most of these capillary stalls contained red blood cells (~62%). While investigating possible pathological markers induced by AngII we found a striking increase in microglia activation measured by cortical Iba-1 area (p<0.001), decreased occludin coverage (p<0.05), and a trend for increased matrix metalloproteinase-9 (p=0.09).
Preliminary findings show a clear interaction between hypertension and ApoE status with regards to cerebrovascular and cognitive function whereby mice with the ε4 allele experience more deleterious consequences brought on by hypertension compared to ε3 counterparts. This work implicates the possibility of a vasoconstriction stalling mechanism involving pericytes or other perivascular cells that may release constricting agents and provides us with a clinically

relevant model to further study the cellular mechanisms underlying capillary stalls. We are currently exploring the involvement of cyclophilin A and platelet activity as possible contributors.

Disclosures: L.J. Trigiani: None. **N.E. Chernavsky:** None. **R. Kim:** None. **A.A. Mistry:** None. **D.A. Rivera:** None. **K.E. McGarty:** None. **N.H. Allan-Rahill:** None. **M. Lamont:** None. **N. Nishimura:** None. **C.B. Schaffer:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant EB002019
NIH R21AG066001

Title: In vivo, label-free measurement of blood oxygen concentration using third-harmonic generation spectroscopic imaging

Authors: *N. CHERNAVSKY[1], K. MCGARTY[1], N. H. ALLAN-RAHILL[1], N. NISHIMURA[2], C. B. SCHAFFER[2];
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Abstract: Detecting blood oxygen changes in the cerebrovascular network is important for increasing our understanding of neurodegenerative diseases and cerebrovascular dysfunction, but existing methods are complex or lack temporal resolution. Previous work in our lab demonstrated the ability to perform label-free *in vivo* imaging of red blood cells (RBCs), based on the nonlinear optical process of third-harmonic generation (THG), which highlights optical interfaces, such as the RBC/blood plasma boundary, and can be resonantly enhanced by electronic transitions. Interestingly, the linear absorption spectra of oxyhemoglobin and deoxyhemoglobin have separately distinct peaks in the range of 400 nm to 450 nm. Clay, et al. (2006) demonstrated the ability to detect hemoglobin oxygenation using THG intensity ratios at a few excitation wavelengths for hemoglobin in solution, and measuring signal from a hemoglobin-glass interface. Chang et al. (2010) further showed a ~30 nm shift in the wavelength that produced peak THG intensity between oxyhemoglobin and deoxyhemoglobin across wavelengths ranging from 390 nm to 460 nm (fundamental wavelengths of 1170 nm to 1380 nm).Similar to pulse oximetry, wherein linear spectroscopy is used to quantify blood oxygen concentration, we have demonstrated the use of THG spectroscopy to quantify blood oxygenation, but now with the natural 3D sectioning capabilities and scattering insensitivity of all nonlinear microscopies. By tuning the fundamental wavelength from 1200 nm to 1320 nm while maintaining constant power and a stable laser focus, we can reproducibly detect a ~10-15 nm shift in the wavelength for maximum THG intensity between cerebral arterioles and venules

in an anesthetized mouse. Further, we have calibrated this measurement using linear spectroscopy and THG spectroscopy of varying oxygenation levels in ovine whole blood cells. From this, we are working to derive blood oxygen concentration of arterioles, venules, and capillaries on a vessel-by-vessel basis through a three-dimensional image stack with the potential for 1 Hz temporal resolution.Blood oxygen concentration maps are useful for answering questions regarding neurovascular dysfunction. Potential future experiments include evaluating changes in blood oxygen concentration caused by capillary stalls in neurodegenerative disease mouse models, quantifying blood oxygen concentration changes in the capillary network surrounding an induced penetrating arteriole stroke, and correlating changes in blood oxygen concentration with neural activity (using calcium signaling) to evaluate the breakdown of neurovascular coupling in mouse models of dementia.

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Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.26

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH, AG057622

Title: Effects of anti-Ly6G antibody treatment on hippocampal memory-associated activity in APP/PS1 mouse model of Alzheimer's disease

Authors: *L. BERKOWITZ, R. MORTON, N. NISHIMURA, C. SCHAFFER;
Cornell Univ., Ithaca, NY

Abstract: Spatial memory impairment is an early symptom of Alzheimer's disease and is associated with the progressive degeneration of entorhinal-hippocampal circuits. The APP/PS1 mouse model mimics features of amyloid pathology, including amyloid plaques, inflammation, and cerebrovascular dysfunction. In addition, hippocampal CA1 activity is altered starting at 6 - 8 months of age, including reductions in basal synaptic transmission, LTP, and reductions of hippocampal sharp-wave ripples. Furthermore, APP/PS1 mice exhibit amyloid-induced hyperexcitability, including increased rates of interictal epileptiform discharges (IEDs) which are negatively associated with rates of hippocampal sharp-wave ripples. Recently, work in our lab found that administration of anti-Ly6G antibodies rapidly increased cerebral blood flow in APP/PS1 mice. Notably, while object-location memory improves rapidly following anti-Ly6G antibody administration (< 1 day), it is unclear if anti-Ly6G antibody treatment also leads to changes in hippocampal memory associated-activity. To address this question, 8 to 12 month APP/PS1 mice and littermate controls were implanted with 64-channel laminar probes in hippocampal area CA1 and dentate. Neural activity was recorded while the mice slept in their

home cage or engaged in the object-location task. Anti-Ly6G antibody treatment (4mg/kg I.P.) was administered 30-60 minutes before or directly after object learning. IEDs and sharp-wave ripples were detected in both groups, though the rate of IEDs was markedly increased in APP/PS1 mice. Features of sharp-wave ripples, including rate and duration, were examined during pre-task and post-task slow-wave sleep. Preliminary results indicate that sharp-wave ripple rate and duration were similar between pre-task and post-task sleep in APP/PS1 mice, whereas increases in both measures were observed during post-task sleep of control mice. Additionally, the rate of IEDs decreased in APP/PS1 following anti-Ly6G injection compared to isotype control injection. Overall, these early results are consistent with previous literature showing alterations of hippocampal CA1 activity in APP/PS1 mice. The results of this study will be discussed in light of the hypothesis that vascular changes in APP/PS1 mice contribute to disruptions of hippocampal memory associated-activity.

Disclosures: L. Berkowitz: None. **R. Morton:** None. **N. Nishimura:** None. **C. Schaffer:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.27

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant grant RO1NS094201

Title: Brain proteomics reveals mechanistic insight to pathologies in specific disease stages in the rTg-DI rat model of cerebral amyloid angiopathy.

Authors: *J. SCHRADER, F. XU, W. E. VAN NOSTRAND;
Col. of Pharm., Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral amyloid angiopathy (CAA), characterized by cerebral vascular amyloid accumulation, cerebral microbleeds, intracerebral hemorrhages and cerebral infarction, is a prevalent comorbidity in Alzheimer’s disease (AD) and prominent contributor to vascular cognitive impairment and dementia (VCID). Despite the obvious clinical relevance, underlying mechanisms for the development of CAA are poorly understood, and there remains no effective treatment nor validated biomarkers for the disease. rTg-DI rats, a preclinical model of CAA, develop regionally distinctive CAA related brain pathologies including age-dependent progressive microvascular amyloid deposition and neuroinflammation in several brain regions, whereas thrombotic events of small vessel occlusions and microbleeds are mainly restricted to the thalamic region. Here, we investigated proteomic changes in rTg-DI rat brain regions and compared differentially expressed proteins from rats with emergent stages of CAA to rats in late stages of CAA where extensive vascular amyloid and severe vasculopathies are present. The cortex, hippocampus, and thalamus regions were isolated from 4M and 12M old rats via laser

capture microdissection and subjected to data independent acquisition protein mass spectrometry to identify differentially expressed proteins. ANXA3, APOE, and HTRA1 were elevated in every region in both age groups, while S100A4, SDC4 and HSPB1 were elevated in the hippocampus and thalamus at both ages. Pathway analysis using Ingenuity Pathway Analysis, indicated TNFα and TGFβ activation in multiple regions, and thrombin activation in the thalamus in the 12M age group. Proteomics analysis and immunolabeling also revealed the presence of neutrophil extracellular traps (NETs) specifically in the thalamus, a potentially important mechanism in the progression of the thrombotic vasculopathies observed in that region. Thus, we report new and significant insight linking specific proteomic changes to differential and progressive CAA-pathologies, and present potential mechanisms for the regionally distinct disease progression. Furthermore, we report several uniquely elevated proteins in early and late stages of disease in the rTg-DI rat brains with potential as early disease stage diagnostic biomarkers of CAA.

Disclosures: J. Schrader: None. **F. Xu:** None. **W.E. Van Nostrand:** None.

Poster

277. Alzheimer's Disease: Roles of the Cerebrovasculature

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 277.28

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH GRANT R01AG062738 to A.Y. S
NIH GRANT F32NS117649-01 to S.K.B.

Title: Characterization of mural cells and perivascular fibroblasts in the Tg-SwDI mouse model of cerebral amyloid angiopathy

Authors: *M. J. SOSA, S. K. BONNEY, A. Y. SHIH;
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Abstract: Cerebral Amyloid Angiopathy (CAA) is a common small vessel disease that leads to pathological alterations in the cerebral vasculature and is often associated with Alzheimer’s disease (AD). CAA is characterized by the accumulation and deposition of amyloidogenic proteins (amyloid-beta) along the vascular basement membrane of cerebral vessels, which is where mural cells and perivascular fibroblasts (PVFs) reside. However, little is known about how these perivascular cell types respond to increased CAA burden. Multiple mouse models of CAA have been developed over the years, and researchers have shown that there is a reduction in mural cells at late stages of CAA, but there is still little known about the progression of CAA and cerebrovascular pathology in earlier stages of disease in these mouse models. Our goal was to understand how mural cells (pericytes and smooth muscle cells (SMCs)) and PVFs are affected during the progression of CAA in the Tg-SwDI mouse model. This mouse line exhibits CAA buildup along parenchymal arterioles and capillaries, similar to that seen in some human cases. We used histological approaches to visualize the deposition of amyloid-beta and to study the

populations across whole brain sections from 6, 9 and 12 month Tg-SwDI mice in relation to CAA progression. We found that cerebrovascular amyloid deposition occurs along arterioles, venules, and capillaries starting at 6 months in this model. This deposition increases with age, and exhibits regional variance, with highest burden in the thalamus, followed by the hippocampus, and the cortex. We identified a reduction of PVFs on arterioles in the cortex and hippocampus at 6 months, while SMC coverage remained stable through early stages of CAA. Further analysis will reveal how pericytes are affected in relation to the progression of the disease. These findings lead us to hypothesize that PVFs are uniquely sensitive to amyloid (particularly soluble amyloid or diffuse amyloid plaques), as the presence of CAA was not seen in the cortex in the earlier time points. This opens the possibility that PVF loss is a triggering event that leads to impaired mural cell function, vascular stability, and amyloid-beta clearance.

Disclosures: M.J. Sosa: None. **S.K. Bonney:** None. **A.Y. Shih:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.01

Topic: H.08. Learning and Memory

Support: CIHR

Title: The effect of early life environmental enrichment on multisensory integration in the triple transgenic alzheimer's disease mouse model

Authors: *S.-E. WEBER[1], S. D. MCGRAW[2], K. LADOUCEUR[3], S. PATEL[3], B. L. MCNAUGHTON[4], B. D. WINTERS[2];
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Abstract: Alzheimer’s Disease (AD) is a neurodegenerative disorder associated with several cognitive deficits. The cognitive reserve (CR) hypothesis posits that individuals who have a high education, higher occupational attainment, and exercise regularly will be less susceptible to cognitive deficits associated with aging and dementia. CR can be modeled with a standard Environmental enrichment (EE) protocol which places mice in a large cage with cage-mates, a running wheel, and objects that can be substituted daily. Unfortunately, the standard EE procedure has a limit on quantifying the enrichment each individual mouse obtains from being in an enriched cage. Thus, we developed a novel EE procedure to enable better control and quantification of daily enrichment. In the present study, we used male triple transgenic Alzheimer’s disease (3xTg-AD) mice and male wildtype mice to evaluate the potential for modeling CR using this procedure. Mice were assigned to each of the four following conditions

immediately after weaning at 5 weeks of age: Environmental Enrichment homecage (EH), which is modeled after 'conventional' EE protocols; Enrichment Track (ET), in which mice run laps on an obstacle track 6 days/week with novel obstacles daily; Exercise Control Track (CT), in which mice run laps but are not exposed to complex obstacles; and Standard Housing (SH). Following 10 weeks in these conditions, we tested all mice on tasks assessing multisensory integration abilities, an understudied but potentially important aspect of AD cognitive impairment. These tasks were the tactile-visual Cross-Modal Object Recognition (CMOR) task with a 5-minute or 'immediate' retention delay, as well as the olfactory-tactile Multisensory Oddity (MSO) task. At 3 months of age, only the wildtype and 3xTG-AD groups in the ET condition successfully performed the CMOR task with both delays. All mice were re-tested at 6 months, but only the wildtype ET mice displayed intact performance (immediate delay). To complement the CMOR findings with a potentially easier task, and to minimize memory demands, we turned to the MSO task. Here, the wildtype and 3xTg-AD ET mice were the only groups that demonstrated intact multisensory perceptual discrimination when tested at three and six months of age. Thus, our early results suggest that enrichment track training early in life may confer cognitive benefits related to multisensory integration and that this effect can reverse deficits on the CMOR and MSO tasks that relate to AD-like pathology.

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Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Tau transgene drives aberrant hippocampal dynamics throughout life

Authors: *J. MCGREGOR[1], S. ENSLEY[1], C. A. FARRIS[1], K. RONAYNE[1], C. WANG[2], R. WESSEL[3], D. M. HOLTZMAN[2], K. B. HENGEN[1];
[1]Biol., [2]Neurol., [3]Physics, Washington Univ. in St. Louis, St. Louis, MO

Abstract: Neuronal activity is homeostatically-regulated around set points. This process is believed to maintain stable circuit function over long timescales and in the face of perturbations. The interplay between neurodegenerative disease progression and homeostatic set points is unclear. Disease-related degradation of function presumably involves a progressive inability to maintain setpoints at multiple levels of neuronal and network organization. Further, it has been suggested that aberrant set points early in life predict and facilitate the onset of disease. Here, we address these questions by performing chronic, multi-month electrophysiological recordings of ensembles of single units in CA1 of freely behaving mice. We recorded neuronal spiking from wild-type mice (WT) and P301S/E4 mice (TE4), which are a mouse model of tauopathy that

overexpress a human tau transgene (1N4R) containing the P301S mutation. We found that first-order measurements of neuronal activity, such as mean firing rate and coefficient of variation, could not meaningfully differentiate genotypes at any age. On the other hand, homeostatic set-points in emergent properties of network dynamics, such as network structure and criticality, were significantly different between genotypes as a function of disease progression. Crucially, we found that emergent properties in early life were sufficient to delineate healthy (WT) mice and TE4 mice destined to develop disease months later. In other words, network dynamics were tuned to an aberrant set-point prior to the onset of insoluble tau accumulation. Together, these results suggest that homeostatically-regulated set points of network dynamics are sensitive to disease states, and that aberrant set points early in life are poised to contribute to the development of future disease.

Disclosures: **J. McGregor:** None. **S. Ensley:** None. **C.A. Farris:** None. **K. Ronayne:** None. **C. Wang:** None. **R. Wessel:** None. **D.M. Holtzman:** None. **K.B. Hengen:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.03

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: CIHR
NSERC

Title: Early changes in firing properties of VIP interneurons and abnormal CA1 inhibition in asymptomatic 3xTg-AD mice

Authors: *F. MICHAUD[1,2], R. FRANCAVILLA[3,2], D. TOPOLNIK[2], P. ILOUN[1,2], S. TAMBOLI[1,2], F. CALON[1,2], L. TOPOLNIK[1,2];
[1]Univ. Laval, Quebec, QC, Canada; [2]CRCHUQ-CHUL, Quebec, QC, Canada; [3]Dept. of Neurosci., Sainte-Justine, Montreal, QC, Canada

Abstract: The 3xTg-AD mouse model is used to study Alzheimer’s disease (AD), as at advanced stage, mice display some cognitive deficits and neuropathological hallmarks similar to those seen in human AD patients. AD is characterized by a progressive memory loss, with hippocampal hyperactivity being considered as one of the earliest pathophysiological processes in both human and animal studies. Various types of GABAergic interneurons are involved in coordination of network activity in the hippocampus. The type 3 interneuron-specific (I-S3) cells co-express vasoactive intestinal peptide and calretinin, and play an important role in memory formation as, by providing disinhibition to principal excitatory cells, they can gate the inputs arriving to the hippocampal CA1 region. Whether the activity of these cells is altered in AD thereby shaping the pathological network motifs remains unknown. Here, we addressed this

question by examining the properties of I-S3 cells and of their target oriens/alveus (O/A) interneurons in young asymptomatic 3xTg-AD mice. Our data indicate that whereas the density and morphological characteristics of IS-3 cells in 3xTg-AD mice remain unaltered, the IS-3 firing output can show significant changes. The latter was associated with a decreased inhibitory drive to O/A interneurons. Furthermore, using wireless fiber photometry calcium imaging in freely behaving mice, we observed changes in the activity of CA1 O/A interneurons in 3xTg-AD mice during different behavioural states. Together, these data indicate that the altered I-S3 cells' firing output in 3xTg-AD mice may be responsible for abnormal activity of hippocampal CA1 interneurons, and can potentially lead to excitation/inhibition imbalance and further mnemonic dysfunction.

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Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.04

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NIH R01 AG057767
NIH R01 AG061937
Dale and Deborah Smith Center for Alzheimer's Research and Treatment
Kenneth Stark Endowment

Title: Abnormal Glutamatergic Neurotransmission and Cognitive Impairment in $APP^{NL\text{-}F/NL\text{-}F}$ Mice

Authors: *C. A. FINDLEY, S. A. MCFADDEN, K. N. HASCUP, E. R. HASCUP; Southern Illinois Univ. Sch. of Med., Springfield, IL

Abstract: Evidence supports the essential role of glutamatergic neurotransmission in learning and memory. In Alzheimer's disease (AD), previous studies indicate that glutamate dynamics shift with disease progression, inciting cognitive impairment and perpetuating disease pathology. Here, we build on prior work by utilizing a knock-in mouse model of AD ($APP^{NL\text{-}F/NL\text{-}F}$) to characterize hippocampal glutamate signaling and cognition in aged AD mice. At 18-24 months old, male and female $APP^{NL/NL}$, $APP^{NL\text{-}F/NL\text{-}F}$, and genetic background control (C57BL/6) mice underwent cognitive assessment through Morris water maze (MWM) and novel object recognition (NOR) tasks. Following NOR, basal and 70 mM KCl stimulus-evoked glutamate release was measured in the CA3, CA1, and DG of the hippocampus using a glutamate-selective microelectrode in isoflurane anesthetized mice. Female $APP^{NL\text{-}F/NL\text{-}F}$ mice exhibited impaired performance during the initial MWM trial days compared to control female mice. No differences

were observed between male mice. Probe challenge results indicated diminished spatial long-term memory for female $APP^{NL-F/NL-F}$ mice on platform proximity compared to $APP^{NL/NL}$ and control female mice. $APP^{NL-F/NL-F}$ male mice also displayed impairment compared to control male mice on platform entries. NOR findings failed to show differences in discrimination index for either sex. Hippocampal glutamate recordings support decreased basal glutamate levels for $APP^{NL-F/NL-F}$ male and female mice compared to sex-matched controls. The observed loss was exhibited in the CA3 subregion for male $APP^{NL-F/NL-F}$ mice and CA1 for female mice. Female $APP^{NL-F/NL-F}$ mice alone showed increased CA1 stimulus-evoked glutamate release compared to sex-matched $APP^{NL/NL}$ mice. No differences were observed for stimulus-evoked glutamate release in male mice. No differences were found for clearance of the released glutamate for either sex. These findings support impaired spatial cognition and altered glutamatergic neurotransmission in aged male and female $APP^{NL-F/NL-F}$ mice. Lack of glutamate activity at this time point may underlie cognitive decline in $APP^{NL-F/NL-F}$ mice. Our laboratory previously observed similar findings in a transgenic AD model. However, unlike $APP^{NL-F/NL-F}$ mice, those mice displayed increased basal glutamate levels. This divergence could be attributed to differences between the mouse models, considering transgenic overexpression versus knock-in and mutations utilized to achieve amyloid pathology, and warrants further investigation. Studies are ongoing to elucidate glutamate dynamics at a pre-plaque deposition time point for comparative analysis.

Disclosures: C.A. Findley: None. **S.A. McFadden:** None. **K.N. Hascup:** None. **E.R. Hascup:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.05

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF DMS-1555237

Title: Deep hybrid modeling of neuronal dynamics using generative adversarial networks

Authors: *S. SAGHAFI[1], T. H. RUMBELL[2], K. WEDGWOOD[3], F. TAMAGNINI[4], V. GUREV[2], J. KOZLOSKI[2], C. DIEKMAN[1];
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Abstract: Mechanistic modeling and machine learning methods are powerful techniques for approximating biological systems and making accurate predictions from data. However, when used in isolation these approaches suffer from distinct shortcomings: model and parameter

uncertainty limit mechanistic modeling, whereas machine learning methods disregard the underlying biophysical mechanisms. To address these shortcomings, we build Deep Hybrid Models (DeepHMs) that combine deep learning with mechanistic modeling to identify the distributions of mechanistic modeling parameters coherent to the data [1]. One type of DeepHM uses Generative Adversarial Networks (GANs) to provide the inverse mapping from data to mechanistic model parameters [2].
We employed GANs in a DeepHM to identify ion channel conductances coherent with altered excitability properties of hippocampal neurons in mouse models of Alzheimer's disease [3]. Although the underlying cause of Alzheimer's disease (AD) remains poorly understood, it is believed to occur when abnormal amounts of amyloid beta and tau proteins aggregate in the brain, forming extracellular plaques (amyloidopathy) and intracellular tangles (tauopathy) that result in a progressive loss of neuronal function. In transgenic mice with amyloidopathy, neurons in the hippocampus exhibit altered intrinsic excitability properties, such as action potentials with reduced peaks and widths. Hippocampal neurons in transgenic mice with tauopathy also show altered excitability, such as increased hyperpolarization-activated membrane potential sag and rebound and decreased action potential threshold.
We applied the DeepHM to map experimental data recorded from hippocampal CA1 neurons in transgenic AD mice and age-matched wild-type littermate controls [3] to the parameter space of a conductance-based CA1 model [4]. The corresponding parameter distributions enable us to infer the full set of ion channel disruptions in the model that can explain altered excitability in the amyloidopathy and tauopathy mice.
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Disclosures: S. Saghafi: None. **T.H. Rumbell:** A. Employment/Salary (full or part-time):; IBM Research. **K. Wedgwood:** None. **F. Tamagnini:** None. **V. Gurev:** A. Employment/Salary (full or part-time):; IBM Research. **J. Kozloski:** A. Employment/Salary (full or part-time):; IBM Research. **C. Diekman:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant RF1AG065675
NIH Grant RF1MH120020
NIH Grant R35 GM127102

Title: Hippocampal neural circuit connectivity alterations in an Alzheimer's disease mouse model revealed by monosynaptic rabies virus tracing

Authors: *Q. YE[1], G. GAST[1], X. SU[1], T. SAITO[2], T. C. SAIDO[3], T. C. HOLMES[1], X. XU[1]; [1]Univ. of California Irvine, Irvine, CA; [2]Nagoya City Univ., Nagoya, Japan; [3]RIKEN Ctr. for Brain Sci., Wako, Japan

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder with growing major health impacts, particularly in countries with aging populations. The examination of neural circuit mechanisms in AD mouse models is a recent focus for identifying new AD treatment strategies. We hypothesize that age-progressive changes of both long-range and local hippocampal neural circuit connectivity occur in AD. Recent advancements in viral-genetic technologies provide new opportunities for semi-quantitative mapping of cell-type-specific neural circuit connections in AD mouse models. We applied a recently developed monosynaptic rabies tracing method to hippocampal neural circuit mapping studies in AD model mice to determine how local and global circuit connectivity to hippocampal CA1 excitatory neurons may be altered in the single amyloid precursor protein knock-in (APP-KI) AD mouse model. To determine age-related AD progression, we measured circuit connectivity in age-matched littermate control and AD model mice at two different ages (3-4 vs. 10-11 months old). We semi-quantitatively mapped the connectivity strengths of neural circuit inputs to hippocampal CA1 excitatory neurons from brain regions including hippocampal subregions, medial septum, subiculum and entorhinal cortex, comparing different age groups and genotypes. We focused on this brain region because of its clear relationship with learning and memory and that the hippocampal formation shows clear neuropathological changes in human AD. Our results reveal alterations in circuit connectivity of hippocampal CA1 in AD model mice. Overall, we find weaker extrinsic connectivity CA1 input strengths in AD model mice compared with control mice. Unexpectedly, we find a connectivity pattern shift with an increased proportion of inputs from the CA3 region to CA1 excitatory neurons when comparing young and old AD model mice, as well as old wild-type mice and old AD model mice. These unexpected shifts in CA3-CA1 input proportions in this AD mouse model suggest the possibility that compensatory circuit increases may occur in response to connectivity losses in other parts of the hippocampal circuits. We expect that this work provides new insights into the neural circuit mechanisms of AD pathogenesis.

Disclosures: Q. Ye: None. **G. Gast:** None. **X. Su:** None. **T. Saito:** None. **T.C. Saido:** None. **T.C. Holmes:** None. **X. Xu:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant R01NS118442

Title: State dependent network alterations in a mouse model of Alzheimer's Disease

Authors: *S. J. BRUNWASSER[1], C. FARRIS[3], K. BHASKARAN-NAIR[2], J. D. WHITESELL[4], J. A. HARRIS[4], K. B. HENGEN[2];
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Abstract: Alzheimer's Disease (AD) has been extensively studied at a cellular level, yet it remains poorly understood how neurodegenerative pathology produces systems level impairments of memory. Whereas neurodegeneration is widespread across the brain in late disease, studies of early disease reveal restricted pathology within specific networks important for stable memory. These findings suggest that impairments produced by AD are not a consequence of pathology in single brain regions, but rather due to failures of communication between regions that are part of a memory network. Additionally, AD patients show disruptions in stages of sleep important for memory consolidation, suggesting that functional deficits may be both spatially and temporally restricted. We sought to determine if communication impairments in a mouse model of early AD (APP/PS1) are restricted to networks functionally involved in memory, and whether such impairments are more pronounced in a sleep/wake dependent manner. We performed chronic in vivo electrophysiology across natural sleep and wake states in three cortical sites - retrosplenial cortex (RSP), dorsal anterior cingulate cortex (ACAd), and visual cortex (VISp) - which form two monosynaptic pathways - one involved in memory (RSP - ACAd) and one involved in vision (RSP - VISp) with the hypothesis that the RSP - ACAd pathway would show selective communication deficits in APP/PS1 mice. We found that local single unit statistics, neuron yield, and firing rate activity failed to differentiate vulnerable pathways in APP/PS1 mice. However, increased feedforward drive was seen from RSP to ACAd selectively during sleep, while RSP - VISp communication was preserved. This finding led us to the hypothesis that increased unidirectional activity within the RSP-ACAd circuit is a compensation for noisy communication. Subsequent analyses revealed that communication between RSP and ACAd has high variance in APP mice which is especially pronounced during sleep. Taken together, these findings suggest that functional pathology in APP/PS1 mice is specific to memory pathways and depends on sleep state. Identifying circuits that are selectively impaired early in AD will spur the development of systems-based treatment approaches.

Disclosures: S.J. Brunwasser: None. **C. Farris:** None. **K. Bhaskaran-Nair:** None. **J.D. Whitesell:** A. Employment/Salary (full or part-time):; Cajal Neuroscience. **J.A. Harris:** A. Employment/Salary (full or part-time):; Cajal Neuroscience. **K.B. Hengen:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG066171
A2020833S
AARG-18-52336

Title: Optogenetic targeting of astrocytes restores sleep-dependent brain rhythm function and slows Alzheimer's disease

Authors: Y. E. LEE[1], A. N. RUSS[2], Q. ZHAO[2], M. MACI[2], M. R. MILLER[2], S. S. HOU[2], M. ALGAMAL[2], A. ARAQUE[3], E. GALEA[4], B. J. BACSKAI[2], ***K. KASTANENKA**[2]; [1]Boston Univ., Boston, MA; [2]Massachusetts Gen. Hosp., Charlestown, MA; [3]Univ. of Minnesota, Minneapolis, MN; [4]ICREA, Barcelona, Spain

Abstract: Patients with Alzheimer's disease (AD) exhibit sleep disturbances, specifically deficits in deep non-rapid eye movement (NREM) sleep. Disruption of NREM slow waves occurs early in the disease progression and is recapitulated in transgenic mouse models of beta-amyloidosis. However, the mechanisms underlying slow-wave disruptions remain unknown. Also, it is not clear if these sleep disturbances are a cause or effect of AD. Because astrocytes contribute to slow-wave activity, we used multiphoton microscopy and optogenetics to investigate whether they contribute to slow-wave disruptions in APP mice. We monitored astrocytic calcium transients expressing genetically encoded calcium reporter Yellow Cameleon 3.6 (YC3.6) using multiphoton microscopy. The power but not the frequency of astrocytic calcium transients associated with slow waves was reduced. Optogenetic activation of astrocytes at the endogenous frequency of slow waves restored slow-wave power, reduced amyloid deposition, prevented neuronal calcium elevations, and improved memory performance. Our findings revealed malfunction of the astrocytic network driving slow-wave disruptions. Thus, targeting astrocytes to restore circuit activity underlying sleep and memory disruptions in AD could ameliorate disease progression.

Disclosures: **Y.E. Lee:** None. **A.N. Russ:** None. **Q. Zhao:** None. **M. Maci:** None. **M.R. Miller:** None. **S.S. Hou:** None. **M. Algamal:** None. **A. Araque:** None. **E. Galea:** None. **B.J. Bacskai:** None. **K. Kastanenka:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH U24 AG072701

Title: The influence of social isolation on the fronto-insular circuits and behaviors in a mouse model of Alzheimer's Disease

Authors: *H. WNUK[1], Y. ZUO[2], K. H. WANG[3];
[1]Dept. of Biol., Univ. of Rochester, Rochester, NY; [2]Dept. of Molecular, Cell and Developmental Biol., UC Santa Cruz, Santa Cruz, CA; [3]Dept. of Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Social isolation has been identified as a risk factor for developing Alzheimer's Disease (AD), a dementia that impairs an individual's cognitive functions. Recent research has implicated the fronto-insular cortical circuits in socioemotional processing and regulation of cognitive decisions. However, how social isolation influences AD pathology and cognitive deficits associated with fronto-insular cortices is unknown. Here, using the APP/PS1 mouse model of amyloidosis in AD, we investigated the interaction between AD genetic risk factors and social isolation on fronto-insular pathology and cognitive behaviors. We adopted the commonly used rodent attentional set-shifting task (AST) to assess how social isolation influences cognitive flexibility in APP/PS1 mice. Adult APP/PS1 and wild-type (WT) mice starting at 3-months of age were housed in group or isolation for more than a month and then tested in AST. Our preliminary data showed that these four groups of mice did not show statistical differences in their performance during early stages of AST, during which they learned to distinguish compound olfactory and tactile cues for food reward and shifted their choices to new relevant cues within a single sensory dimension. However, at the last and most advanced stage of AST when the relevant cues changed to a different sensory dimension, i.e., extradimensional shifting (EDS), socially isolated APP/PS1 mice were more persistent in their responses to the irrelevant sensory dimension and showed the worst performance among the four groups, whereas the group-housed WT control showed the best performance. These results suggest that social isolation exacerbates cognitive rigidity that would develop in AD models. Because previous studies have suggested that cognitive flexibility measured by EDS depends on the medial prefrontal cortex and potentially interconnected anterior insular cortex, we established a set of molecular histological assays to assess potential changes in beta-amyloid deposition as well as neuronal and microglial activation in response to social isolation. Ongoing experiments aim to quantify the impacts on fronto-insular circuits resulting from the interaction between AD genetic factors and social isolation, which may provide mechanistic insight into how social isolation, as perceived through fronto-insular cortices, influence AD progression.

Disclosures: H. Wnuk: None. **Y. Zuo:** None. **K.H. Wang:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RF1 AG064859

Title: Longitudinal behavioral characterization of 5xFAD (C57BL/J6) mice confirms sexual dimorphism and finds novel differences in motivational/apathy behaviors

Authors: *V. A. DAVIS, D. J. BRAUN, L. J. VAN ELDIK;
Sanders-Brown Ctr. on Aging, Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: The 5xFAD model is a commonly used transgenic mouse line co-expressing five familial Alzheimer's disease (5xFAD) mutations that are inherited together and lead to accelerated amyloid plaque formation. Many publications report behavioral studies of 5xFAD mice; however, most of these studies test only one sex or do cross-sectional testing at a specific age. Discerning the temporal progression of AD-like learning and memory deficits in both sexes of 5xFAD mice and identifying behavioral assays that reveal subtle impairments would be useful as a guide to select appropriate ages and behavioral tests for specific research questions. Some longitudinal batteries have been reported, but they have not included frequently used water mazes. Here we describe a longitudinal battery of behavioral tests developed to chart the normal aging impairment expected in both male and female 5xFAD (C57BL/6J background) mice as compared to wildtype (WT) littermates. At approximately 9 months of age, 32 mice (evenly balanced for sex and genotype), began the first of three cycles of a 3-week battery, each cycle separated by 3 months. The battery consisted of four consecutive days of dry maze testing in week 1 (Open Field, Spontaneous Alternation, Novel Spatial Recognition, Rotarod). The following week, a 2-day 8-arm Radial Arm Water Maze (RAWM) test was done, followed by one day of Open Pool testing. The next day, mice were assessed using the Frailty Index, then singly housed with free access to running wheels for 7 days before being returned to normal housing. The resulting data provide a temporal progression of behavioral deficits in 5xFAD males and females and reveal a sexual dimorphism in behavior consistent with that seen in AD-like brain pathology, wherein females show more impairment than males at similar ages. This sexual dimorphism suggests different tests may be necessary at different ages for males and females, providing important information to investigators working with this model. This work also provides the novel incorporation of a water maze in a longitudinal study of the 5xFAD model. Interestingly, the RAWM data indicate females may express motivational/apathy behaviors sooner than in males (as measured by errors of omission).

Disclosures: V.A. Davis: None. **D.J. Braun:** None. **L.J. Van Eldik:** None.

Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: PRIN 2017A9MK4R_004

Title: Role of IL-1β in synaptic and memory deficits in Herpes Simplex Virus Type-1-infected mice

Authors: D. D. LI PUMA[1,2], B. BANDIERA[1], M. RINAUDO[1,2], F. PACIELLO[1,2], G. PULIATTI[1], C. RIPOLI[1,2], G. DE CHIARA[3], A. PALAMARA[4,5], R. PIACENTINI[1,2], *C. GRASSI[1,2];
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Abstract: Experimental and epidemiological data suggest that repeated infections of the central nervous system with Herpes Simplex Virus Type-1 (HSV-1) are a risk factor for Alzheimer's disease (AD). We recently developed a mouse model of HSV-1 replications within the brain, triggered by thermal stress (TS), exhibiting typical AD hallmarks including accumulation of amyloid-β and phosphorylated tau proteins, increased levels of the proinflammatory cytokine interleukin 1β (IL-1β) and impaired hippocampal neurogenesis (De Chiara et al., *PLoS Pathogens*, 2019; Li Puma et al., *Stem Cells*, 2019). By exploiting this model, we investigated the role of IL-1β in the HSV-1-induced synaptic dysfunction. Our results showed that synaptic plasticity at the hippocampal CA3-CA1 synapse, assessed by long-term potentiation (LTP) paradigm, was significantly reduced in brain slices from HSV-1-infected mice undergone two TSs compared to mock-infected ones (fEPSP amplitude potentiation: 58.2±7.9 *vs.* 97.6±10.8%, respectively; $p<0.05$). Accordingly, we found downregulated expression of pre- and postsynaptic proteins in hippocampi of infected mice (-60% *vs*. mock), along with a significant reduction of spine density in both apical dendrites (from 1.67±0.06 spines/µm in the mock to 1.34±0.03/µm in infected mice) and basal ones (from 1.64±0.06 to 1.43±0.04/µm in mock- and HSV-1-infected mice, respectively; $p<0.05$). In HSV-1-infected mice we also found significantly greater expression of two epigenetic repressors, MeCP2 and REST, at both protein (+59% and +95% *vs*. mock, respectively; $p<0.05$) and mRNA (+4.8 and +3.8 folds *vs*. mock, respectively; $p<0.05$) levels that negatively impinge on the expression of plasticity-related genes. All these effects were associated with increased levels of IL-1β in HSV-1-infected mice (20.9±1.1 *vs*. 5.8±2.1 pg/mg in controls; $p<0.05$) and were significantly rescued by blocking the IL-1 receptor with Anakinra. Indeed, this treatment: i) increased LTP from 58.2±7.9 to 87.2±10.5% ($p<0.05$); ii) rescued the expression of synaptic proteins and dendritic spine density to values similar to controls; iii) counteracted the HSV-1-induced increases in MeCP2 and REST at both mRNA and protein levels; iv) improved memory assessed by novel object recognition test performed two weeks after 2TS (the preference index being 63.0±2.7% in Anakinra-treated, HSV-1-infected mice *vs*. 57.1±.2.14% of HSV-1 mice; $p<0.05$). Collectively, our results suggest that IL-1β-activated pathways triggered by HSV-1 reactivation contribute to an AD-like phenotype probably acting via upregulation of MeCP2 and REST.

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Poster

278. Mouse Models of Alzheimer's Disease: Molecular, Cellular, Circuit, and Behavioral Studies

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 278.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH 5P20GM109025
NIH 5R01AG062762-02

Title: Evaluation of sex differences in a novel mouse model with loss of $GABA_B$receptors

Authors: *A. M. L. OSSE, A. A. ORTIZ, E. N. STROM, J. W. KINNEY;
Dept. of Brain Hlth., Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Alzheimer's Disease (AD) is a neurodegenerative disease that is clinically described as progressive learning and memory deficiencies. Pathologically, AD is characterized by the presence of three core features, beta-amyloid plaques (Aβ), neurofibrillary tangles (NFT), and chronic neuroinflammation. The activation of the immune response through glia cells is associated with neuroinflammation and has been shown to promote and exacerbate both Aβ and NFT pathologies. Alterations in gamma amino butyric acid (GABA), the principle inhibitory neurotransmitter in brain, shown to be involved in several processes, have been demonstrated in AD patients. In addition, GABAergic signaling markers, including the metabotropic receptor $GABA_B$, have also been shown to be altered in an AD animal model (Salazar 2021). $GABA_B$ receptors are expressed on glia and have been shown to have anti-inflammatory properties. Loss of this receptor could play an important role in exacerbating the disease. To investigate how the decrease in the $GABA_B$ receptor modulates neuroinflammation, we developed a novel mouse model with the loss of this receptor restricted to glia (GAB/CX3ert) that results in alterations in amyloid processing as well as network activity. Currently, two-thirds of AD patients are women. Sex differences within neuroinflammation have been identified and may contribute to the higher prevalence. In this study, we aimed to evaluate the alterations between male and female GAB/CX3ert mice and investigate sex differences on inflammatory mechanisms, as well as AD related pathology, in mice with a reduction of $GABA_B$ receptors on glia, as well as how they differ from control mice. These differences were investigated through cellular and molecular techniques, including flow cytometry, Luminex multiplex assay, immunohistochemistry, and cell culture. This study demonstrates how sex can influence neuroinflammation, potentially contributing to higher rates of AD in women, and will help in the development of possible sex-specific therapeutic treatments for AD.

Disclosures: **A.M.L. Osse:** None. **A.A. Ortiz:** None. **E.N. Strom:** None. **J.W. Kinney:** None.

Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/Washington University Medical School Grant 1RF1AG071706-01A1

Title: Alzheimer's Disease-associated circHomer1 can inhibit the expression of long APP and MAPT mRNA isoforms in the frontal cortex via competing for binding to HuD.

Authors: ***M. OTERO**[1], G. PAPAGEORGIOU[1,2], S. ECKEL[1], M. R. WESTENSKOW[1], I. EL-SHARKAWY[1], N.-P. NYKÄNEN[3], B. A. BENITEZ[3,4], C. CRUCHAGA[3,5], N. MELLIOS[1,2]; [1]Univ. of New Mexico Dept. of Neurosciences, Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM; [2]Autophagy Inflammation and Metabolism Ctr. of Biomed. Res. Excellence, Univ. of New Mexico Hlth. Sci. Ctr., Albuquerque, NM; [3]Dept. of Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO; [4]Dept. of Neurology, Harvard Med. Fac. Physicians at Beth Israel Deaconess Med. Center, Harvard Med. Sch., Boston, MA; [5]Dept. of Neurology, Hope Ctr. for Neurolog. Diseases, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Circular RNAs (circRNAs) are a novel category of non-coding RNAs derived from the back-splicing and covalent joining of exons or introns. Recent studies have suggested that circRNAs are preferentially generated from synaptic plasticity-related genes and are particularly enriched in the brain. Although some circRNAs have been found to sequester microRNAs and others to associate with RNA-binding proteins (RBPs), the mechanism of action of most circRNAs remains poorly understood. Moreover, little is known about the potential involvement of circRNAs in Alzheimer's disease (AD). Using circRNA-specific quantification, we had previously found that *circHomer1*, a neuronal-enriched circRNA derived from Homer protein homolog 1 (*HOMER1*) capable of regulating cognitive function, is significantly downregulated postmortem brains of patients with AD and robustly associated with clinical dementia ratings and AD-associated neuropathology. Here we show that *in vivo* knockdown (KD) of *circHomer1* in mouse frontal cortex results in a significant upregulation of long Amyloid precursor protein (*APP*) and microtubule-associated protein tau (*MAPT*) mRNA isoforms, of which accumulation of their respective encoded proteins are the pathological hallmark of AD. Furthermore, we show that *circHomer1* is predicted to directly bind to both of these mRNA isoforms, potentially competing for binding with ELAV-like protein 4 (*ELAVL4* or HuD), an RBP associated with AD and known to both bind to *circHomer1* and associate with the long *APP* mRNAs, to promote their stability. Lastly, we demonstrate that *circHomer1* is reduced in iPSC-derived neurons from subjects with AD and in the cortex of the 5xFAD model of AD. Ongoing experiments are aimed at further investigating the role of *circHomer1* in *APP* and *MAPT* gene regulation and AD-associated pathogenesis and examining the effects of different drugs on brain *circHomer1*

expression. Taken together, our work introduces novel molecular networks with potential importance for AD.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant 1RF1AG058674-01
The JPB Foundation
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FRQS Postdoctoral Training Fellowship
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NIH Grant RF1AG063755

Title: Rate of tau propagation is a heritable disease trait in genetically diverse mouse strains

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Abstract: One of the most important risk indicators for developing Alzheimer's disease (AD) is a family history of dementia. Despite growing interest in understanding how AD-associated genetic polymorphisms lead to pathological outcomes, it remains unclear how polygenic factors contribute to the formation and expansion of tau neurofibrillary lesions, which are the closest correlate of neural degeneration and clinical symptoms during AD. While clinical data indicate that severity of tau pathology is likely influenced by heritable factors, studying this phenomenon in an experimental setting is especially difficult. We tested whether the rate of tau propagation is a heritable disease trait using a large, well-characterized cohort of 19 genetically diverse mouse strains, based on the BXD genetic reference panel. P301L-mutated human tau (hTau) was introduced into the entorhinal cortex using an AAV-based model system of tau propagation, distinguishing hTau-producing cells from recipient neurons of tau protein transfer. Interestingly, some mouse strains were relatively resistant to tau spread despite strong AAV expression, while others were particularly vulnerable. Importantly, the heritability (h^2_{RIX}) of tau propagation was calculated to be 0.435; that is, ~43.5% of the observed variance in tau propagation could be

explained by heritable factors. Total fluorescence and percent area of microglia- and astrocyte-specific markers, Iba1 and GFAP respectively, varied significantly across BXD groups. However, there was no correlation between glial abundance and tau propagation. Given that the risk of developing AD has a strong heritable component, identifying genetic variants that influence tau propagation may help uncover novel molecular targets to prevent or slow the pace of tau spread and cognitive decline.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

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Program #/Poster #: 279.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: P01 AG014449
P30 AG072931
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R01 AG060731
R01AG051086
R21AG056007
R21AG074539

Title: Microrna dysregulation in the default mode network during the progression of ad

Authors: J. S. BECK[1], M. MALEK AHMADI[3], J. KOCHMANSKI[1], S. D. GINSBERG[4,5], E. J. MUFSON[6], D. K. LAHIRI[7], ***S. E. COUNTS**[2];
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Abstract: Perturbations in non-coding microRNAs (miRNAs) regulating diverse brain physiological pathways are linked to Alzheimer's disease (AD). For instance, we recently reported a role for miR-298 and miR-20b in AD neurophysiology and disease probability. Notably, altered functional connectivity of resting-state networks such as the default mode network (DMN) is increasingly appreciated as a critical component of prodromal AD and may even play a role in cognitive resilience. However, the extent to which miRNA dysregulation plays a role in these phenomena is unclear. To address this question, we sequenced miRNA transcripts in posterior cingulate cortex (PCC), a DMN hub that underlies autobiographical

memory retrieval and shows hypometabolic changes early in AD. Postmortem PCC samples were obtained from Rush ROS/MAP participants who died with a diagnosis of a) no cognitive impairment and low pathology (NCI-LP, Braak stage I/II, n = 12), b) NCI with high pathology (NCI-HP, Braak stage IV, representing cognitive resilience, n = 8), c) mild cognitive impairment (MCI, n = 10), or d) dementia due to AD (n = 9). Differential expression analysis (FDR = 0.04) revealed 42 miRNAs that were significantly dysregulated among the NCI (independent of Braak stage), MCI, and AD groups. Two miRNAs, miR-99a (p = 0.01) and miR-664b (p = 0.005), were downregulated in AD compared to NCI and MCI, whereas miR-30a (p = 0.006), miR-374a (p = 0.004), and miR-501 (p = 0.005) were significantly upregulated in MCI compared to NCI and AD. Post hoc comparisons revealed three miRNAs significantly downregulated in NCI-HP compared to NCI-LP: miR-103a (p= 0.04), miR-211 (p = 0.03), and miR-4443 (p = 0.03). These miRNAs may be operating in resilience-related pathways in the DMN. Correlation analysis revealed that decreasing PCC levels of miR-664b levels (see above) were associated with poorer performance on antemortem tests of episodic memory (r = 0.41, p = 0.009), semantic memory (r = 0.44, p = 0.004), and visuospatial ability (r = 0.45, p = 0.004). Furthermore, levels of miR-4634, which were not significantly dysregulated among the groups, were nonetheless associated with episodic memory (r = 0.41, p = 0.01), working memory (r = 0.45, p = 0.005), and the global cognitive *z* score for the subjects (r = 0.45, p = 0.005), as well as Braak (r = 0.47, p = 0.003) and NIA-Reagan (r = 0.36, p = 0.03) diagnostic criteria. Current data analysis is focused on functional pathway enrichment and mRNA target identification to elucidate potential pathogenic miRNA-related mechanisms contributing to PCC and DMN dysfunction in AD, which may inform biomarker and intervention strategies.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: The JPB Foundation
The Cockrell Foundation

Title: Studies of the functional consequences of the Alzheimer's disease associated variant CIC^{P36L}

Authors: *H. LEE[1], M. A. DURHAM[1], K. LEE[1], O. LICHTARGE[1], H. Y. ZOGHBI[2]; [2]Howard Hughes Med. Inst., [1]Baylor Col. of Med., Houston, TX

Abstract: Late-onset Alzheimer's disease (LOAD) is a highly heritable disorder. However, despite the large-scale sequencing effort, most of the genetic risk factors for LOAD remain to be

identified. Identifying such risk variants will be critical for understanding the pathogenesis and potentially discovering therapeutic targets for AD. Recently, we and our collaborators demonstrated that loss of Ataxin1 (ATXN1), a gene previously identified to be associated with AD risk but without clear functional evidence, potentiates AD pathology mediated by the impaired ATXN1-CIC complex function in mice. Capicua (CIC) encodes a transcriptional repressor which forms a co-repressor complex with ATXN1, and this interaction is critical for CIC protein stability. Knock out of ATXN1 in mice, leads to reduced CIC protein level, which, in turn, activates a transcriptional cascade of ETV4/5 and β−secretase (BACE1). An increase in BACE1 accelerates generating pathogenic amyloid-beta (Aβ) species which ultimately exacerbates AD pathology. Interestingly, while CIC is critical for survival, heterozygous loss of CIC by truncating variants cause intellectual disability in patients. Given the link between loss of CIC protein function and increase in BACE1 mediated AD pathology, we hypothesized that less severe mutations in CIC contribute to AD pathology. We searched the AD databases for CIC variants and identified a rare heterozygous missense variant, CIC^{P36L}, which appeared only in four LOAD patients but not in the controls. The CIC^{P36L} variant is located within the highly conserved ATXN1 binding domain of CIC which suggests it could affect the interaction with ATXN1. We demonstrated that CIC^{P36L} reduces ATXN1-CIC interaction by 60% in cells. Thus, we hypothesize that this variant leads to a partial loss of function of the ATXN1-CIC complex and could potentiate AD pathology via upregulation of BACE1. To evaluate the functional consequences of the variant in vivo, we generated a Cic^{P36L} knock-in mouse. With the novel $Cic^{P36L/P36L}$ mouse model, we showed that Cic^{P36L} reduces CIC protein stability and leads to de-repression of the downstream *Etv4/5-Bace1* axis without affecting survival or body weight phenotype. These data suggest that CIC^{P36L} leads to partial loss-of-function of CIC, subsequent upregulation of *Bace1*, and vulnerability to AD. This work underscores the need to search for rare variants that could be missed in population-based association studies and potentially demonstrate a mechanism for how less severe variants of a gene that cause intellectual disability could contribute to AD pathology.

Disclosures: H. Lee: None. **M.A. Durham:** None. **K. Lee:** None. **O. Lichtarge:** None. **H.Y. Zoghbi:** None.

Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Electromagnetized gold nanoparticles improve neurogenesis and cognition in the aged brain

Authors: *S. AN, J. KIM;
Chem., Dongguk Univ., Seoul, Korea, Republic of

Abstract: Adult neurogenesis is the lifelong process by which new neurons are generated in the dentate gyrus. However, adult neurogenesis capacity decreases with age, and this decrease is closely linked to cognitive and memory decline. Our study demonstrated that electromagnetized gold nanoparticles (AuNPs) promote adult hippocampal neurogenesis, thereby improving cognitive function and memory consolidation in aged mice. According to single-cell RNA sequencing data, the numbers of neural stem cells (NSCs) and neural progenitors were significantly increased by electromagnetized AuNPs. Additionally, electromagnetic stimulation resulted in specific activation of the histone acetyltransferase Kat2a, which led to histone H3K9 acetylation in adult NSCs. Moreover, in vivo electromagnetized AuNP stimulation efficiently increased hippocampal neurogenesis in aged and Hutchinson-Gilford progeria mouse brains, thereby alleviating the symptoms of aging. Therefore, our study provides a proof-of-concept for the in vivo stimulation of hippocampal neurogenesis using electromagnetized AuNPs as a promising therapeutic strategy for the treatment of age-related brain diseases.

Disclosures: S. An: None. **J. Kim:** None.

Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

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Program #/Poster #: 279.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Lieber Institute for Brain Development
10x Genomics

Title: Influence of amyloid and tau pathology on local microenvironment gene expression in the human inferior temporal cortex in Alzheimer's disease

Authors: *S. KWON[1], M. TIPPANI[2], S. PARTHIBAN[3], J. S. LOBANA[4], C. BRUCE[5], S. WILLIAMS[5], M. MAK[5], G. YU[5], J. AVALOS-GRACIA[5], T. H. HYDE[2], S. C. PAGE[2], S. C. HICKS[3], L. COLLADO-TORRES[2], K. MARTINOWICH[1], K. R. MAYNARD[1];
[1]Johns Hopkins Sch. of Med., Baltimore, MD; [2]Lieber Inst. for Brain Develop., Baltimore, MD; [3]Johns Hopkins Bloomberg Sch. of Publ. Hlth., Baltimore, MD; [4]Johns Hopkins Krieger Sch. of Arts and Sci., Baltimore, MD; [5]10x Genomics, Pleasanton, CA

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder marked by senile plaques of β-amyloid (Aβ) and neurofibrillary alterations of phosphorylated tau (pTau). Accumulating evidence suggests that Aβ and tau act in concert to orchestrate complex pathobiological events underlying AD, but their impact on the local microenvironment remains poorly understood. Here, we profiled spatial gene expression patterns surrounding local amyloid and tau pathology within the human inferior temporal cortex (ITC) of individuals with AD. We employed 10x Genomics Visium spatial transcriptomics coupled with immunofluorescence (Visium-IF) to generate an integrated proteomic and transcriptomic spatial map of the human ITC in AD.

Visium-IF pairs IF staining with on-slide cDNA synthesis across 5000 oligonucleotide-barcorded spots to create spatial maps of gene and protein expression in the same tissue section. For this study, tissue blocks containing ITC were dissected from 3 brain donors diagnosed with late-onset AD (Amyloid C/Braak III) and 1 age-matched neurotypical adult donor. Cryosections from each block were collected in duplicate on Visium arrays and immunostained for Aβ and pTau Ser202/Thr205 to visualize amyloid plaques and neurofibrillary structures. Multispectral images were acquired and libraries were prepared for whole transcriptome sequencing. Following segmentation of Aβ and pTau fluorescent signals, gene expression spots were annotated by pathological burden in the following categories: no pathology, Aβ+, Aβ-adjacent, pTau+, pTau-adjacent, both Aβ/pTau, and both-adjacent. Within the gray matter region, we pseudobulked these spots by category for AD donors and performed differential gene expression analysis to identify transcriptional signatures associated with each pathological combination. Candidate differentially-expressed genes were validated by IF-combined RNAscope in situ hybridization technology and characterized by gene ontology analysis to implicate their molecular functions in putative disease mechanisms and pathways. Finally, we performed multi-marker analysis of genomic annotation (MAGMA) to identify which pathological domains in this study harbored aggregated genome-wide association study (GWAS) risk for neurodegenerative diseases and dementia and further investigated spatial enrichment of genes associated with AD using publicly available bulk and single-nucleus RNA-seq datasets. Our novel approach can disentangle molecular dynamics and regional heterogeneity in gene expression while refining annotation of genes that are functionally linked to AD-afflicted local microenvironments in the human brain.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.07

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Transcriptional activation with Cas9 activator nanocomplexes rescues Alzheimer’s disease pathology

Authors: *Y. KIM, J. KIM;
Chem., Dongguk Univ., Seoul, Korea, Republic of

Abstract: N^6-methyladenosine (m^6A), a conserved epitranscriptomic modification of eukaryotic mRNA (mRNA), plays a critical role in a variety of biological processes. Here, we report that m^6A modification plays a key role in governing direct lineage reprogramming into induced neuronal cells (iNs). We found that m^6A modification is required for the remodeling of specific mRNAs required for the neuronal direct conversion. Inhibition of m6 A methylation by Mettl3 knockdown decreased the efficiency of direct lineage reprogramming, whereas increased m^6A methylation by Mettl3 overexpression increased the efficiency of iN generation. Moreover, we found that transcription factor Btg2 is a functional target of m^6A modification for efficient iN generation. Taken together, our results suggest the importance of establishing epitranscriptomic remodeling for the cell fate conversion into iNs.

Disclosures: Y. Kim: None. **J. Kim:** None.

Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: New insights on Bridging integrator 1 protein isoforms as a risk increasing gene in Alzheimer's disease

Authors: *M. HU[1], P. REINHARDT[2], M.-T. WEIL[2], L. BAHNASSAWY[2], L. GASPARINI[2], J. F. WARING[1], A. VASANTHAKUMAR[1];
[1]Genomics Res. Ctr., Abbvie, North Chicago, IL; [2]Neurosci. Res., Abbvie, Ludwigshafen, Germany

Abstract: New insights on Bridging integrator 1 protein isoforms as a risk increasing gene in Alzheimer's disease Min Hu[1], Peter Reinhardt[2], Marie-Theres Weil[2], Lamiaa Bahnassawy[2], Laura Gasparini[2], Jeffrey F. Waring[1], Aparna Vasanthakumar[1 1] AbbVie Inc., 1 North Waukegan Rd., North Chicago, IL 60064 [2] AbbVie Deutschland GmbH & Co. KG, Neuroscience Research, Knollstrasse, 67061 Ludwigshafen
Genome-wide association study (GWAS) identified the Bridging integrator 1 (BIN1) as the second -most-prevalent genetic risk factor for late-onset of Alzheimer's disease (AD). However, not much is known about the specific SNPs that drive this increased risk and the functional impact of the SNP. BIN1 gene has 20 exons and is transcribed into 14 alternate transcripts, of which 11 are protein-coding. The role of genetics and cell type specificity on the expression of these 11 BIN1 isoforms is largely unknown, as is the main roles that they play in AD disease susceptibility or progression. To address these questions, protein coding isoform specific droplet digital polymerase chain reactions (ddPCR) were performed on induced pluripotent stem cells (iPSC) and lymphoblastoid cell lines (LCL) carrying the susceptibility variants. During the differentiation from iPSCs to neurons, expression levels of neuron-specific BIN1-isoforms (isoform 1-5 and 7) are significantly increased in a time-dependent manner. Microglia-specific

BIN1-isoforms (isoform 6, 9, 10 and 12) are decreased. Opposite expression patterns are observed in iPSCs microglia. Our LCL data indicates that the presence of certain BIN1 SNPs might correlate with the expression of BIN1 isoforms. Recent literature suggests that BIN1 affects tau aggregation by modulating many cellular functions including endocytosis/trafficking, inflammation, calcium homeostasis, and apoptosis. Given the strong association with susceptibility to AD, exploring the molecular mechanisms of cell-type specific BIN1 expression could serve as a valuable resource for selection of promising genes for drug development and stratification approaches.

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Disclosures: **M. Hu:** A. Employment/Salary (full or part-time):; Abbvie. **P. Reinhardt:** A. Employment/Salary (full or part-time):; Abbvie. **M. Weil:** A. Employment/Salary (full or part-time):; Abbvie. **L. Bahnassawy:** A. Employment/Salary (full or part-time):; Abbvie. **L. Gasparini:** A. Employment/Salary (full or part-time):; Abbvie. **J.F. Waring:** A. Employment/Salary (full or part-time):; Abbvie. **A. Vasanthakumar:** A. Employment/Salary (full or part-time):; Abbvie.

Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Microscoop, a Discovery-Based Image-Guided Proteomics Technology, Reveals Novel Factors on Amyloid-Beta Aggregates in Differentiated SH-SY5Y Cells

Authors: *C.-C. HUANG, W.-M. CHONG, C.-K. HUANG, Y.-D. CHEN, C.-W. CHUNG, H.-J. CHANG, J.-C. LIAO;
SYNCELL(Taiwan) Inc., Syncell, Inc., Taipei, Taiwan

Abstract: Aggregation of amyloid-β peptides (Aβ) is a hallmark of Alzheimer's disease (AD). Subcellular distribution of Aβ is well recognized under a microscope, but its pathological and physiological functions remain unclear, partially due to our limited understanding of interacting proteins and corresponding signaling pathways associated with Aβ. Existing spatial proteomics technologies focus on mapping of known proteins using antibody panels/arrays, hindering hypothesis-free proteome discovery. In this study, we used Microscoop^{TM}, a fully-automated microscopy-guided subcellular photolabeling with a machine learning-based precision recognition to enable discovery-based image-guided proteomics. We applied Aβ1-42 deposition in human neuroblastoma, SH-SY5Y, differentiation cells as an AD model. Multiple images of Aβ1-42 aggregates were applied to segment to locations of specific Aβ1-42 aggregates of interest using convolutional neural networks (CNN)-based deep learning. Microscoop^{TM} was

used to illuminate these segmented regions to induce photochemical reactions of proprietary photosensitive probes and trigger spatial covalent labeling of proteins adjacent to Aβ1-42 aggregates. This spatial-specific photochemical labeling process was repeated automatically on thousands of microscopic fields of view to accumulate enough Aβ1-42-associated proteins for LC/MS-MS-based proteome identification. A series of novel factors were discovered to be associated with Aβ aggregation in SH-SY5Y differentiated cells. We further validated these newly identified proteins using antibody staining and found that these proteins indeed colocalized with Aβ1-42. The finding of these novel factors opens a door to reveal associated signaling pathways related to AD. They may also serve as new diagnostics biomarkers or new AD drug target. Our study not only reveals the Aβ-associated spatial proteome, but also demonstrates its possible broad applications on discovery-based spatial proteomics in neuroscience.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG071859-01A1

Title: Vitamin A Deficiency Drives Alzheimer's Disease Progression through Dysregulation of Glucocorticoid Signaling

Authors: A. G. RUDD[1,2], M. MAHMUD SYED[1], B. LIU[6], ***J. J. LAWRENCE**[1,3,4,5]; [1]Pharmacol. and Neurosci., [2]MD/PhD Program, [3]Garrison Inst. on Aging, [4]Ctr. of Excellence for Translational Neurosci. and Therapeut., [5]Ctr. of Excellence for Integrated Hlth., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [6]Psychology, Texas Tech. Univ., Lubbock, TX

Abstract: Glucocorticoid (GC) signaling plays a central role in cellular activity, stress, and immune response. GCs bind to mineralocorticoid and glucocorticoid receptors, and because of their involvement in the stress response, they play a significant role in CNS functions like learning and memory. Previous research has shown that there are strong interrelationships between dysregulation of the hippocampal-pituitary-adrenal axis, GC overexposure, increased serum levels of pro-inflammatory cytokines, and the pathogenesis of Alzheimer's disease (AD) and major depressive disorder (MDD). There is also increasing evidence linking Vitamin A (VA) deficiency in AD. All-trans retinoic acid (ATRA) is the bioactive derivative of VA. Research connecting VA with glucocorticoid expression has shown that VA deficiency leads to an increase in the levels of glucocorticoids in serum of rats but retinoic acid (RA) treatment is able to decrease those levels to baseline. In rodents, VA treatment improves symptoms of AD in vitro

and in vivo by reducing proinflammatory cytokines and amyloidogenesis. Moreover, VA deprived mice exhibit impaired learning. In this study, we investigated the most dysregulated ATRA-sensitive pathways in the human hippocampus in AD. We performed an in silico experiment via Ingenuity Pathway Analysis (IPA)from the publicly available human AD hippocampal transcriptomics data generated by van Rooij and colleagues (2019) using 673 ATRA-sensitive genes. This dataset consisted of 18 AD cases (including both male and female) and 10 control samples. The top canonical pathway was glucocorticoid receptor (GR) signaling (p=4.86E-34). The most dysregulated ATRA-sensitive gene in the Pathway was UQCRC2 (FDR=9.86E-14), which was downregulated in complex III located within mitochondria. A total of 36 genes, including NDUFA genes, in the Mitochondrial Dysfunction pathway were dysregulated (p=2.27E-21), further linking ATRA deficiency to mitochondrial dysregulation inhuman AD. Several previous studies have implicated the importance of dysregulated GR signaling in AD. Our analysis also reveals that ATRA-sensitive neuroinflammatory genes (p=1.63E-22) are upregulated in AD. Combining these results with work done by others, it's very likely that NF-kB1upregulation in MDD may be a major pathway for the development of AD later in life. Finally, our IPA analysis highlighted that the top Upstream Regulator was tretinoin (ATRA itself), validating the ATRA sensitivity of our enriched gene set and the regulation of the GR pathway. Our study provides a wealth of new knowledge regarding interactions between ATRA availability, GR signaling, and mitochondrial function.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

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Program #/Poster #: 279.11

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: CIHR
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CRC
APRI
Genome Canda

Title: Differential adenosine to inosine RNA editing of SINE B2 non-coding RNAs in mouse unveils a novel type of epi-transcriptome response to amyloid beta neuro-toxicity

Authors: L. MITCHELL, L. SAVILLE, M. MOHAJERANI, ***A. ZOVOILIS**;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Alzheimer’s disease (AD) is a multifactorial severe neurodegenerative disorder, whose underlying molecular pathology is largely unclear. Editing of RNA involving conversion

from adenosine to inosine (A-to-I) has been connected with cellular function, bringing epi-transcriptomics to the spot-light. Among the RNAs that have been reported to harbor a large percentage of A-to-I edits are non-coding RNAs generated from Short Interspersed Nuclear Elements (SINEs), such as B2 RNAs in mouse and Alu RNAs in human. We have recently shown that B2 RNAs can act as riboswitches, regulating gene expression though self-cleavage, and they are abnormally processed during amyloid beta pathology in hippocampal cells, contributing to the transcriptome de-regulation observed in this condition. Here, we present a novel A-to-I editing analysis approach, which is customized for repetitive elements, and we report that changes in A-to-I editing of SINE B2 RNAs is associated with epi-transcriptome response to amyloid beta neuro toxicity and pathology. We show that A-to-I editing at specific B2 RNA positions is modified as an early response to amyloid neural toxicity in both the hippocampi of a mouse model of amyloid beta pathology and a hippocampal cell culture model of amyloid beta toxicity. This data suggests that the recently described mode of regulation of gene expression through B2 RNA processing may be intertwined with RNA editing and that the cell may be employing RNA editing as a regulation mechanism of increased B2 RNA processing during response to amyloid beta toxicity. Our findings unveil RNA editing of SINE RNAs as an additional level of epi-transcriptome response to amyloid beta neuro pathology, with potential implications for the role of RNA editing of SINE RNAs also in human and Alzheimer's disease (AD).

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

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Program #/Poster #: 279.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Eclipse, an automated CRISPR platform for the large-scale generation of cell models for the iPSC Neurodegenerative Initiative (iNDI)

Authors: *P. DENG, A. KING, B. SAAVEDRA, M. MORELL, A. CHAIR, D. SAILOR, A. G. SOMMER, K. HOLDEN;
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Abstract: The National Institutes of Health led iPSC Neurodegenerative Disease Initiative (iNDI) is the largest iPSC genome engineering project attempted with the goal of generating a widely available and standardized set of diseased cell models for over 100 single nucleotide variants (SNV) mutations associated with Alzheimer's disease and related dementias (ADRD) in isogenic IPSC lines. The standardization of cell models is of vital importance for the generation of reproducible and actionable data in therapeutic development. As part of a multi-institution collaboration, Synthego was selected for the generation of 25 SNVs in the candidate KOLF2.1 iPSC line. Toward these goals, we describe the use of our automated, high throughput CRISPR

editing platform, ECLIPSE, for the rapid generation of knock-in iPSC models of ADRD. We leveraged our state-of-the-art knock-in methods and automated pipelines for the design, experimental optimization, and clonal isolation of 23 of the candidate target mutations in iPSCs. For each SNV target, at least 3 clonal homozygous and 6 clonal heterozygous mutation lines were generated for a total of 264 clonal cell lines over a 6-month period. The utilization of automated systems such as our ECLIPSE platform are critical catalysts for the rapid development of relevant cell models in large scale disease initiatives such as iNDI.

Disclosures: **P. Deng:** A. Employment/Salary (full or part-time):; Synthego. **A. King:** A. Employment/Salary (full or part-time):; Synthego. **B. Saavedra:** A. Employment/Salary (full or part-time):; Synthego. **M. Morell:** A. Employment/Salary (full or part-time):; Synthego. **A. Chair:** A. Employment/Salary (full or part-time):; Synthego. **D. Sailor:** A. Employment/Salary (full or part-time):; Synthego. **A.G. Sommer:** A. Employment/Salary (full or part-time):; Synthego. **K. Holden:** A. Employment/Salary (full or part-time):; Synthego.

Poster

279. Therapeutic Strategies: Preclinical Model

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Program #/Poster #: 279.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grat R01 AG073826-01A1

Title: A machine learning analysis on a recent human hippocampal transcriptome elucidates multivariate differentiating genes in Alzheimer's Disease

Authors: *C. SANCHEZ-VILLALOBOS[1], K. HALBGEWACHS[2,4,3], R. ZHANG[1], J. J. LAWRENCE[4,5,6,7], R. PAL[1];
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Abstract: Alzheimer's Disease (AD) is the most common cause of dementia and one of the leading causes of death in the United States. The molecular mechanisms underlying AD remain unclear, complicating the development of effective treatments. In this novel work, we apply machine learning (ML) algorithms to transcriptomic data obtained from 28 subjects in a publicly available dataset from van Rooij et al. (2018). The data consisted of 14564 genes from 30 post-mortem hippocampi (18 AD, 10 age-matched controls). We implemented data-driven feature selection algorithms such as ReliefF and Sequential Forward Search to reduce the number of predictors to 40 genes. We also created a Random Forest (RF) Classifier to discriminate among AD and control groups, finding a cross-validation accuracy of 100%. Among the top dysregulated genes was KCNIP1, which has no previous association with AD. KCNIP1 is a

voltage-gated potassium channel interacting protein that assists with trafficking Kv4-containing voltage-gated potassium channels (Kvs) and contributes to its function. Although widely expressed across the CNS, KCNIP1 is shown to be enriched in presynaptic terminals of parvalbumin-positive GABAergic neurons (Xia et al. 2010; Xiong et al., 2009). In transgenic mice, manipulation of KCNIP1 expression bidirectionally alters synaptic transmission dynamics and cellular potassium channel density. There is growing appreciation that GABAergic synaptic dysfunction and excitation-inhibition imbalance contribute to AD, implicating downregulation of KCNIP1 expression in GABAergic dysfunction in human AD. Consistent with this role, there is growing evidence that hippocampal hyperexcitability contributes to AD pathophysiology. KCNIP1 could be a novel predictor of GABAergic dysfunction in AD and a target for therapeutic intervention. Interestingly, KCNIP1 contains a retinoic acid response element, potentially connecting altered vitamin A homeostasis in human AD-related GABAergic dysfunction. Although these are promising results that implicate exciting new cell-type-specific molecular mechanisms in AD, the existing dataset was limited to 28 subjects. We intend to expand this analysis to cross-validate these results with additional human hippocampal transcriptomics datasets and investigate the broader applicability of KCNIP1 and other genes highly expressed in GABAergic neurons as generalizable biomarkers in AD. Finally, we will integrate different data sets to explore relationships between gene expression and hippocampal performance metrics.

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Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.14

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: CIHR PJT-156414

Title: Accumulation and cognitive effect of the histone variant H2A.Z in Alzheimer's Disease

Authors: *S. D. CREIGHTON[1], F. MENG[4], G. STEFANELLI[2], M. A. BRIMBLE[6], L. HATEGAN[1], J. ZAKARIA[1], E. A. COLLINS[1], N. A. GAJEWSKA[1], A. REDA[1], T. A. B. MCLEAN[1], B. WALTERS[1], M. FAHNESTOCK[7], E. OROUJI[8], P. MURPHY[5], I. B. ZOVKIC[3]; [2]Dept. of Psychology, [3]Psychology, [1]Univ. of Toronto Mississauga, Mississauga, ON, Canada; [4]Univ. of Rochester, Rochester, NY; [5]Univ. of Rochester, Rochester t, NY; [6]St. Jude Children’s Res. Hosp., Memphis, TN; [7]Psychiatry & Behavioural Neurosciences, McMaster Univ., Hamilton, ON, Canada; [8]Princess Margaret Genomic Ctr., Toronto, ON, Canada

Abstract: The dysregulation of gene expression has a role in cognitive deficits and neuropathology in Alzheimer’s disease (AD), thus prompting interest in epigenetic factors as

mechanisms of neurodegeneration and memory loss. Here, we assess the therapeutic potential of the histone variant H2A.Z, a memory suppressor that is actively removed from DNA during learning to promote gene expression and memory formation. In the aged brain, H2A.Z levels increase and may act as a prelude to age-related memory decline. We hypothesize that H2A.Z also accumulates in AD and that depletion of H2A.Z is an effective therapy for memory deficits. To characterize H2A.Z levels in the AD brain, we assessed genome wide (ChIP-seq) binding of H2A.Z to DNA as well as mRNA expression of genes encoding H2A.Z in the human post-mortem and 5xFAD mouse hippocampus. mRNA expression of genes encoding H2A.Z increased in the human AD patient hippocampus. Similarly, genome wide binding of H2A.Z increased in both the male and female 5xFAD hippocampus. This increase in H2A.Z was paralleled by the remediation of hippocampal-dependent spatial memory following H2A.Z depletion. This first investigation of histone variants as regulators of memory in AD revealed changes in H2A.Z that generalize across species and provides promising support for H2A.Z depletion as a therapeutic strategy.

Disclosures: S.D. Creighton: None. **F. Meng:** None. **G. Stefanelli:** None. **M.A. Brimble:** None. **L. Hategan:** None. **J. Zakaria:** None. **E.A. Collins:** None. **N.A. Gajewska:** None. **A. Reda:** None. **T.A.B. McLean:** None. **B. Walters:** None. **M. Fahnestock:** None. **E. Orouji:** None. **P. Murphy:** None. **I.B. Zovkic:** None.

Poster

279. Therapeutic Strategies: Preclinical Model

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 279.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH U01 NS110453

Title: Single cell epigenetic, transcriptional, and gene-regulatory dissection of Alzheimer's Disease and Related Dementias (ADRDs)

Authors: *A. BERG[1], B. JAMES[1], N. SUN[1], K. GALANI[1], D. A. BENNETT[2], L.-H. TSAI[1], M. KELLIS[1];
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Abstract: Alzheimer's Disease and Related Disorders (ADRD) are debilitating neurodegenerative disorders afflicting 47 million individuals globally. They include Alzheimer's Disease (AD), Frontotemporal Dementia (FTD), Lewy Body Disease (LBD), and Vascular Contributions to Cognitive Impairment and Dementia (VCID), which are highly heterogeneous, frequently co-occur, and share numerous clinical, pathological, and molecular manifestations, raising the question as to whether more etiologically-salient subdivisions exist. Previous attempts at cell-resolution molecular profiling and de novo subtyping were limited by the lack of single-cell, multi-omic datasets encompassing multiple ADRD subtypes. To address this challenge, we

generated scRNA-seq and scATAC-seq data for 540,000 cells from the prefrontal cortex of 102 patients with AD, FTD, LBD, VCID, and neurotypical diagnoses.We used these data to derive cell type-specific functional descriptions of ADRD pathologies at the transcriptional, epigenomic, and TF-regulatory levels, including TF-regulon activation scores derived by SCENIC. Of all these levels, TF-regulons were particularly distinct between ADRDs and highly interpretable, revealing multiple shared and distinct GO-enrichments between the diseases. We trained a cell-type-specific logistic regression model for disease classification, showing a consistently superior performance than gene expression or epigenomic information alone. We used the model coefficients to weight target genes in further biological enrichment analyses, and to deconvolve and identify shared and distinct cell-level regulatory patterns across the diseases, and as a basis for de novo subtyping across the ADRD spectrum. We also used flow, centrality, and community analyses of our TF-regulatory networks to reveal network-level cell-type-specific and disease-specific regulatory disruption. Lastly, we validated our biological enrichments and de novo ADRD subtypes using transcriptional, epigenomic, and phenotypic data. Overall, our cell-resolution profiling across ADRDs demonstrates a novel, algorithmically-amenable and biologically-informed dimensionality reduction technique for single-cell data analysis in complex traits, and reveals etiologically salient de novo subtypes in ADRD.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH grant R01

Title: Cell-specific TorsinA mutation in the striatum and cerebellum and effects on brain microstructure and connectivity

Authors: *R. ADURY[1], B. J. WILKES[1], L. R. CONCEPCION[1], D. L. BERRYMAN[1], Y. LI[2], D. E. VAILLANCOURT[3];
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Abstract: DYT1 dystonia is a movement disorder that causes involuntary, twisting movements and abnormal postures. It is the most common inherited form of primary dystonia, caused by mutation of the DYT1 gene, encoding protein torsinA. Our limited understanding of which cell types are most vulnerable to torsinA mutation and its contribution to the pathophysiology of DYT1 dystonia has slowed down the development of targeted therapies. To address this key question, we investigated the effects of cell-specific mutation of the torsinA gene separately in two cell types that have been implicated in DYT1 dystonia: medium spiny neurons of the

striatum and Purkinje cells in the cerebellum. We assessed fine motor deficits and performed *in-vivo* neuroimaging at 11.1T to investigate brain microstructure and function in key motor circuitry**.** We compared sensory-evoked brain function and connectivity, and whole-brain microstructural changes. The rationale for using *in-vivo* sensory-evoked functional Magnetic Resonance Imaging (fMRI) is that there is prior clinical and neurophysiological evidence demonstrating somatosensory processing deficits in dystonia patients. In the medium spiny neuron model, we found evidence of behavioral deficits along with altered resting-state functional connectivity and microstructure. In the Purkinje cell model, we found no behavioral deficits but observed altered sensory-evoked brain activation and microstructure. These data suggest differential contribution of medium spiny neurons and Purkinje cells to the behavioral and pathophysiological signatures of DYT1 dystonia.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Wellcome Trust Investigator Grant 110193
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Sir Henry Wellcome Postdoctoral Fellowship 221610/Z/20/Z
Royal Society Newton International Fellowship NIF\R1\192316

Title: Pathophysiology of Dyt1 dystonia is mediated by spinal cord dysfunction

Authors: A. M. POCRATSKY[1], F. NASCIMENTO[1], M. ÖZYURT[1], I. WHITE[2], R. SULLIVAN[3], C. C. SMITH[1], S. SURANA[4], M. BEATO[5], R. M. BROWNSTONE[1]; [1]Dept. of Neuromuscular Dis., [2]MRC Lab. for Mol. Cell Biol., [3]Dept. of Mol. Neurosci., [4]Dept. of Neuromuscular Diseases; UK Dementia Res. Inst., [5]Dept. of Neuroscience, Physiology, and Pharmacol., Univ. Col. London, London, United Kingdom

Abstract: Dystonia, a neurological disorder defined by abnormal postures and disorganised movements, is thought to be a neural disorder with dysfunction arising within and between brain regions. Given that spinal circuits are the *de facto* final common pathway for motor control, we sought to determine their contribution to dystonia. We developed a new flippase-sensitive Tor1a-

frt mouse in which exons 3-5 are flanked by frt sites and crossed this strain with the established Cdx2::FlpO mouse in which flippase expression is restricted to the developing spinal cord. Through multigenerational breeding, we generated a spinal-restricted biallelic *Tor1a* conditional knockout (cko). qPCR and Western blot analyses confirmed that spinal *Tor1a* cko mice showed normal expression in the brain but was virtually absent in the spinal cord. Ultrastructure analysis of *Tor1a* deleted spinal neurons revealed overt nuclear envelope abnormalities. Unbiased analysis of postnatal video recordings revealed a striking early-onset dystonic-like phenotype that emerged in the hindlimbs, spread rostrally during postnatal maturation, then became fixed below the head. Electromyogram recordings revealed persistent involuntary hindlimb muscle contractions at rest and disorganised muscle activity - including antagonist coactivation - during locomotion. Fictive locomotion experiments in isolated postnatal spinal cords showed similar findings, indicating that spinal circuits are the principal source of excessive activity and disorganised motor output. Testing *in vitro* spinal sensory-motor reflexes revealed a caudal-to-rostral progression in monosynaptic reflex impairments, including reflex dispersion across time and increased latencies. Patch-clamp recordings from lumbar motoneurons showed altered excitatory post-synaptic currents following dorsal root stimulation, including reduced amplitude, prolonged duration, and multiple asynchronous peaks. Microstimulation of afferent fibre bundles revealed that the multiple peaks observed may be due to increased time dispersion of the incoming afferent volley. Ongoing experiments in the monoallelic spinal *Tor1a* cko mice also reveal spinal circuit dysfunction. In summary, the spinal-restricted knockout of *Tor1a* reproduces the pathophysiology of the human *TOR1A*-DYT1 condition, uncovering a key role for spinal circuit dysfunction in early onset generalised dystonia. These findings highlight the key role that the spinal cord plays in organising movement, and the importance of considering spinal circuits in the pathophysiology of movement disorders.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.03

Topic: C.04. Movement Disorders other than Parkinson’s Disease

Support: NIH Grant R00NS110878
Tyler's Hope for Dystonia Foundation

Title: Striatal GNAL knockdown leads to dystonic motor phenotypes

Authors: *N. E. CHAMBERS[1], M. MILLETT[2], S. BARSOUM[1], D. NABERT[2], S. POUNDERS[1], K. HUTCHINSON[1], M. S. MOEHLE[3];

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Abstract: Dystonia is one of the most common movement disorders characterized by involuntary muscle contractions of individual muscles or muscle groups. DYT-25 is an adult-onset form of dystonia that is caused by loss of function mutations in *GNAL*, which encodes G-alpha olf, an alpha subunit of the heterotrimeric G-proteins. Expression of Galpha olf is restricted to the striatum, cerebellum, olfactory bulb, and dentate gyrus, which suggests that *GNAL* may play a unique role in the control and regulation of basal ganglia function. To investigate how loss of *GNAL* leads to dystonia, we created a new *GNAL* floxed mouse line to allow for cre-mediated removal of *GNAL* in specific cell types and nuclei. Thereafter, we investigated how striatal knockdown of GNAL affects dystonia symptoms in our mouse model. We employed a battery of motor assays to assess dystonia symptoms. Additionally, given evidence that non-specific cholinergic antagonists are effective at alleviating dystonia symptoms, we also tested whether pharmacological manipulation of the M4 muscarinic acetylcholine receptor would modulate dystonia symptoms using a selective antagonist and positive allosteric modulator of M4R. To further characterize our model, we tested how striatal neuronal activity is affected during movement using fiber photometry with a genetically encoded calcium indicator. Finally, we interrogated striatal neuronal activity, plasticity, and downstream outputs to the globus pallidus externa and substantia nigra pars reticulata via ex vivo electrophyioslogy. Overall, our findings suggest that our mouse model produces a dystonic motor phenotype and that striatal neuronal activity and output is lessened by loss-of-function of GNAL. Furthermore, our findings suggest that inhibition the M4 muscarinic acetylcholine receptor may be a promising therapy for treatment of dystonia symptoms.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.04

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: PRSS - Research Centre of Geriatric Institute of Montreal

Title: Interaction between the proprioceptive and attentional demands of dynamic postural control in cervical dystonia

Authors: K. SKOTHOS[1,2], M.-J. VERMETTE[1,2], S. CHOUINARD[3], F. PRINCE[1], *J. MESSIER[1,2];
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Abstract: Cervical dystonia (CD) is a movement disorder associated with a dysfunction of a complex neural network that comprises the basal ganglia-thalamic-frontal cortex, as well as the cerebellum. Abnormal postures and movements of the neck and head characterize this movement disorder. Postural instabilities in CD have been associated with impaired neck proprioceptive processing. The present study used a dual task paradigm to explore the interaction between the proprioceptive and attentional demands of dynamic postural control in CD. Healthy (n=17) and CD participants (n=10) performed a postural stability limit task, with and without vision, as well as a secondary cognitive subtraction task. These two tasks were performed alone (single task) or concurrently (dual-task). Ground reaction forces were collected using an AMTI force platform and center of pressure (COP) displacements were analysed. The functional limits of stability were quantified as the maximum COP excursion during voluntary leaning in four different directions. Our results revealed systematically smaller mean postural stability limits in CD patients compared to healthy controls in all sensory-attentional conditions; however, this difference reached significance only for the mediolateral axis ($p < 0.05$). In the anteroposterior axis, the stability limits of CD patients were smaller relative to controls when vision was removed, particularly in the single task condition ($p < 0.05$). Furthermore, interestingly, patients with CD degraded their stability limits relative to healthy controls when concurrently performing the attentional task under the visual condition ($p < 0.05$). These findings suggest a higher reliance on visual information for dynamic postural control in CD patients and thus, increases the attentional-cognitive processing. A deeper understanding of sensorimotor deficiency in CD is crucial to elucidate the neuropathophysiology of CD and to develop strategies to improve sensory-attentional challenges imposed in daily life activities.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: OHSU-OFDIR fellowship
NIH grant 5R01AG006457
NIH grant 5R00HD078492
NIH grant R01MH115357
NIH grant K99HD07849

Title: Salience network as a predictor of responsiveness to cueing in people with Parkinson's disease

Authors: *C. SILVA-BATISTA[1], O. MIRANDA-DOMINGUEZ[4], A. RAGOTHAMAN[5], D. A. FAIR[7], A. MANTOVANI[2], S. STUART[8], J. G. NUTT[9], F. B. HORAK[3], M. MANCINI[6];

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Abstract: We previously showed that both open-loop (beat of a metronome) and closed-loop (phase-dependent tactile feedback) cueing may be similarly effective in reducing Freezing of Gait (FoG), assessed with a quantitative FoG Index, while turning in place in the laboratory in a group of people with Parkinson's disease (PD). Despite the similar changes on the FoG Index, it is not known whether both cueing responses require attentional control, which would explain FoG Index improvement. The mechanisms underlying cueing responses are poorly understood. Here, we tested the hypothesis that the salience network would predict responsiveness (*i.e.*, FoG Index improvement) to open-loop and closed-loop cueing in people with and without FoG of PD, as salience network contributes to tasks requiring attention to external stimuli in healthy adults. Thirteen people with PD with high-quality imaging data were analyzed to characterize relationships between resting-state MRI functional connectivity and responses to cues. The interaction of the salience network and retrosplenial-temporal networks was the best predictor of responsiveness to open-loop cueing, presenting the largest effect size (d=1.16). The interaction between the salience network and subcortical as well as cingulo-parietal and subcortical networks were the strongest predictors of responsiveness to closed-loop cueing, presenting the largest effect sizes (d=1.06 and d=0.84, respectively). Salience network activity was a common predictor of responsiveness to both cueing, which suggests that auditory and proprioceptive stimuli during turning may require some level of cognitive and insular activity, anchored within the salience network, which explain FoG Index improvements in people with PD.

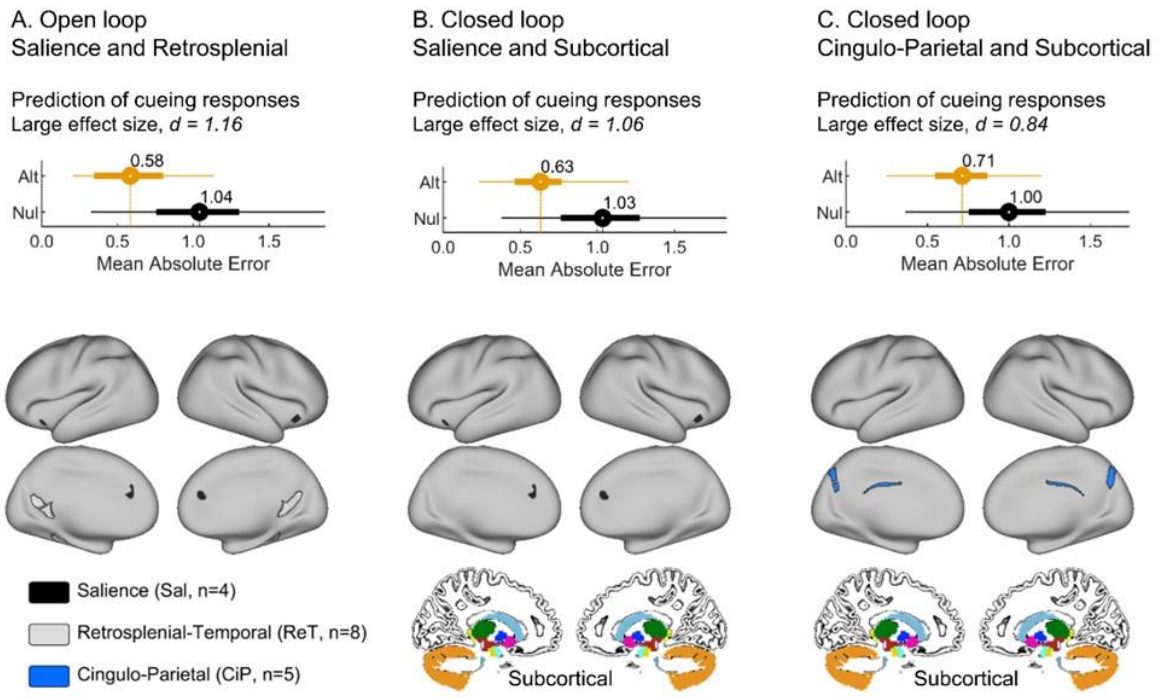

Figure 1. Each column displays the functional system pairs that show the strongest association with improvement after cueing. ***A***. Salience and Restrosplenial for open loop. ***B.*** Salience and subcortical for closed-loop. ***C.*** Cingulo-Parietal and Subcortical for closed-loop. Each column shows the distribution of the mean absolute error (*mae*) when predicting out-of-sample cueing responses (yellow lines) and the out-of-sample performance of models trained with shuffle data, *i.e.,* Null hypothesis (Black line). Thin lines show the percentiles 5 to 95, thick lines the interquartile rang,e and circles indicate mean values. 286 partitions were made splitting the data randomly leaving-3-out on each run. PLSR models were fit using 4 components on each "modeling" partition. *mae* are the absolute value of the difference between the predicted and observed scores calculated by using the PLSR model obtained in the modeling subsample and using it to predict scores in the validation subsample. The approach was repeated by permuting data in the modeling partition each time to generate null-hypothesis data (n=10,000). Cohen effect size is also indicated. This figure also indicates the location of the functional system pairs in a very-inflated projection of the cerebral cortex.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Feasibility of Vibro-tactile Stimulation as a Non-invasive Treatment for Cervical Dystonia

Authors: *J. XU[1], J. OH[1], S. STANDAL[2], P. SALEHI[2], D. MARTINO[3], L. AVANZINO[4], A. CONTE[5,6], J. KONCZAK[1];
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Abstract: Cervical dystonia (CD) is a form of focal dystonia associated with involuntary cervical muscle contractions that lead to sustained or intermittent abnormal, painful head movements or postures, which severely affect a CD patient's daily life. Current treatment opportunities for CD are limited and mainly consist of Botulinum toxin injections (Botox) in the dystonic muscles or deep brain stimulation. Both methods are invasive, and Botox is not tolerated by all CD patients. This study examined if superficial, vibro-tactile stimulation (VTS) of the cervical muscles can be an alternative method to provide temporary symptom relief for people with CD. **Method:** A total of 21 CD patients (mean age ± standard deviation: 62.1 ± 9.6 years, 15 females, 6 males) participated in the study. All participants were seen within two weeks of their next Botox injection (i.e., their symptomatic period). The most often affected cervical muscles, sternocleidomastoid and trapezius, were targeted and stimulated. Participants completed up to 9 treatment sessions (stimulating a single muscle or the combination of two muscles) in randomized order and based on their clinical manifestations (e.g., torticollis, laterocollis). In each treatment session, VTS was applied continuously for 5 minutes. The planar tilt angles of head posture were recorded with a 16-camera optoelectronic motion capture system. The self-reported pain level and the effort level (both 100-point scales) of holding the head in a neutral position were collected throughout the entire experiment as well. **Results:** First, approximately 29% (6/21) of participants responded in at least one of the VTS treatment sessions with visible and measurable changes in head position that restored near-normal, upright head position. Second, 76% (16/21) of CD participants reported reduced pain level during, or past VTS application in at least one session. Mean pain level was reduced by 52% (52.3% ± 28.3%) during VTS and by 21% (20.9% ±62.7%) post-VTS. Third, 79% (15/19) of participants reported that it took them less effort to hold the head in a neutral position post-VTS. The mean effort level was reduced by 51% (50.9% ± 33.3%) post-VTS. Fourth, the effectiveness of VTS in alleviating dystonic muscle spasms depended on the site of vibration. Typically, for those who responded, we found one optimal vibration site or vibration site combination with other sites showing milder effects. **Conclusion:** These initial proof-of-concept data demonstrate that VTS of cervical muscles can potentially serve as a form of non-invasive neuromodulation in CD and indirectly relieve the symptoms caused by CD.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.07

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Benefits of conditional knockout of NADC3 in Canavan disease mice

Authors: *A. DEHGHANI[1], V. HULL[2], Y. WANG[3], S. STERNBACH[6], J. MCDONOUGH[7], F. GUO[4], D. E. PLEASURE[5];
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Abstract: Canavan disease (CD) is a recessively inherited neurodegenerative disease caused by inactivating ASPA mutations and characterized by prominent astroglial and intramyelinic vacuolation in forebrain, cerebellum, and brainstem. This "spongy" leukodystrophy most often presents in infancy with hypotonia, ataxia, and failure to acquire normal developmental milestones, frequently in association with macrocephaly and seizures. ASPA encodes aspartoacylase, an enzyme required for cleavage of the abundant brain amino acid N-acetyl-L-aspartate (NAA). As a consequence of aspartoacylase deficiency, brain NAA levels are markedly elevated in Canavan disease patients. Prior studies by our laboratory and others demonstrated that knocking out or knocking down the neuronal NAA synthesizing enzyme Nat8l in aspartoacylase-deficient Canavan disease model mice ("CD mice") prevents or reverses brain NAA elevation, ataxia, and brain vacuolation. More recently we've documented that constitutive deletion of *Slc13a3*, which encodes NaDC3, a sodium-coupled plasma membrane dicarboxylic acid transporter with high affinity for NAA, also prevents brain NAA elevation, ataxia and brain vacuolation in CD mice. Molecular and immunohistological studies show that *Slc13a3* and NaDC3 are expressed by astroglia, meninges, and renal proximal tubular epithelium. Our most recent studies show that astroglial conditional *Slc13a3* deletion in 2 month old CD mice improves motor performance and partially reverses brain vacuolation, thus suggesting that NaDC3-mediated astroglial NAA accumulation is a major contributor to spongiform leukodystrophy in Canavan disease and identifying astroglial *SLC13A3* and NaDC3 as promising Canavan disease therapeutic targets.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.08

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: University Medicine Essen Clinician Scientist Academy (UMEA)/ German Research Foundation (Deutsche Forschungsgemeinschaft; DFG; FU356/12-1)
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Mercator Foundation/ Mercator Research Center Ruhr (MERCUR)
German Research Foundation (Deutsche Forschungsgemeinschaft; DFG; 441409627)
European Union (European Joint Programme on Rare Diseases)

Title: Cerebellar Cognitive Affective/ Schmahmann Syndrome Scale: Need for adjusted cut-off values

Authors: *A. THIEME[1], K. RUBARTH[3,4], J. FABER[5,6], P. SULZER[7,8], K. REETZ[9,10], I. DOGAN[9,10], M. BARKHOFF[5], J. S. KRAHE[9,10], H. JACOBI[12], J.-E. AKTORIES[12], M. MINNEROP[13,14,11], S. ELBEN[13,14], D. HUVERMANN[1,13,14], F. ERDLENBRUCH[1], R. VAN DER VEEN[1], J. MÜLLER[1], G. BATSIKADZE[1], B. FRANK[1], M. KÖHRMANN[1], E. WONDZINSKI[15], M. SIEBLER[15], S. HETZE[2], O. MÜLLER[16,2], U. SURE[2], J. KONCZAK[17], T. KLOCKGETHER[5,6], M. SYNOFZIK[7,8], F. KONIETSCHKE[3,4], S. RÖSKE[5], D. TIMMANN[1];
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Abstract: Patients with cerebellar disorders frequently present with cognitive and affective symptoms termed as Cerebellar Cognitive Affective or Schmahmann Syndrome (CCAS). A brief bedside test (CCAS Scale) consisting of twelve test items has been introduced to screen for a CCAS by Hoche et al. (Brain 2018; 141: 248-70). The presence of a CCAS is defined based on the number of failed test items: one failed item = "CCAS possible", two failed items = "CCAS probable", ≥ three failed items = "CCAS definite". The original CCAS Scale has been validated in an US-American cohort of adults with cerebellar disorders and healthy controls. The aim of this multi-center study was to validate a recently published German version of the CCAS Scale (Thieme et al. Neurol Res Pract 2020; eCollection 2:39). More than 200 adult patients with various cerebellar disorders and an equal number of sex-, age- and education-matched healthy controls participated. Test-retest and interrater reliability were good. Internal consistency was

acceptable. Sensitivity (i.e., the portion of patients correctly identified as patients) was high for the category “CCAS possible” and moderate for the categories “CCAS probable” and “CCAS definite”. Selectivity (i.e., the portion of controls correctly identified as controls) was high for the category “CCAS definite”, moderate for the category “CCAS probable” and low for “CCAS possible”, yielding a substantial number of false-positive test results. Sensitivity and selectivity were best in patients with known extracerebellar involvement. Patients with spinocerebellar ataxia type 3 (SCA3) performed worse on the CCAS Scale than patients with SCA6 or patients with chronic cerebellar stroke. Age, level of education and, to a lesser degree, sex influenced performance. Results in controls were fitted to a Poisson regression model: Y= e^(0.89 - 0.26 * x_sex + 0.02 * x_age - 0.09 * x_edu) where Y corresponds to the predicted number of failed items for a healthy participant of a given sex, age and level of education. x_sex is 1 if participant is female and x_sex is 0 if participant is male, x_age is the age of the participant in years and x_edu is the number of educational years. A number of failed test items larger than Y in a cerebellar patient is indicative of CCAS. The word fluency test items differentiated best between patients and controls. Given the substantial overlap between the performance of healthy controls and patients, especially regarding patients with milder forms of CCAS, other weighting or exclusion of items, or the introduction of additional items may be necessary to further improve the diagnostic value of the CCAS Scale.

Disclosures: **A. Thieme:** A. Employment/Salary (full or part-time):; holds a position that is funded in part by the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG) and the University Medicine Essen Clinician Scientist Academy (UMEA). 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 grants from the University Medicine Essen Clinician Scientist Academy (UMEA), the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG), Freunde und Förderer der Neurologie der Universitätsmedizin Essen, the German Heredo-Ataxia-Society (Deutsche Heredo-Ataxie Gesellschaft e.V.), and the Mercator Foundation/ Mercator Research Center Ruhr (MERCUR). E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); holds stocks of Viatris Pharmaceuticals. **K. Rubarth:** None. **J. Faber:** None. **P. Sulzer:** None. **K. Reetz:** 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 grants from the German Ministry of Education and Research (BMBF 01GQ1402, 01DN18022), the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG; IRTG2150), the Alzheimer Forschung Initiative e.V. (NL-18002CB), and Friedreich's Ataxia Research Alliance (FARA). F. Consulting Fees (e.g., advisory boards); received honoraria for presentations or advisory board from Biogen and Roche. **I. Dogan:** None. **M. Barkhoff:** None. **J.S. Krahe:** None. **H. Jacobi:** None. **J. Aktories:** None. **M. Minnerop:** None. **S. Elben:** None. **D. Huvermann:** None. **F. Erdlenbruch:** None. **R. Van der Veen:** None. **J. Müller:** None. **G. Batsikadze:** None. **B. Frank:** None. **M. Köhrmann:** None. **E. Wondzinski:** None. **M. Siebler:** None. **S. Hetze:** None. **O. Müller:** None. **U. Sure:** None. **J. Konczak:** None. **T. Klockgether:** None. **M. Synofzik:** 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 grants from the German Research

Foundation (Deutsche Forschungsgemeinschaft; DFG) and the European Union. Other; received consultancy honoraria from Ionis Pharmaceuticals (Carlsbad, California, USA), Jansen Pharmaceuticals (Beerse, Belgium) and Orphazyme Pharmaceuticals (Copenhagen, Denmark). **F. Konietschke:** None. **S. Röske:** None. **D. Timmann:** 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 grants from the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG), the European Union, and the Bernd-Fink-Foundation.

Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.09

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: MOST Grant 109-2326-B-002-013-MY4
MOST Grant 111-2321-B-002 -016

Title: Desynchronizing the Olivo-Cerebellar Loop: A Computational Study of Pharmacological Intervention for Climbing Fiber Overgrowth Induced Essential Tremor.

Authors: *A. WHITE[1], Y.-M. WANG[2], W.-C. LIU[4], L.-Y. LU[5], J.-C. LEE[5], Y.-F. CHEN[5], M.-K. PAN[3], C.-C. LO[6];
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Abstract: Although essential tremor is the most common cause of tremor, first line medications are not always effective for a significant portion of affected individuals [1]. Previous studies have suggested that essential tremor may be caused by two separate mechanisms [1,2]. One mechanism centers on pathological synchronization in the inferior olive (IO), and can be chemically induced by harmaline increasing the synchrony between IO neurons [3]. The second mechanism centers on overgrowth of climbing fiber terminals to nearby Purkinje cells that generates synchronized complex spikes between Purkinje cells, and is associated with a decrease in GluRdelta2 as seen in the mutant Grid2dupE3 [2].
Our work sets out to build a computational model of these two mechanisms by constructing a detailed electrophysiological neural model of the olivo-cerebellar loop. As expected, we are able to confirm that the Grid2dupE3 has a much higher spectral power at the tremor frequency than the harmaline induced tremor. Moreover, we show that the higher spectral power is a consequence of an increase in global synchronization in the Grid2dupE3 tremor model as compared to the harmaline induced tremor. Most importantly, we are able to show that

traditional pharmacological agents, such as GABA agonists, work in treating the harmaline induced subtype. We next use the model to predict the effectiveness of different pharmacological agents on the climbing fiber overgrowth subtype. We tested a P-type calcium antagonist and a BK agonist to attenuate complex spikes, and an antagonist of muscarinic glutamate receptors that inhibits the synaptic transmissions from the climbing fibers. Interestingly, a muscarinic antagonist, P-type calcium antagonist, and a BK agonist were effective at desynchronizing the olivo-cerebellar loop. Thus, our model makes relevant predictions for the next generation of medications for essential tremor treatment.

[1] Louis, E.D., Faust, P.L. Essential tremor pathology: neurodegeneration and reorganization of neuronal connections. Nat Rev Neurol 16, 69-83 (2020).
[2] Pan, Ming-Kai et al. "Cerebellar oscillations driven by synaptic pruning deficits of cerebellar climbing fibers contribute to tremor pathophysiology." Science translational medicine vol. 12,526 (2020).[3]Llinas, R., Volkind, R. A. The olivo-cerebellar system: functional properties as revealed by harmaline-induced tremor. Exp. Brain Res. 18, 69-87 (1973).

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Myotonic Dystrophy Foundation Doctoral Research Fellowship
National Science Foundation Graduate Research Fellowship Program under Grant No. DGE-2038238

Title: RNA subcellular mislocalization in myotonic dystrophy type 1 (DM1) patient iPSC-derived neurons

Authors: *M. L. GOSZTYLA, A. LI, K. H. MORELLI, G. W. YEO;
Ctr. for Mol. and Cell. Med., Univ. of California San Diego, La Jolla, CA

Abstract: Myotonic dystrophy type 1 (DM1) is a multisystem disorder caused by an expanded CTG triplet repeat in the 3' untranslated region of the dystrophia myotonic protein kinase (DMPK) gene. CUG repeat expansions in *DMPK* RNA transcripts form ribonuclear foci that dysregulate RNA-binding proteins. In muscles, this causes abnormal splicing, which gives rise to progressive myopathy and myotonia. However, DM1's cognitive symptoms do not appear to be fully explained by missplicing of neuronal transcripts. We hypothesized RNA mislocalization contributes to neuronal phenotypes in DM1. We differentiated neurons from DM1 patient-derived iPSCs and neurotypical controls. Then we utilized a version of subcellular fractionation modified for neuronal cells, followed by RNA sequencing, to characterize RNA subcellular

localization. When comparing DM1 to control neurons, we observed hundreds of mislocalized transcripts, most of which became more cytosolic in DM1. These changes could not be fully explained by changes in overall gene expression. We observed that GC content is positively correlated with more nuclear enrichment in DM1. Nuclear-shifted transcripts included many genes important for muscle and/or cardiac development, as well as components of the extracellular matrix, a result that is consistent with DM1 phenotypes. In contrast, cytosolic-shifted transcripts were highly enriched for the "plasma membrane" GO cellular component term and included multiple families of cell surface receptors. Many of these are G protein-coupled receptors, whose translation and transport to the plasma membrane are known to be highly dependent on specific RNA localization, suggesting these changes may have functional consequences at the protein level as well. To our knowledge, this is the first report of receptor dysregulation in DM1 neurons. Furthermore, cell fractionation of iPSCs revealed distinct patterns of mislocalization compared to neurons, suggesting that these changes derive from a neuron-specific mechanism. Our results highlight neuronal RNA subcellular localization defects as an important layer of dysregulation in DM1.

Disclosures: M.L. Gosztyla: None. **A. Li:** None. **K.H. Morelli:** None. **G.W. Yeo:** A. Employment/Salary (full or part-time):; National University of Singapore.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Locanabio, Eclipse Bioinnovations. F. Consulting Fees (e.g., advisory boards); Locanabio, Eclipse Bioinnovations.

Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.11

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Rna and protein mediated neurodegeneration in fxtas

Authors: *S. GRUDZIEN[1], S. E. WRIGHT[2], B. N. FLORES[4], A. KRANS[3], S. J. BARMADA`[3], P. K. TODD[3];
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Abstract: Fragile X-associated tremor/ataxia syndrome (FXTAS) is a relatively common neurodegenerative ataxia that arises from a trinucleotide CGG repeat expansion that ranges from 55 to 200 repeats in the 5' UTR of FMR1. CGG repeats may drive neurodegeneration through RNA mediated mechanisms or repeat-associated non-AUG (RAN) translation. As a complicating factor, while the major RAN translation product from CGG repeats in a polyglycine-containing peptide, FMRpolyG, RAN translation can potentially generate multiple protein products that could contribute to neurodegeneration. Data in transgenic models (Drosophila and mice) suggests that FMRpolyG synthesis is required for neurotoxicity, but whether FMRpolyG is

sufficient to elicit maximal toxicity remains unclear. In rodent neurons and through intracerebroventricular injection of adeno-associated viruses (AAVs) expressing vectors in neonatal mice, we expressed vectors that generate both FMRpolyG and CGG repeat RNA from the native FMR1 5'UTR; just FMRpolyG in the absence of the repeat (through alternative codon usage and AUG initiation codon), or just the repeat RNA (CGG-RNA) but with little FMRpolyG production (through near-cognate start codon removal). Primary hippocampal rodent neurons that express 100 CGG repeats in the native FMR1 5'UTR context exhibit greater toxicity than constructs that solely express FMRpolyG, despite greater FMRpolyG production from the AUG initiated alternative codon construct. Compared to control GFP constructs, expression of the CGG repeat as RNA alone did not evoke toxicity. Preliminary data in AAV injected mice that permit expression of both the CGG-RNA and FMRpolyG exhibit robust FMRPolyG inclusions in conjunction with impaired motor behavioral deficits that mirrors the neuronal culture results. Our results in rodent cultures implies potential synergy between FMRpolyG production and CGG-RNA toxicity that we are evaluating in vivo. Future work will determine how this synergy is achieved, and whether its selective abrogation is feasible.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.12

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: ataxia charlevoix-saguenay foundation

Title: Metabolic rewiring in a cellular model of ARSACS

Authors: *L. PERNA, O. HAWORTH, G. A. SALSBURY, J. P. CHAPPLE;
Endocrinol., Queen Mary Univ. of London, London, United Kingdom

Abstract: Autosomal Recessive Spastic Ataxia of Charlevoix Saguenay (ARSACS) is an early onset neurodegenerative disease that also has a neurodevelopmental component. It is caused by loss of function of sacsin, a 520 kDa protein with multiple domains linked to protein quality control systems. Impaired mitochondrial health is a feature of cellular models of ARSACS. This includes altered mitochondrial network organisation, reduced oxidative phosphorylation and increased levels of superoxide in sacsin deficient cells and patient fibroblasts (Bradshaw et al., 2016). These phenotypes maybe a consequence of impaired recruitment of the mitochondrial fission factor dynamin-related protein 1 (Bradshaw et al., 2016). In this study we investigate if the mitochondrial dysfunction caused by loss of sacsin impacts on cellular metabolism. Using CRISPR/Cas9 we generated a sacsin knockout SH-SY5Y (neuroblastoma-derived) cell line. Then, to compare the metabolite profiles of wild-type control and sacsin knockout cell lines, we

performed mass spectrometry-based metabolomic flux analysis with both glucose and glutamine traced carbon**.** This revealed that sacsin knockout cells have increased lactate production and alterations in the glutaminolysis pathway, suggesting an increased reliance on aerobic glycolysis in the absence of sacsin. Our analysis also revealed decreased levels of GABA in sacsin knockout cells, which given its neurotransmitter function may be directly relevant to neuronal dysfunction in ARSACS. Further analysis of our metabolomic data set will increase understanding of the molecular consequences of sacsin loss and may identify metabolic deficiencies that could potentially be targeted to treat ARSACS. Bradshaw, T. Y., Romano, L. E., Duncan, E. J., Nethisinghe, S., Abeti, R., Michael, G. J., . . . Chapple, J. P. (2016). A reduction in Drp1-mediated fission compromises mitochondrial health in autosomal recessive spastic ataxia of Charlevoix Saguenay. Hum Mol Genet, 25(15), 3232-3244. doi:10.1093/hmg/ddw173

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.13

Topic: C.04. Movement Disorders other than Parkinson’s Disease

Title: Brief, synchronous, sensory peripheral electrical stimulation does not suppress essential tremor effectively, independent of stimulation frequency

Authors: *C. METZNER[1], A. STRINGHAM[2], B. HISLOP[2], J. BONHAM[2], L. CHATTERTON[2], R. DEFIGUEIREDO[2], S. K. CHARLES[1,2];
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Abstract: Peripheral electrical stimulation below motor neuron threshold (sensory PES) has shown potential as a non-invasive treatment for Essential Tremor (ET). Researchers have identified two hypothetical mechanisms by which sensory PES could suppress ET: first, PES could activate neural signaling that disrupts supraspinal tremor networks and potentially changes the tremor frequency, or, second, appropriately timed PES could activate the reciprocal inhibition reflex to reduce tremorgenic muscle activity. Studies using an asynchronous PES strategy, in which antagonist muscles are stimulated separately out-of-phase with the tremor signal, tentatively support the second mechanism. To understand the mechanisms better, we explored the effects of different sensory PES strategies and frequencies on ET power and frequency. We studied the effects of brief, synchronous, sensory PES on tremor power and frequency of the antagonistic flexor and extensor carpi radialis muscles, testing a range of 15 stimulation frequencies from 10 to 150 Hz on 21 ET patients. We compared tremor power and frequency from wrist acceleration (WA) and EMG data between pre-, per-, and post-stimulation phases across all test subjects using a mixed-model ANOVA and a post-hoc Tukey HSD test. We found that our stimulation paradigm did not result in changes in tremor power or frequency at

any of the tested stimulation frequencies. There was no statistically significant interaction between phase and stimulation frequency for tremor power measured by either WA (p=0.24) or EMG (p=0.45). Similarly, there was no statistically significant interaction between phase and stimulation frequency for tremor frequency measured by either WA (p=0.30) or EMG data (p=0.81). Since the supraspinal mechanism should work for either stimulation strategy, the lack of change in tremor power or frequency is consistent with the hypothesis that brief sensory PES uses the reciprocal inhibition reflex to suppress tremor. Still, our study does not rule out the supraspinal tremor network disruption hypothesis since this may require longer stimulation times. We conclude that an asynchronous stimulation strategy or a longer stimulation duration are required for effective tremor suppression.

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Poster

280. Dystonias, Dystrophies, and Degenerative Disorders

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 280.14

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH 1R01 DC016315-01A1

Title: Electrocortical responses characterizing different frequencies of laryngeal vibration

Authors: *N. ELANGOVAN[1], G. SAMPSON[2], J. KONCZAK[3];
[1]Univ. of Minnesota, [3]Human Sensorimotor Control Lab., [2]Univ. of Minnesota, Minneapolis, MN

Abstract: Laryngeal vibration is considered as a form of non-invasive neuromodulation for improving voice quality in people with Spasmodic dysphonia (SD), a voice disorder resulting in choked or strained speech. Earlier work showed that 100 Hz laryngeal vibration induced speech quality improvements on people with SD that persisted for 20 minutes past VTS. Additionally, the application of laryngeal vibration induced a significant rise of gamma rhythm over right somatosensory-motor cortex and suppression of theta band power over the left somatosensory-motor cortex indicating that laryngeal vibration reduces atypical patterns of cortical activity in people with SD during voice production. However, the optimal laryngeal vibration frequency required to maximize these positive effects on SD is unknown. To obtain a better understanding of the underlying cortical neural mechanism of two different frequencies (high vs low) of laryngeal vibration, we analyzed electroencephalographic (EEG) signals in a sample of healthy adults. Participants were randomly assigned to one of the two frequencies of laryngeal vibration and received two blocks (17 minutes each) of the assigned intervention. EEG signals were recorded during vowel */a/* vocalization (epoch duration: 2500ms) before and after the intervention. During vocalization, gamma band event-related spectral power (ERSP) in the right

hemisphere was observed to be greater in the high frequency vibration group than the low frequency group. Theta band ERSP appears to have less differential activity between the two frequencies. An increase in right hemispheric gamma activity indicates the modulatory effect of laryngeal vibration on speech cortical networks. These findings suggest that high frequency of laryngeal vibration is optimal for maximizing symptom improvements in people with SD. Our results inform the optimal frequency of laryngeal vibration needed for the development of wearable, non-invasive, voice-activated, user-programmable medical devices that could apply vibration on laryngeal muscles while monitoring its effect on speech production in real-time.

Disclosures: N. Elangovan: None. **G. Sampson:** None. **J. Konczak:** None.

Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.01

Topic: C.06. Neuromuscular Diseases

Support: Interdisziplinäres Zentrum für Klinische Forschung, Universitätsklinikum Erlangen (J88)
BaCaTeC (A5[2019-02])
Bayerisches Staatsministerium für Bildung und Kultus, Wissenschaft und Kunst (ForInter)
Bundesministerium für Bildung und Forschung (01GM1905B)
Deutsche Forschungsgemeinschaft (WI 3567/2-1)
Deutsche Forschungsgemeinschaft (270949263/GRK2162)
Interdisziplinäres Zentrum für Klinische Forschung, Universitätsklinikum Erlangen (E30)

Title: Aberrant NOVA1 functions disrupts alternative splicing in early stages of ALS

Authors: *F. KRACH[1], E. WHEELER[2], M. REGENSBURGER[1], T. BOERSTLER[1], H. WEND[1], A. VU[2], R. WANG[2], S. REISCHL[1], K. BOLDT[3], R. BATRA[2], S. AIGNER[2], J. RAVITS[2], J. WINKLER[1], G. YEO[2], B. WINNER[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by aberrant alternative splicing (AS). Nuclear loss and cytoplasmic accumulation of the splicing factor TDP-43 in motor neurons (MN) are hallmarks of ALS at late stages of the disease. However, it is unknown if altered AS is present before TDP-43 pathology occurs. Here, we investigate altered AS and its origins in early stages of ALS by using human induced pluripotent stem cell derived motor neurons (MNs) from sporadic and familial ALS patients. We find high levels of the RNA-binding proteins NOVA1, NOVA2, and RBFOX2 in the insoluble protein fractions and observe

that AS events in ALS-associated MNs are enriched for binding sites of these proteins. Our study points to an early disrupted function of NOVA1 that drives AS changes in a complex fashion, including events caused by a consistent loss of NOVA1 function. NOVA1 exhibits increased cytoplasmic protein levels in early stage MNs without TDP-43 pathology in ALS postmortem tissue. As nuclear TDP-43 protein level depletes, NOVA1 is reduced. Potential indications for a reduction of NOVA1 also came from mice overexpressing TDP-43 lacking its nuclear localization signal and iPSC-MN stressed with puromycin. This study highlights that additional RBP-RNA perturbations in ALS occur in parallel to TDP-43.

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Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.02

Topic: C.06. Neuromuscular Diseases

Title: Interactions of TDP-43 with endomembrane and nucleocytoplasmic transport proteins in FTD/ALS models

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Abstract: Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) comprise a devastating continuum of progressive neurodegenerative diseases with the shared hallmark of TDP-43 pathology in 45% and 97% of cases, respectively. TDP-43 pathology is defined by the cytoplasmic mislocalization of the predominantly nuclear RNA-binding protein TDP-43, where it forms hyperphosphorylated and ubiquitinated detergent-insoluble aggregates. The pervasiveness of this pathology, in conjunction with the genetic link between TDP-43 and FTD/ALS, suggests it plays a critical role in the disease pathogenesis; however, the causes of TDP-43 proteinopathy remain largely unknown. Previously published modifier screens in disease models and neuropathological studies in patients have established a link between nucleocytoplasmic transport defects and FTD/ALS caused by C9orf72 repeat-expansions. Proximity-proteomics data from our lab indicate that this may apply to the majority of sporadic and familial cases because pathological TDP-43 co-aggregates with a subset of nucleoporins, proteins that comprise the nuclear pore complex. These finding suggest a positive feedback loop, where TDP-43 aggregates cause nucleocytoplasmic transport dysfunction, contributing to further TDP-43 mislocalization. In addition to nucleoporins, we found that TDP-43 also co-aggregates with components of all major pathways in the endomembrane system. We hypothesize that the

sequestration of endomembrane system components into TDP-43 aggregates leading to dysfunction in endomembrane trafficking and neurotoxicity in FTD/ALS. While investigating these pathways we identified specific nucleoporins that can decrease insoluble TDP-43 levels *in vitro*. Using cellular and organotypic models of TDP-43 proteinopathy we are investigating the interactions between these proteins and insoluble TDP-43. Establishing this mechanism of action may reveal new therapeutic targets to reverse TDP-43 pathology in FTD/ALS.

Disclosures: **C. Smith:** None. **B. Khalil:** None. **W. Rossoll:** None.

Poster

281. Amyotrophic Lateral Sclerosis

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Topic: C.06. Neuromuscular Diseases

Support: NIH R33 NS110960
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DOD W81XWH-19–1-0193
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NIH P30 AG062677
NIH R01 NS097542
NIH P30 AG072931

Title: Nuclear import receptors reduce hallmarks of TDP-43 proteinopathy in cellular and animal models of ALS/FTD

Authors: B. KHALIL[1], D. CHHANGANI[2], M. C. WREN[1], C. L. SMITH[1], J. H. LEE[1], X. LI[3], C. PUTTINGER[1], C.-W. TSAI[1], G. FORTIN[1], D. MORDERER[1], J. GAO[1], F. LIU[1], C. KIM LIM[4], J. CHEN[1], C.-C. CHOU[5], C. L. CROFT[6], A. M. GLEIXNER[7], C. J. DONNELLY[7], T. E. GOLDE[6], L. PETRUCELLI[1], B. OSKARSSON[1], D. W. DICKSON[1], K. ZHANG[1], J. SHORTER[8], S. H. YOSHIMURA[4], S. J. BARMADA[3], D. E. RINCON-LIMAS[2], ***W. ROSSOLL**[1];
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Abstract: Cytoplasmic mislocalization and aggregation of TAR DNA-binding protein-43 (TDP-43) is a hallmark of the amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) disease spectrum. While most ALS cases are sporadic, mutations in TDP-43 can directly cause ALS, likely via a combination of nuclear loss of function and cytoplasmic toxic gain of function phenotypes. Here we show that karyopherin beta-1 (KPNB1) and other members of the nuclear import receptor (NIR) protein family can rescue the hallmarks of TDP-43 proteinopathy, by

restoring its solubility and nuclear localization, and reducing neurodegeneration in cellular and animal models of ALS/FTD. Our findings suggest a novel mechanism where analogous to its canonical role in dissolving the diffusion barrier formed by phenylalanine and glycine-rich nucleoporins (FG-Nups) in the nuclear pore, KPNB1 is recruited into TDP-43 aggregates present in TDP-43 proteinopathies and therapeutically reverses their deleterious phase transition, mitigating neurodegeneration.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.04

Topic: C.06. Neuromuscular Diseases

Support: NIH R33 NS110960
W81XWH-19–1-0193

Title: Proximity proteomics of poly-GA aggregates in C9-FTD/ALS models

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Abstract: The most common inherited cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) is the presence of expanded GGGGCC intronic hexanucleotide repeats in the C9orf72 gene. Aside from haploinsufficiency and toxic RNA foci, another non-exclusive disease mechanism is the non-canonical translation of the repeat RNA into five different dipeptide repeat proteins (DPRs), which form neuronal inclusions in affected patient brains. While evidence from cellular and animal models supports a toxic gain-of-function of pathologic poly-GA, poly-GR, and poly-PR aggregates in promoting deposition of TDP-43 pathology and neurodegeneration in affected brain areas, the relative contribution of DPRs to the disease process in c9FTD/ALS patients remains unclear. Previously, we have used the proximity-dependent biotin identification (BioID) proximity proteomics approach to investigate the formation and collective composition of poly-GA aggregates using cellular models and

mouse organotypic brain slice cultures (BSCs). In cellular models, poly-GA aggregates demonstrated a distinct association with proteasomal components, molecular chaperones (HSPA1A/HSP70, HSPA8/HSC70, VCP/p97), co-chaperones (BAG3, DNAJA1A) and other factors that regulate protein folding and degradation (SQSTM1/p62, CALR, CHIP/STUB1). The involvement of molecular chaperones and co-chaperones in the pathologic process was confirmed in autopsy brain tissue, where HSPA8, BAG3, VCP, and its adapter protein UBXN6 show a close association with poly-GA aggregates in the frontal cortex, temporal cortex, and hippocampus of c9FTLD and c9ALS cases. We identified HSP40 co-chaperones of the DNAJB family as potent modifiers that increased the solubility of poly-GA, highlighting a possible novel therapeutic avenue and a central role of molecular chaperones in the pathogenesis of human C9orf72-linked diseases. To further investigate poly-GA related pathology in the CNS, we have established BioID in a brain slice culture (BSC) as a more physiological model. The ex vivo culture maintains the 3D brain organization and combines advantages of in vitro and in vivo models. The identification of novel poly-GA interactors and affected pathways will contribute to a better understanding of C9-FTD/ALS relevant disease processes and therapeutic targets.

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Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.05

Topic: C.06. Neuromuscular Diseases

Support: FightMND
Motor Neurone Disease Research Australia
National Health and Medical Research Council of Australia
Macquarie University

Title: Divergent cerebellar transcriptome in amyotrophic lateral sclerosis cases with greater burden of pTDP-43 neuropathology

Authors: *N. GRIMA[1], C. E. SHEPHARD[2], D. B. ROWE[1], M. C. KIERNAN[3], S. MAZUMDER[3], I. P. BLAIR[1], K. L. WILLIAMS[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative disease characterised by the progressive degeneration of upper and lower motor neurons. Intraneuronal

aggregates of phosphorylated TDP-43 protein are present in the brain and spinal cord of ~97% of ALS patients. While pTDP-43 aggregates are considered the hallmark pathology of ALS, their incidence is variable across central nervous system regions and between patients. Four stages have been defined by the neuroanatomical location of pTDP-43 aggregates, representative of the lowest (stage I) to highest (stage IV) pathology burden. This neuropathological staging suggests a spread of pathology from regions that always exhibit pTDP-43 pathology to regions that less frequently show pathology.

We aimed to investigate the transcriptome profile of ALS patients with different stages of pTDP-43 pathology. Previous transcriptomic studies of ALS post-mortem brain tissue have neglected pTDP-43 pathology staging; a critical gap in our knowledge considering the established role of TDP-43 in ALS. We obtained post-mortem brain tissue for a cohort of sporadic ALS cases (n=22) and neurologically normal controls (n=11). ALS cases were subclassified as having stage I (n=8), stage II-III (n=7) or stage IV (n=7) pTDP-43 pathology. For each individual, RNA-Seq was performed on 5 brain regions varying in their presentation of pTDP-43 pathology (n=165 samples): motor cortex (pathology in stages I-IV), prefrontal cortex (pathology in stages II-IV), hippocampus (pathology in stage IV), and occipital cortex and cerebellum (no pTDP-43 pathology). Differentially expressed genes (DEGs) were identified using DESeq2 (FDR adjusted p-value <0.05).

Strikingly, despite not presenting with pTDP-43 pathology, cerebellum demonstrated the greatest difference in gene expression between stages. While only 11 DEGs were identified between stage I ALS and controls, this increased to 544 for stage II-III ALS and 1842 for stage IV ALS. This suggests that increased cerebellar transcriptome alterations may directly correlate with greater pTDP-43 pathology burden. Gene Ontology analysis identified “detoxification of copper ion” as the most significantly enriched biological process in stage II-III ALS cerebellum. This was driven by the upregulation of metallothionein genes, a change also evident in stage IV ALS cerebellum. Of the DEGs detected in ALS cerebellum, >90% were not identified in other brain regions, highlighting a divergent gene expression profile for the cerebellum. We propose that cerebellar gene expression changes provide insight into protective or compensatory mechanisms in ALS neurodegeneration.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.06

Topic: C.06. Neuromuscular Diseases

Support: NIEHS/NIH Grant P30ES010126

Title: Environmental inducers of TDP-43 pathology linked to amyotrophic lateral sclerosis

Authors: *G. FRAGOLA[1], J. WOLTER[2], R. WEEKS[3], A. WALL[3], A. PLANCHART[3], T. J. COHEN[4];
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Abstract: Altered functionality and solubility of Transactive response DNA binding Protein 43 kDa (TDP-43) is a hallmark of amyotrophic lateral sclerosis (ALS), a devastating neurodegenerative disease affecting upper and lower motor neurons. The overwhelming majority of ALS cases is sporadic, and the etiology likely stems from the interaction between age, genetics, and the environment. Recently, many environmental factors, including potentially damaging toxicants, have been linked to ALS through epidemiological studies, but a comprehensive identification of all risk compounds that we as humans are exposed to has not been done. There is, therefore, an urgent need to identify environmental toxins causing TDP-43 pathology. To address this need, we performed a high-content imaging screen by exposing cells expressing a GFP-tagged TDP-43 to a library of over 1,000 environmental compounds with high potential for human and ecosystem exposure and denoted as “at risk”. This screen led to the identification of novel inducers of TDP-43 aggregation, which were validated in mouse primary neurons, hiPSC-derived neurons, and adult zebrafish. Mechanistic studies indicate that these novel compounds induce TDP-43 aggregation via new cellular and biochemical pathways and highlight unanticipated targets for therapeutic intervention. Moreover, the detection of these environmental risk compounds identifies human populations at high risk of developing ALS and other TDP-43 related diseases.

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Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.07

Topic: C.06. Neuromuscular Diseases

Title: Characterization of the molecular mechanisms underlying neuromuscular junction defects and cell death in FUS and sporadic ALS

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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neurons (MNs) death in the spinal cord and brain, leading the loss of skeletal muscle mass. Previous data collected in our lab highlight an interesting aberrant crosstalk between the ALS-linked FUS protein and the RNA-binding protein (RBP) HuD, leading to upregulation of HuD

levels. As a consequence, some HuD targets such as the axonal proteins GAP43 and NRN1 are upregulated in FUS mutant MNs. Moreover, we have found NRN1-dependent aberrant increase in neurite branching and axonal outgrowth in these cells. Based on these findings, we aimed to assess whether such altered molecular circuitry, besides neurite alteration, can lead to neuromuscular junction (NMJ) disruption. To recapitulate the NMJ circuit in vitro we took advantage of human induced pluripotent stem cells (hiPSCs) to obtain a neural-muscle model system by 2D co-cultures. We found that FUS-mutant MNs were less able to establish NMJs with FUS-WT muscle despite the presence of a significant increase of the neurite network. Moreover, mutant MNs co-cultures show degeneration of both cellular components suggesting that this extensive neurite sprouting, as a part of a compensatory reinnervation process, could have a detrimental effect on neuromuscular endplate maturation, leading to axonal and motor unit degeneration. Interestingly, we observed similar phenotypes with HuD overexpressing MNs in a FUS WT genetic background, with implications for sporadic ALS. Indeed, recent studies revealed an increase of HuD expression in sporadic ALS patients devoid of mutations in known ALS-linked genes. Notably, FUS-WT MNs co-cultures exposed to oxidative stress showed upregulation of HuD levels, suggesting that a stressful condition, similar to that observed in ALS sporadic patients, is sufficient to trigger HuD dysregulation. Finally, we found that HuD and FUS can synergistically induce GAP43 increased expression by direct mRNA binding. Taken together, these observations suggest that a gain of function of HuD, due to mutant FUS or oxidative stress, might lead to altered NMJ in ALS.

Disclosures: B. Silvestri: None. **M. Garone:** None. **V. De Turris:** None. **M. Medici:** None. **A. Rosa:** None.

Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.08

Topic: C.06. Neuromuscular Diseases

Support: DBT

Title: C9orf72 interaction with cofilin modulates actin dynamics in motor neurons

Authors: *R. SIVADASAN;
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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease caused by motoneuron degeneration in the cerebral cortex, lower medulla, and spinal cord. Frontal temporal dementia (FTD) is the second most common form of dementia after Alzheimer's disease affecting an age group under 65-age. Even though ALS and FTD present with distinct clinical features, recent genetic analyses have revealed overlapping genetic and pathological mechanisms. A number of genetic mutations have been found to cause both ALS and FTD,

namely mutations in FUS-TLS, TDP-4, SQSTM1, UBQLN2, CHCHD, VCP, and C9ORF72. The identification of aberrant GGGGCC (G4C2) intronic repeat expansions in the C9ORF72 gene as the most common genetic cause of familiar ALS and FLTD in Europe has reinforced the relation between ALS and FTLD. Observations of population frequencies of the expansion are consistent with a common founder effect. But it is unknown whether loss of function, toxicity by the expanded RNA or dipeptides from non-ATG- initiated translation are responsible for the pathophysiology. Deep proteomics analysis determined the interactome of C9ORF72 in motor neurons and found that C9ORF72 was present in a complex with cofilin and other actin-binding proteins. Phosphorylation of cofilin was enhanced in C9ORF72-depleted motor neurons, in patient-derived lymphoblastoid cells, induced pluripotent stem cell-derived motor neurons and post-mortem brain samples from ALS patients. Regulating the activity of the small GTPases Arf6 and Rac1 modulated the phosphorylation of cofilin by C9ORF72. This modulation resulted in enhanced activity of LIM-kinases 1 and 2 (LIMK1/2). This results in reduced axonal actin dynamics in C9ORF72-depleted motor neurons. Dominant-negative Arf6 rescues this defect, suggesting that C9ORF72 acts as a modulator of small GTPases in a pathway that regulates axonal actin dynamics. Further analysis the show that C9ORF72 regulate the activity of Arf1and Arf6.

Disclosures: R. Sivadasan: None.

Poster

281. Amyotrophic Lateral Sclerosis

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Topic: C.06. Neuromuscular Diseases

Support: ALS Association (18-11A-418)
NIH/NINDS (R01NS116143)

Title: Effects of nuclear pore disruption on mRNA processing in C9ORF72 mutant iPSC derived cortical neurons

Authors: *M. GREGOIRE, C. FALLINI;
Cell and Mol. Biol., Univ. of Rhode Island, Kingston, RI

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are part of a broad neurodegenerative disease spectrum characterized by the degeneration of motor neurons and frontal and temporal cortex neurons, which leads to muscle paralysis and cognitive decline. Shared neurodegenerative pathways, clinical, pathological, and genetic features contribute to commonly observed cell phenotypes. These phenotypes include synapse loss, dendrite retraction, axonal degeneration, excitotoxicity, cytoskeletal abnormalities, protein aggregation, and altered nuclear membranes, nuclear pore complexes (NPC), and nucleocytoplasmic transport (NCT). The cause and consequence of these pathological observations are not clear. We hypothesize that

alterations to the NPC and NCT result in changes in mRNA transcription, splicing, and translation of proteins that may drive neurodegeneration. Which disease-relevant transcriptional pathways may be affected by NCT impairment is not well defined. This research investigates the effects of NPC disruption on RNA transcription in cortical neurons derived from C9ORF72 mutant and isogenic induced pluripotent stem cell (iPSC) using unbiased and candidate-based approaches. Our results demonstrate that mutant neurons display a reduced activation of the transcription factor CREB, an essential regulator of gene expression in response to neuronal stimulation. Collectively, our research supports the hypothesis that NPC/NCT impairment alters the ability of neurons to regulate gene expression and RNA metabolism, which may be early drivers of ALS/FTD pathology. This work was supported by the ALS Association (18-11A-418) and NIH/NINDS (R01NS116143).

Disclosures: M. Gregoire: None. **C. Fallini:** None.

Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.10

Topic: C.06. Neuromuscular Diseases

Support: VA Merit awards BX003625 and BX005585 to SD

Title: Single-nucleus transcriptome and epigenome profiling of human brain reveals the contribution of neuronal and glial cell types to C9orf72-associated ALS/FTD

Authors: *J. LI[1], M. K. JAISWAL[3], J. CHIEN[2], A. KOZLENKOV[3], V. V. BELZIL[4], E. A. MUKAMEL[1], S. DRACHEVA[3,5];
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Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal human neurodegenerative disorders that share many clinical and genetic characteristics. Mutations in several genes can lead to ALS, FTD, or mixed phenotypes. The most common mutation is a hexanucleotide repeat expansion in the first intron of C9orf72, which is found in around 12% of ALS and FTD patients. These disorders are conventionally considered to be caused by degeneration of motoneurons in the primary motor cortex and spinal cord of ALS patients, and von Economo neurons in the frontal and temporal lobes of FTD patients. However, mounting evidence indicates that non-neuronal cells may play a role in the pathogenesis of ALS/FTD. Moreover, the contributions of other neuronal populations are unknown.
Here we analyzed autopsied motor cortex and medial frontal cortex specimens from 6 ALS and 6 FTD cases with C9orf72 (C9) mutation, and 6 age-matched control individuals. We performed transcriptome and epigenomic profiling in these samples using single-nucleus RNA sequencing

(snRNA-seq) and Assay for Transposase-Accessible Chromatin with sequencing (snATAC-seq). We found that among the 14 major classes of neurons and glia, astrocytes and upper layer excitatory neurons had the most profound transcriptional disruptions in both motor cortex and frontal cortex of the diseased brains. Disease-associated differentially expressed genes were functionally enriched in cytoskeleton and cell-surface protein remodeling in astrocytes, as well as mitochondrial function and cellular proteostasis in excitatory neurons. These transcriptome alterations were accompanied by concordant changes in chromatin accessibility in the gene body of differentially expressed genes. We further used chromatin immunoprecipitation sequencing (ChIP-seq) for H3K27ac to assay active enhancers and promoters in purified cell populations that were separated by fluorescence-activated nuclei sorting (FANS). We found that the active histone mark was correlated with disease-associated differential mRNA expression. We further confirmed these findings using immunofluorescence and Western blot detection of selected differentially expressed genes.
In summary, our study represents a comprehensive characterization of molecular alterations in C9orf72-associated ALS and FTD at single-cell resolution. Our findings of considerable ALS/FTD-associated perturbations in astrocytes provide insights on novel biological pathways relevant to the pathology of the diseases.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.11

Topic: C.06. Neuromuscular Diseases

Title: Dysregulated mir-18b by mitf inhibits myogenic differentiation via the activation of ctgf/trka/erk1,2 signaling pathway in duchenne muscular dystrophy

Authors: *K. CHOI, J. SUNG;
Seoul Natl. Univ. Sch. of Med., Seoul, Korea, Republic of

Abstract: Duchenne muscular dystrophy (DMD) is a degenerative disorder caused by the absence of the cytoskeletal protein dystrophin. Dystrophin is localized in the extracellular matrix and the actin cytoskeleton. Connective tissue growth factor (CTGF/CCN-2) is involved in the induction of fibrosis in muscle degenerative diseases. In *mdx* mouse, the mouse model of DMD, the level of CTGF is significantly increased whereas the level of dystrophin is decreased. Importantly, the expression of CTGF is negatively regulated by microRNA-18b (miR-18b). Microphthalmia-associated transcription factor (MITF) is involved in the regulation of melanocyte, development, and survival. Interestingly, we discovered MITF binding motif in the promoter region of miR-18b, which regulates the miR-18b activity. Therefore, MITF is associated with the regulation of both CTGF and miR-18b. Moreover, miR-18b further regulates

myogenesis, especially myogenic differentiation, via CTGF/TrkA/Erk1,2 signaling pathway. Taken all together, our data indicate that miR-18b is involved in the regulation of CTGF and mediated by MITF. miR-18b modulated by MITF at its promoter region plays a significant role in the regulation of CTGF expression, which would not only promote the development of fibrosis but also inhibit the myogenesis. Our study suggests a novel mechanism to understand the pathophysiology of DMD.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.12

Topic: C.06. Neuromuscular Diseases

Support: DFG-Emmy Noether Research Group DA 1657/2-1

Title: Epigenetic Chromatin Accessibility Alterations in Amyotrophic Lateral Sclerosis Peripheral Blood Cells

Authors: *J. K. KÜHLWEIN[1], W. RUF[1], V. GROZDANOV[1], K. M. DANZER[1,2];
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Abstract: The synergistic interplay of genetic predisposition and environmental impacts is believed to play a central role in complex neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS). Both genetic predisposition and environmental impacts leave specific epigenetic signatures in the cells of the affected tissues which in turn affect transcriptional programs. The combined effect of these factors can be assessed by assaying the genome-wide epigenetic landscape of cells. Studying the epigenome is powerful for the discovery of novel and targetable disease mechanisms in affected tissues. However, the central nervous system (*CNS*) is hardly accessible in living ALS patients. Therefore, the possibility to investigate CNS-relevant mechanisms from the periphery is of utter importance for the progress in ALS research. We employ a multi-omic approach combining simultaneous ATAC-seq and RNA-seq with single-cell sequencing in PBMCs and motor cortex from ALS patients and integrate the findings by thorough data science analysis. By applying ATAC-seq to PBMCs from 23 sporadic ALS patients and 18 matched healthy controls, we identify a robust ALS-relevant epigenetic signature (*'epiChromALS'*) that compromise 729 differentially accessible peaks (FDR<0.05) that are annotated to 668 unique genes. Most of these peaks (80 %) were less accessible and enriched in enhancer and promoter regions. A functional enrichment analysis of these differentially accessible peaks showed a relation to neuronal functions and neuron differentiation suggesting that neurodevelopmental processes are epigenetically impaired in ALS. By combining ATAC-seq and RNA-seq from the same sample, we show that the chromatin accessibility signature of ALS was largely independent from the dysregulation of PBMC subtypes which we found in the

transcriptome profile of ALS. Further, the comparison of *epiChromALS* to GWAS databases show a clear overlap as well as a correlation to clinical parameters indicating its functional relevance for the disease. Integrating single-nuclei ATAC-seq data from post-mortem cortex of an ALS patient show that our signature is also detectable in the CNS. Together, these findings strongly suggest that robust epigenetic disease mechanisms can be studied and monitored in peripheral blood and will strong outreach in the study not only of ALS, but also other neurodegenerative diseases.

Disclosures: J.K. Kühlwein: None. **W. Ruf:** None. **V. Grozdanov:** None. **K.M. Danzer:** None.

Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.13

Topic: C.06. Neuromuscular Diseases

Support: National Science Foundation, Graduate Research Fellowship Program
All NYGC ALS Consortium activities are supported by the ALS Association (ALSA, 19-SI-459) and the Tow Foundation

Title: Transcriptomic based stratification identifies molecular subtypes of ALS with differences in prognosis

Authors: *J. ESHIMA[1], S. A. O'CONNOR[1], E. MARSCHALL[1], N. Y.G.C. ALS CONSORTIUM[2], R. P. BOWSER[3], C. L. PLAISIER[1], B. S. SMITH[1];
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a highly heterogeneous neurodegenerative disease, evidenced by clinical variability in patient survival, age of symptom onset, site of symptom onset, and therapeutic response. ALS heterogeneity has traditionally been poorly understood and, as a consequence, disease-specific diagnostic and prognostic markers remain elusive[1]. In this study, we consider ALS heterogeneity by leveraging systems-based strategies for patient stratification. Utilizing frontal and motor postmortem cortex transcriptomes, derived from 208 unique ALS patients[2], we identified three distinct molecular subtypes and first report subtype-driven differences in patient survival. Following enrichment, these distinct molecular subtypes were defined by i) glial activation (ALS-Glia), ii) oxidative stress and altered synaptic signaling (ALS-Ox), and iii) transcriptional dysregulation (ALS-TD). Building off previous works[3], we help to clarify the role of transposable elements in the context of ALS by considering these genomic elements at the locus specific level, leading us to redefine one of the three subtypes previously established. Taken together, our results suggest that independent molecular

mechanisms drive some of the clinical heterogeneity observed in ALS. Many of the genes and transcripts in this study have not been previously associated with ALS neurodegeneration. Therefore, these findings offer new insight into disease mechanisms and potential targets for diagnostic or personalized therapeutic development.
[1]Bowser, R., Turner, M.R., Shefner, J., Biomarkers in amyotrophic lateral sclerosis: opportunities and limitations. *Nature Reviews Neurology* 7, 631-638 (2011).[2]Prudencio, M. et al. Truncated stathmin-2 is a marker of TDP-43 pathology in frontotemporal dementia. *The Journal of clinical investigation* 130, e139741 (2020).[3]Tam, O.H. et al. Postmortem cortex samples identify distinct molecular subtypes of ALS: retrotransposon activation, oxidative stress, and activated glia. *Cell reports* 29, 1164-1177 (2019).

Disclosures: J. Eshima: None. **S.A. O'Connor:** None. **E. Marschall:** None. **N. Y.G.C. ALS Consortium:** None. **R.P. Bowser:** A. Employment/Salary (full or part-time):; Iron Horse Diagnostics. **C.L. Plaisier:** None. **B.S. Smith:** None.

Poster

281. Amyotrophic Lateral Sclerosis

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Support: NIH Grant R01-NS121125-01
Mayo Clinic Ventures Innovation Program Award

Title: Examining the role of PABPC4 as a TDP-43 modifier in FTLD-TDP

Authors: *U. SHETH[1,2], N. FINCH[2], A. VEIRE[2], L. DAUGHRITY[2], K. JANSEN-WEST[2], M. BAKER[2], N. GRAFF-RADFORD[3], B. F. BOEVE[4], R. PETERSEN[4], K. JOSEPHS[4], B. OSKARSSON[3], L. PETRUCELLI[2], R. RADEMAKERS[2], D. W. DICKSON[2], Y. ZHANG[2], M. VAN BLITTERSWIJK[2], T. F. GENDRON[2];
[1]Mayo Clin. Grad. Sch. of Biomed. Sci., [2]Neurosci., [3]Neurol., Mayo Clin., Jacksonville, FL; [4]Neurol., Mayo Clin., Rochester, MN

Abstract: Background: Approximately 50% of Frontotemporal lobar degeneration (FTLD) cases are characterized by the abnormal cleavage, phosphorylation, and aggregation of TDP-43. Reducing toxic TDP-43 accumulation thus represents a promising therapeutic approach. Our preliminary data show that cytoplasmic polyadenylate-binding protein 4 (PABPC4) decreases phosphorylated TDP-43 (pTDP-43) in cultured cells. **Methodology:** We expressed GFP-tagged TDP-43 species [wild-type TDP-43, TDP-43 with mutations in the nuclear localization signal ($TDP\text{-}43_{NLSmut}$), or a C-terminal TDP-43 fragment ($TDP\text{-}43_{220\text{-}414}$)] in HEK293T or M17 cells that expressed exogenous PABPC4 or not. We measured soluble and insoluble pTDP-43 and total TDP-43 by Western Blot, and immunofluorescence (IF). To examine whether PABPC4 modulates TDP-43 *in vivo*, we are using a mouse model that inducibly expresses $TDP\text{-}43_{NLSmut}$.

Post-natal day 0 (P0) pups are being injected with an adeno-associated virus (AAV) encoding PABPC4. **Results:** Through transcriptomic studies, we discovered that higher levels of frontal cortex *PABPC4* mRNA associate with greater survival in patients with FTLD-TDP. Further, we found a significant inverse correlation between frontal cortex *PABPC4* and pTDP-43 (N = 71, r = -0.4089, p = 0.0004). We found that PABPC4 overexpression decreased pTDP-43 and insoluble TDP-43 levels; the converse was true when PABPC4 was knocked-down. Consistent with these findings, IF revealed more diffuse, non-aggregated TDP-43_{NLSmut} in cells overexpressing PABPC4 compared to controls. Our *in vivo* studies will help us determine whether PABPC4 reduces TDP-43 pathology, rescues motor deficits, and enhances survival compared to control mice. **Conclusions:** Based on our present findings, PABPC4 is a novel modifier of TDP-43 pathology; studies are underway to decipher the underlying mechanisms.

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Poster

281. Amyotrophic Lateral Sclerosis

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.15

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS107347

Title: Pathomechanisms of distinct repeat RNA species in C9ORF72 ALS

Authors: B. L. ZAEPFEL[1], J. D. ROTHSTEIN[2], *A. COYNE[2];
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Abstract: The most common genetic cause of Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) is a GGGGCC (G_4C_2) hexanucleotide repeat expansion (HRE) in the *C9orf72* gene. Bidirectional transcription and subsequent repeat associated non-ATG (RAN) translation results in the production of molecular hallmarks of disease: two repeat RNA and five dipeptide repeat protein (DPR) species. While numerous reports suggest that DPRs are neurotoxic, the contribution of repeat RNA toxicity to neurodegeneration remain understudied. Recently, we have shown that G_4C_2 (sense) repeat RNA, but not DPRs, can trigger a significant injury to the nuclear pore complex (NPC) ultimately culminating in TDP-43 dysfunction and neuronal death. However, the contribution of G_2C_4 (antisense) repeat RNA to disease pathogenesis and the mechanism by which repeat RNA species elicit NPC injury cascades remains unknown. Here, using induced pluripotent stem cell (iPSC) derived neurons (iPSNs) we show that G_2C_4 and G_4C_2 repeat RNA expression is sufficient to trigger the molecular event that

initiates NPC injury and subsequent TDP-43 dysfunction in iPSNs: aberrant nuclear accumulation of the ESCRT-III protein CHMP7. Notably, G_4C_2 repeats impair the association of CHMP7 with XPO1, thereby retaining CHMP7 in the nucleus to initiate this pathogenic cascade. Using strand-specific antisense oligonucleotides (ASOs) we show that G_2C_4 but not G_4C_2 repeat RNA significantly contributes to NPC injury and downstream deficits in TDP-43 function. Additionally, we demonstrate that synthetic G_4C_2 and C_4G_2 oligonucleotides bind distinct subsets of neuronal proteins *in vitro.* Collectively, these studies indicate that pathogenic repeat RNAs play important and previously underappreciated role in *C9orf72* HRE-mediated neurodegeneration.

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Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.16

Topic: C.06. Neuromuscular Diseases

Title: Organelle contact dysregulation in neurons with familial ALS cytoskeleton mutations.

Authors: *S. RHOADS[1], D. CAMPISI[2], S. HSU[2], V. DONG[2], S. COHEN[2], T. J. COHEN[3]; [1]Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Univ. of North Carolina, Chapel Hill, NC; [3]Neurol., Univ. of North Carolina - Chapel Hill, Chapel Hill, NC

Abstract: Organelle contacts are physiological interactions between two or more membrane-bound organelles. They facilitate critical lipid, protein, and metabolite transfer in all cells and have recently been implicated in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). ALS is a polygenic neurodegenerative disease with largely unknown etiology. Of the approximately 50 causative genes, several encode proteins related to the cytoskeleton, organelle contact sites, organelle trafficking, and multi-organelle processes (e.g., autophagy). Mutations in such genes could lead to alterations in organelle contacts. However, the extent of organelle contact dysfunction in ALS is not fully understood. Here, we utilized multispectral imaging to simultaneously visualize contacts between six fluorescently tagged organelles in live neurons and astrocytes. Using pharmacological perturbations to mimic ALS phenotypes *in vitro,* we have identified unique organelle signatures between cell types and drug treatments. These findings confirm that neurons and astrocytes respond to cellular stresses in distinct ways that can be observed through organelle contacts and morphology. In future experiments, we will utilize this technique to investigate organelle contact dysregulation from familial ALS mutations in actin and microtubule cytoskeleton genes. We hope to identify putative organelle contact sites disrupted in ALS and determine their associated pathological mechanisms.

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Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

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Program #/Poster #: 281.17

Topic: C.06. Neuromuscular Diseases

Support: Target ALS

Title: Development of improved immunoassays for TDP-43 and pathologic species of TDP-43 in human biofluids.

Authors: *V. ORTEGA[1], J. AN[2], J. SAUL[2], R. P. BOWSER[2];
[1]BARROW NEUROLOGICAL INSTITUTE, PHOENIX, AZ; [2]Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Development of improved immunoassays for TDP-43 and pathologic species of TDP-43 in human biofluids **Authors**: Vanessa Ortega, Jiyan An, Justin Saul, Robert Bowser, PhD. Department of Translational Neuroscience, Barrow Neurological Institute, Phoenix, AZ, United States, 85013
Abstract Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that includes the aggregation of multiple proteins in neurons and glia. The most prevalent protein aggregate includes the TAR DNA-binding protein (TDP-43). TDP-43 aggregation and cytoplasmic inclusions are observed in approximately 97% of post-mortem ALS cases. TDP-43 proteinopathies also includes frontotemporal lobar dementia (FTLD), limbic-predominant age-related TDP-43 encephalopathy (LATE), multisystem proteinopathy (MSP), and Alzheimer's disease. Accurate and reliable measures of TDP-43 (both full-length and pathologic species) in human biofluids may be valuable biomarkers for all TDP-43 proteinopathies. Current commercial immunoassays to measure TDP-43 have generated conflicting data in the literature regarding TDP-43 levels in blood or CSF of ALS and other neurodegenerative diseases. We wished to develop improved TDP-43 immunoassays on the meso scale discovery (MSD) platform for full length and pathologic species of TDP-43 in human biofluids. We tested 14 antibodies using various combinations and assay conditions. We identified the optimal set of anti-TDP-43 for capture and detection antibodies, and further optimized all wash and incubation conditions for detecting full-length TDP-43 in human biofluids. We completed a series of assay validation experiments, and then measured TDP-43 levels in the CSF and blood of both ALS and control subjects. ALS patients exhibited increased levels of full-length TDP-43 in the blood when compared to healthy controls, but reduced levels in the matching CSF samples from ALS patients. There was a strong negative correlation between the TDP-43 levels in the blood and CSF within individual ALS patients. We are currently completing assay conditions to quantify

pathologic species of TDP-43. These biomarker assays will be valuable tools in drug development and clinical trials for TDP-43 proteinopathies.

Disclosures: **V. Ortega:** 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.; IRON HORSE DIAGNOSTICS/VECTOR. **J. An:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Iron Horse Diagnositics/VECTOR. **J. Saul:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Iron Horse Diagnostics/VECTOR. **R.P. Bowser:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Iron Horse Diagnostics/VECTOR.

Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.18

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant T32GM007445
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Title: Assessment of TDP-43 nuclear clearance and functional deficits in immortalized cells and iPSC-derived neurons

Authors: ***B. ZAEPFEL**, A. N. COYNE, J. D. ROTHSTEIN;
Johns Hopkins Med. Inst., Johns Hopkins Sch. of Med., BALTIMORE, MD

Abstract: TAR DNA-binding Protein (TDP-43) is a ubiquitously expressed nuclear DNA- and RNA-binding protein, though it transiently shuttles between the nucleus and cytoplasm. In neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), Frontotemporal Dementia (FTD), and Alzheimer's Disease (AD), TDP-43 becomes cleared from the nucleus where it can aberrantly associate with other proteins and RNA in the cytoplasm to form pathological aggregates in a subset of CNS cells. The nuclear clearance precedes cytoplasmic accumulation and is linked to loss of nuclear TDP-43 function. However, it is not yet known how much TDP-43 must be cleared from the nucleus before its typical regulation of splicing and transcription are altered. Here, we utilize small-interfering RNA (siRNA) and antisense oligonucleotides (ASO) targeting TDP-43 to reduce its abundance in HeLa cells or induced pluripotent stem cell-derived spinal neurons (iPSNs). Following knockdown of TDP-43 to various thresholds, we monitor the total, nuclear, and cytoplasmic abundance of TDP-43 by immunoblot and immunofluorescence. Furthermore, we assess the effect of TDP-43 loss on known transcriptional and splicing targets at these different thresholds. Together, these experiments provide an experimental timeline of when TDP-43 nuclear depletion becomes observable with common assays, as well as a threshold-dependent order of affected mRNA

targets. These results can inform future clinical investigations that monitor TDP-43 targets as indicators of disease status upon therapeutic interventions.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.19

Topic: C.06. Neuromuscular Diseases

Support: MRC New Investigator MR/R024162/1
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Title: Targeting RAN translation and neurodegeneration in C9ORF72-ALS/FTD: moving towards gene therapy approaches in pre-clinical models

Authors: *B. C. BENSON, L. M. CASTELLI, Y.-H. LIN, M. AZZOUZ, P. J. SHAW, L. FERRAIUOLO, G. HAUTBERGUE;
Univ. of Sheffield, Sheffield, United Kingdom

Abstract: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are fatal neurogenerative diseases on a single clinical spectrum, caused by polymorphic G4C2 hexanucleotide repeat expansions in intron-1 of the *C9ORF72* gene. Bi-directional transcription of this locus leads to expression of sense and antisense repeat transcripts which undergo an unconventional form of translation, Repeat Associated Non-AUG (RAN) translation in all frames and in absence of the canonical AUG start codon. This produces five dipeptide repeat proteins, which are aggregate-prone, neurotoxic and comprise one of three key mechanisms relevant to C9ORF72-ALS/FTD neurodegeneration. Here, we investigated the roles of four RNA/DNA helicases in the potential initiation of RAN translation, naming these RAN-translation associated factors (RTFs). We hypothesis that RAN translation of neurotoxic DPRs can be reduced without impacting canonical translation, through partial depletion of RTFs or through inhibition of their helicase activity, to confer neuroprotection in pre-clinical models of C9ORF72-ALS/FTD. We aim to assess the potential of each RTF as a target for gene therapy. Here, we show that shRNA-induced depletion of RTFs or RTF mutants with non-functional RNA helicase activity inhibits RAN translation, reduces DPR production, and rescues DPR-induced cytotoxicity in HEK293T models of C9ORF72-ALS/FTD. Targeting individual RTFs for depletion was found to be non-toxic in this model, while targeting multiple RTFs conferred significant toxicity. As little is known about the role of RTFs in post-mitotic neurons, we next used lentiviral depletion in C9ORF72-ALS patient-derived motor neuron and astrocyte co-cultures. Interestingly, in contrast to the HEK293T model, RTF1 depletion was neurotoxic while depletion of RTF2, 3 and 4 conferred neuroprotection. Overall, these studies advance our

understanding of the regulators of RAN translation, a poorly understood mechanism relevant to several neuromuscular/ neurodegenerative diseases including Huntington's Disease, Spinocerebellar Ataxia, and Myotonic Dystrophy. Further, we highlight the potential of targeting RTFs as a novel therapeutic approach to reduce production of neurotoxic DPRs in C9-ALS/FTD with potential to be taken forward into animal models.

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Poster

281. Amyotrophic Lateral Sclerosis

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Title: Single-cell dissection of ALS and frontotemporal dementia in human motor and prefrontal cortices

Authors: *S. PINEDA[1,3], H. LEE[2], B. FITZWALTER[2], R. LINVILLE[2], E. COOK[4], D. DICKSON[4], V. BELZIL[4], M. KELLIS[1,3], M. HEIMAN[2];
[1]Electrical Engin. and Computer Sci., [2]Brain and Cognitive Sci., MIT, Cambridge, MA; [3]Broad Inst. of MIT and Harvard, Cambridge, MA; [4]Mayo Clin., Jacksonville, FL

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are devastating and fatal neurodegenerative diseases that share many clinical, pathological, and genetic signatures. However, the mechanistic basis of their shared and distinct circuitry remains unknown at the molecular level. To uncover cell type-specific transcriptional changes, underlying biological pathways, and putative upstream regulators, we conducted high-resolution single-cell profiling of transcriptional alterations in the primary motor and dorsolateral prefrontal cortices of 75 sporadic and *C9orf72+* familial ALS and FTLD donor individuals and unaffected controls, providing the most comprehensive-to-date characterization of Brodmann areas 4 and 9, and yielding insights of unprecedented resolution into both ALS and FTLD. Our analysis revealed enhanced cross-region, and cross-phenotypic vulnerability of an extratelencephalic layer Vb population that includes the ALS and FTLD-implicated Betz cells and Von Economo neurons. We identified novel and highly-specific marker genes for these previously ill-defined

populations and found that the majority of these are shared across brain regions and may comprise unique vulnerability factors. Analysis of differentially expressed genes (DEGs) showed that several ALS- and FTLD-associated genes were dysregulated across phenotypes and some with regional and cell type specificity. We observed that a number of high-ranking DEGs belonged to the class of TDP-43 binding targets, and that non-canonically disease-associated cells of the same type showed similar disease signatures across both phenotypes and brain regions. We also discovered disease-associated, but regionally-dependent, changes in gene expression and protein localization of several tight junction proteins and their regulators in vascular populations. Shared across cell types, we observed enrichment of pathway terms associated with oxidative stress, protein localization, and ribosomal dysfunction, and within excitatory neurons, an enrichment for terms associated with axonal growth, maintenance, and repair consistent with reports of axonal integrity deterioration. ALS-enriched pathways showed greater specificity for microtubule maintenance- and organization-associated processes, while FTLD dysregulated genes showed generally amplified enrichment for stress response-associated pathways. Overall, our study represents the largest and most accurate molecular atlas of these two human brain regions to date, and the first cell type-specific molecular characterization of ALS and FTLD in either.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.21

Topic: C.06. Neuromuscular Diseases

Support: Laurence and Sandi Gluck Charitable Foundation

Title: Apolipoprotein B-100 induces neurotoxicity in a novel CSF-mediated animal model of sporadic ALS

Authors: *J. K. WONG, I. GAO, R. P. GRIFFIN, S. A. SADIQ;
Tisch Multiple Sclerosis Res. Ctr. of New York, New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal progressive disease characterized by motor neuron degeneration. Approximately 90% of ALS patients are diagnosed with sporadic ALS (sALS) without a known cause, while 10% have familial ALS (fALS) arising from an identified genetic mutation. Previous in vivo and in vitro studies have demonstrated that ALS cerebrospinal fluid (CSF) is neurotoxic, but it is unclear whether differences in neurotoxicity exist between sALS and fALS CSF, and the neurotoxic candidates in CSF have yet to be identified. Here, our goal was to identify the neurotoxic factor(s) in sALS CSF which trigger motor disability and motor neuron degeneration in our CSF-induced animal model.Adult female

C57BL/6J mice underwent laminectomies at cervical levels 4 and 5, and received 3 µl injections into the subarachnoid space of either: 1) CSF from sALS or fALS (*SOD1*, *C9orf72*, or *TARDBP*) patients, 2) filtered sALS CSF, 3) apolipoprotein B-100 (ApoB)-depleted sALS CSF, or 4) human ApoB protein. Control mice received either saline, CSF from healthy individuals (HC) or multiple sclerosis patients, or human CSF proteins. Forelimb motor deficits were assessed at 1 day post injection, then mice were perfused for histological analyses of the spinal cord. All motor and histological assessments were performed blinded. sALS CSF was passed through a tangential flow filtration system to remove components by molecular weight and unbiased global proteomic profiling was performed on CSF pre- and post-filtration. Immunodepletion of ApoB from sALS CSF was performed using ApoB antibody-coated Dynabeads®.sALS CSF-injected mice exhibited significantly impaired forelimb function associated with increased motor neuron death compared to controls and fALS CSF-injected mice. Filtration studies determined the neurotoxic protein in sALS CSF to be between 300 kDa and 750 kDa in size as removal of components larger than 300 kDa attenuated neurotoxicity, while the 750 kDa filtrate retained neurotoxicity. Proteomic analyses revealed ApoB to be upregulated in sALS CSF compared to HC CSF, downregulated post-filtration, and the only candidate with the appropriate molecular weight (550 kDa). Mice injected with ApoB-depleted sALS CSF did not show motor impairments nor loss of motor neurons, while ApoB protein, but not control proteins, recapitulated the neurotoxic effects of sALS CSF. This study identifies ApoB as the neurotoxic protein in sALS CSF responsible for motor disability and motor neuron degeneration and provides proof-of-concept to support CSF pheresis as a therapeutic strategy for the predominant sporadic form of ALS.

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Poster

281. Amyotrophic Lateral Sclerosis

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Support: NIH 1RO1NS101895-01A1
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The Angel Fund for ALS Research

Title: Protein citrullination marks myelin protein aggregation and disease progression in amyotrophic lateral sclerosis

Authors: *I. O. YUSUF[1], T. QIAO[1,2], P. R. THOMPSON[1,3], Z. XU[1], R. TILVAWALA[1,4], S. PARSI[5];
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of Massachusetts Chan Med. Sch., Worcester, MA; [4]Scorpion Therapeut., Boston, MA; [5]Ctr. for Syst. Biol. Inst. for Innovation in Imaging-i3, Massachusetts Gen. Hosp., Boston, MA

Abstract: Protein citrullination is a posttranslational modification that involves the irreversible conversion of protein-arginine to protein-citrulline and is catalyzed by a family of enzymes known as protein arginine deiminases (PADs). Mammals encode five PADs, and PAD2 is the most dominant and ubiquitous isoform in the central nervous system (CNS). Aberrant protein citrullination and PAD2 dysregulation have been demonstrated in several neurodegenerative diseases. To determine whether this is the case for amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease characterized by motor neurons loss, paralysis, and subsequent death, we investigate protein citrullination and PAD2 in two different transgenic mouse models of ALS expressing human mutant $SOD1^{G93A}$ and $PFN1^{C71G}$, using real-time qPCR, Immunoblotting, Immunohistochemistry, and Immunofluorescent techniques. We report that protein citrullination and PAD2 expression are dynamically altered in the spinal cord during the progression of disease, increasing progressively in astrocytes with reactive astrogliosis, while decreasing in neurons and their axons. Furthermore, in the spinal cord white matter, citrullinated proteins accumulate as protein aggregates containing myelin proteins PLP and MBP. Likewise, citrullinated proteins progressively accumulate in insoluble protein fractions during the disease progression. Interestingly, the increases in PAD2 expression and protein citrullination spatially correlates with CNS areas with the most severe motor neuron degeneration. We also validated our findings in sporadic ALS patient's spinal cord samples and found that protein citrullination and PAD2 expression are increased and correlate with reactive astrogliosis. Also, citrullinated proteins forms aggregates that contain PLP and MBP in patients compared to non-neurological controls. These results suggest that increased protein citrullination and PAD2 dysregulation are critical characteristics of reactive astrogliosis, motor neuron degeneration, and myelin protein aggregation in the pathogenesis of ALS, and may serve as another pathological marker of the ALS disease progression. Further studies are needed to dissect the specific roles that protein citrullination play in the pathogenesis of ALS.

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Poster

281. Amyotrophic Lateral Sclerosis

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Program #/Poster #: 281.23

Topic: C.06. Neuromuscular Diseases

Support: Laurence and Sandi Gluck Charitable Foundation

Title: Upper motor neuron degeneration is induced by apolipoprotein B-100 in a murine model of sporadic ALS

Authors: *R. P. GRIFFIN, I. GAO, J. K. WONG, S. A. SADIQ;
Tisch Multiple Sclerosis Res. Ctr. of New York, New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the progressive onset of motor neuron degeneration in both the brain and spinal cord, leading to motor dysfunction. Sporadic ALS (sALS) is the dominant subtype, accounting for approximately 90% of all ALS cases, but lacks a specific disease model. To better study sALS, we developed a new murine model by intrathecally injecting the CSF of human sALS patients into experimental mice. We previously reported how this method recapitulates many symptoms of ALS, including motor disability, and lower and upper motor neuron loss. CSF filtration and proteomic analyses led to the identification of apolipoprotein B-100 (ApoB) as the neurotoxic factor in sALS CSF. Here, we explored whether ApoB also induces upper motor neuron degeneration in our animal model. Adult C57BL/6J female mice underwent laminectomies at cervical levels 4 and 5, then received an intrathecal injection of 3 µL of either: 1) sALS CSF, 2) ApoB-depleted ALS CSF, 3) human ApoB protein or 4) saline. sALS patient CSF was depleted of ApoB through incubation with ApoB antibody-bound Dynabeads®. At one day post-injection, mice were perfused for histological analysis of the brains. Brains were post-fixed overnight in 4% paraformaldehyde, cryoprotected in 30% sucrose, then cryosectioned at 30 µm for immunostaining with the neuronal marker NeuN. All histological analyses were performed blinded. A significant decrease in the number of $NeuN^+$ motor neurons in the motor cortices was observed in mice injected with sALS CSF, but not in mice injected with ApoB-depleted sALS CSF. Upper motor neuron loss was also observed in ApoB-injected mice. This indicates that ApoB is likely primarily responsible for upper motor neuron degeneration in our animal model. Because the injection is performed at the cervical spinal cord, we question if the observed degeneration is triggered by the diffusion of ApoB from the injection site to the brain or if a retrograde signaling mechanism is triggered by the death of lower motor neurons. We will further investigate this question in order to better understand the pathogenesis of sALS.

Disclosures: R.P. Griffin: None. **I. Gao:** None. **J.K. Wong:** None. **S.A. Sadiq:** None.

Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.24

Topic: C.06. Neuromuscular Diseases

Support: Laurence and Sandi Gluck Charitable Foundation

Title: Apolipoprotein B-100 in sporadic ALS CSF induces human motor neuron degeneration

Authors: *I. GAO, R. P. GRIFFIN, J. K. WONG, S. A. SADIQ;
Tisch Multiple Sclerosis Res. Ctr. of New York, New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron death, with its sporadic form affecting 90% of those with the disorder. We previously showed that cerebrospinal fluid (CSF) from sporadic ALS (sALS) patients induces human motor neuron death and neurotoxic factors can be removed following filtration through a 5 kDa MWCO filter. Additionally, our in vivo studies had identified apolipoprotein B-100 (ApoB) as the neurotoxic factor responsible for inducing motor disability and ALS-like pathology in a CSF-mediated animal model of sALS. Here, we aimed to determine whether ApoB in sALS CSF also triggers death of human motor neurons in vitro. Human iPSC-derived motor neurons were cultured for 8 days, then incubated for 24 hours with either: 1) 50% sALS CSF, 2) 50% filtered sALS CSF, 3) 50% ApoB-depleted sALS CSF, 4) human ApoB protein, or 5) human haptoglobin protein in motor neuron maintenance media. Components larger than 5 kDa, 300 kDa, or 750 kDa were eliminated from sALS CSF through a tangential flow filtration system. ApoB was depleted from sALS CSF using ApoB antibody-coated Dynabeads®. Different concentrations of lipid-free human ApoB were also applied directly to motor neurons, comparing the apolipoprotein's toxicity to media or the control protein haptoglobin. Motor neurons were fixed in 4% paraformaldehyde for ChAT immunocytochemistry. Areas of $ChAT^+$ motor neuron clusters were quantified as a measure of motor neuron death. All analyses were performed blinded. sALS CSF significantly reduced motor neuron cluster sizes compared to media. However, ApoB-depletion and filtration prevented sALS CSF-induced neurotoxicity. Only motor neurons treated with 750 kDa-filtered sALS CSF exhibited smaller clusters than control, since the filter was too large to exclude ApoB (550 kDa). In addition, the direct application of lipid-free ApoB revealed a dose-dependent effect on human motor neuron death, where smaller motor neuron clusters were only seen following treatment with ApoB concentrations similar to that found in sALS CSF or higher. Our studies collectively show that ApoB in sALS CSF induces human motor neuron death in vitro. Future experiments should aim to elucidate the mechanisms of ApoB-induced neurotoxicity and identify therapeutic targets to prevent motor neuron degeneration in sALS.

Disclosures: I. Gao: None. **R.P. Griffin:** None. **J.K. Wong:** None. **S.A. Sadiq:** None.

Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.25

Topic: C.06. Neuromuscular Diseases

Support: Title III Award P031C160209

Title: Impact of phosphatase activity in C. elegans expressing TDP-43, an Amyotrophic Lateral Sclerosis associated disease protein

Authors: *H. PUCCINI DE CASTRO[1], C. VOISINE[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease. Mutations in the TAR DNA-binding Protein of 43 kDa (TDP-43), an RNA binding protein, have been linked to familial cases of ALS. The accumulation of hyperphosphorylated TDP-43 in the cytoplasm of affected neurons is considered a pathological hallmark of the disease. However, the role of phosphorylation in disease progression remains unclear. To understand the impact of phosphorylated TDP-43 on neuronal function, we are using the nematode C. elegans, a transparent worm that has a short lifespan, a simple nervous system and is amenable to genetic manipulation. Deep sequencing of actively translated mRNAs from adult animals that express human TDP-43 pan-neuronally and wild type animals revealed a set of differentially translated genes. Gene Ontology analysis identified an enrichment of the dephosphorylation biological process suggesting that animals expressing neuronal TDP-43 increase phosphatase activity, possibly to reduce the level of phosphorylated TDP-43. I selected two phosphatase-related genes, one gene is expressed in the nervous system of worms and the second gene has a human orthologue, PTPN7, that participates in MAP-kinase signaling. Currently, I am crossing strains that carry deletions of each phosphatase gene into TDP-43 expressing animals. Using western analysis, I will determine if the deleted phosphatases increase levels of phosphorylated TDP-43. Furthermore, behavioral assays will be conducted to measure neuronal functionality in these animals. These studies offer insight into potential therapeutic strategies targeting TDP-43 phosphorylation to alleviate ALS pathology. Funding: Student Center for Science Engagement, College of Arts and Sciences at Northeastern Illinois University, and U.S. Department of Education (USDOE) Title III Award (P031C160209) supported this research.

Disclosures: H. Puccini de Castro: None. **C. Voisine:** None.

Poster

281. Amyotrophic Lateral Sclerosis

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 281.26

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant R01NS107442
NIH Grant R01NS117583
Project ALS

Title: Saturated free fatty acids are unlikely to be the primary astrocyte-secreted neurotoxic factors in ALS

Authors: N. RAMSAMOOJ[1], J. OFOSU NTIRI[2], M. BAPTISTE[2], H. WICHTERLE[3,4,2,5,6], S. E. PRZEDBORSKI[2,3,4,5,6], ***A. E. RUMORA**[2], E. R. LOWRY[3,7];
[1]Project ALS Therapeut. Core, [2]Dept. of Neurol., [3]Dept. of Pathology and Cell Biol., [4]Dept. of Neurosci., [5]Ctr. for Motor Neuron Biol. and Dis., [6]Columbia Stem Cell Initiative, [7]The Project ALS Therapeut. Core, Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is characterized by extensive, but selective, degeneration of motor neurons, leading to a loss of neuromuscular control that progresses rapidly and results in death within 2-5 years of diagnosis. Though motor neurons appear to be intrinsically susceptible to intracellular stresses that arise over the course of the disease, such as the accumulation of mutant, misfolded proteins and the subsequent activation of the unfolded protein response (UPR; Kiskinis et al., 2014; Thams et al., 2018), there is increasing evidence for a vital contribution of astrocytes to motor neuron degeneration. Because treatment with conditioned media from astrocytes carrying ALS-causing mutations is sufficient to trigger apoptotic motor neuron death in culture (Nagai et al., 2007; Giorgio et al., 2007), it has been long been suspected that astrocytes secrete a neurotoxic factor that exacerbates cell-autonomous motor neuron degeneration in ALS. Recent work in primary murine oligodendrocytes treated with conditioned media from wild-type, cytokine-stimulated astrocytes suggested that the identity of the astrocyte-secreted neurotoxic factor may be saturated free fatty acids (SFFAs; Guttenplan et al., 2021), triggering oligodendrocyte degeneration through a lipoapoptotic pathway that shares many components with the UPR such as PERK-mediated EIF2A phosphorylation. We sought to extend these findings and further probe their relevance to ALS by testing the toxicity of SFFAs in mouse and human stem-cell-derived motor neurons. While we were able to demonstrate in mixed cultures that the SFFA palmitate (16:0) was ~2-fold more toxic to motor neurons than other neuronal sub-types, the concentrations at which these effects were achieved (>125 µM) exceeded those in SFFA-treated oligodendrocytes (<5 µM; Guttenplan et al., 2021) and also greatly surpassed the physiological levels that are observed in the serum of ALS patients (<1 µM; Area-Gomez et al., 2021). Furthermore, we found that ISRIB, a small molecule that blocks the downstream effectors of phosphorylated EIF2A, protected motor neurons from a chemical trigger of the UPR that activates PERK, but did not rescue the toxic effects of SFFAs, suggesting that SFFAs in this context do not activate lipoapoptotic pathways. Taken together, these findings call into question the idea that SFFAs are the functional astrocyte-secreted factors that regulate motor neuron degeneration in ALS.

Disclosures: **N. Ramsamooj:** None. **J. Ofosu Ntiri:** None. **M. Baptiste:** None. **H. Wichterle:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ProJenX. F. Consulting Fees (e.g., advisory boards); ProJenX. **S.E. Przedborski:** F. Consulting Fees (e.g., advisory boards); Luciole Pharmaceuticals, Inc.. Other; Receiving Editor for eLife. **A.E. Rumora:** None. **E.R. Lowry:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ProJenX. F. Consulting Fees (e.g., advisory boards); ProJenX.

Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY029360
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Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: Identifying mediators of Retinal Ganglion Cell survival and axon regeneration using scRNA-seq

Authors: *N. M. TRAN[1], A. JACOBI[2], W. YAN[3], I. BENHAR[4], F. TIAN[5], R. SCHAFFER[3], Z. HE[6], J. R. SANES[3];
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Abstract: Purpose: Neurons in the central nervous system have a limited ability to survive and regenerate their axons following injury. While >70 interventions have been identified that alter the regenerative capacity of retinal ganglion cell (RGC) axons, none achieve recovery and typically only a small subset of RGCs respond. We are investigating the molecular pathways associated with RGC populations that have differential responses to regenerative treatments: 1) continued degeneration, 2) survival but not regeneration, 3) and survival plus regeneration. Our goal is to determine the programs regulating each of these processes in order to identify new ways to promote neuroprotection and stimulate axon regeneration. **Methods**: To dissect the molecular pathways associated with differential RGC responses, we applied single-cell RNA-sequencing (scRNA-seq) in two different ways following optic nerve crush (ONC) with or without regenerative interventions. Axon regeneration was stimulated using three established interventions: Pten conditional deletion from RGCs, Pcko + Cntf overexpression, and Pten, Socs3 double conditional deletion from RGCs + Cntf overexpression. First, we applied droplet-based scRNA-seq to profile >130,000 RGCs across these conditions at 0-21 days post-ONC. Second, we specifically profiled regenerative vs. non-regenerative RGCs by retrogradely labelling regenerating RGCs. **Results**: Key results from scRNA-seq expression screens included the following. First, all interventions preserved the expression of cell type markers and suppressed injury-related changes following ONC; much of the effects were observed with *Pten* deletion alone. Second, each intervention resulted in neuroprotection and axon regeneration with distinct type-specificity. Third, we identified multiple population-specific transcriptional modules and gene regulatory networks correlating with degeneration, survival, and axon regeneration. We overexpressed three genes (Crh, Gal, and Wt1) associated with the regenerative program and all promoted neuroprotection and axon regeneration. **Conclusions**: Our results identify gene expression programs correlating with distinct responses by RGC populations to regenerative interventions revealing three novel mediators of axon regeneration. These studies describe core molecular programs associated with regeneration, which could have broad translational implications for the treatment of acute nerve injuries. These studies are detailed in a recent pre-print publication (Jacobi et al BioRxiv doi: https://doi.org/10.1101/2022.01.19.476970).

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Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.02

Topic: B.10. Demyelinating Disorders

Support: European Union's Horizon 2020 Research and Innovation Programme ENDpoiNTs project Grant Agreement number: 825759

Title: Accumulation of endocrine disruptors into the myelin sheath alters remyelination

Authors: ***B. ZALC**[1], P. JUBIN[1], L. BRUTILLE[1], E.-M. MARTIN[1], M.-S. AIGROT[1], C. LUBETZKI[2,3], J. B. FINI[3], B. DEMEINEX[3], S. REMAUD[3];
[1]Sorbonne Université; Inserm, CNRS, ICM, Paris Cedex 13, France; [2]Sorbonne Université; Inserm, CNRS, ICM, AP-HP, Paris Cedex 13, France; [3]CNRS UMR 7221, Sorbonne University, Muséum Natl. d'Histoire Naturelle,, F-75005 Paris France, France

Abstract: Over the past 30 years an unexplained increased incidence in multiple sclerosis (MS) is observed in developed countries, suspected to be exacerbated by environmental factors. Concomitantly over 300 new chemicals have been synthesized and released in the environment. We questioned whether exposure to endocrine disrupting chemicals (EDC), notably flame retardant such as TBBPA or fluorosurfactant (PFAS) such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), could interfere with the process of myelin formation and remyelination. The **overarching aim** of this project was to explore the role of TBBPA and PFAS on oligodendrogenesis, myelination and remyelination. Specific aims were to determine **cellular and behavioural effects of TBBPA and PFAS.** Using *ex vivo* and *in vivo* approaches, we accumulated a set of data showing that TBBPA and among PFAS tested, PFOS, but not PFOA, affects remyelination. To reach these aims, we have combined both ***in vivo*** and ***ex vivo*** **approaches under physiological and pathological conditions.** In rodent demyelination was induced *ex vivo* by lysophosphatidyl choline exposure of explants of cerebellum of Tg(*plp:gfp*) mouse and the effect of EDC was evaluated during the remyelination period. For *in vivo* studies we used the transgenic Xenopus leavis line Tg(*mbp:gfp-NTR*) and shown that at the end of conditional demyelination introduction of either TBBPA or PFOS into the swimming water altered remyelination, measured by counting the number of myelin forming GFP+ oligodendrocytes and the functional consequences evaluated by the distance travelled, the speed of swimming and the visual avoidance of a virtual collision. By investigating the potential incorporation of EDCs into the myelin leaflets our study brings in-depth knowledge on the links between EDCs environmental exposure, the generation of fully remyelinating oligodendrocytes and functional repair of brain lesions, responses that are deficient in Multiple Sclerosis.

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Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Assistant Secretary of Defense for Health Affairs through the Congressionally Directed Gulf War Illness Research Program W81XWH-16-1-0626)
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I01BX005015))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development (I01RX001520))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development (IK2RX003253))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I01BX004561))
The Veterans Bio-Medical Research Institute

Title: Gulf War toxicant exposure dysregulates Arc and dendritic arborization in the mouse hippocampus

Authors: *K. E. MURRAY[1,3], W. A. RATLIFF[4], V. DELIC[1,3,5], K. D. BECK[2,3,5], B. A. CITRON[1,3,5];
[1]Lab. of Mol. Biology, Res. & Develop., [2]Neurobehavioral Res. Laboratory, Res. & Develop., VA New Jersey Hlth. Care Syst., East Orange, NJ; [3]Sch. of Grad. Studies, Rutgers Univ., Newark, NJ; [4]Res. & Develop., Dept. of Veterans Affairs, Bay Pines VA Healthcare Syst., Bay Pines, FL; [5]Pharmacology, Physiol. & Neurosci., Rutgers-New Jersey Med. Sch., Newark, NJ

Abstract: Gulf War Illness (GWI) is a chronic multi-symptom syndrome which affects approximately 30% of the nearly 700,000 Veterans who were deployed to the Persian Gulf from August 1990 to February 1991 during Operations Desert Storm and Desert Shield. These Veterans have reported experiencing a variety of symptoms including learning and memory impairments, depression and anxiety, sleep disturbances, chronic fatigue, and increased incidence of neurodegenerative diseases. Evidence suggests that combined exposure to both reversible and irreversible acetylcholinesterase (AChE) inhibitors is a likely risk factor. We

modeled Gulf War exposure in male C57Bl/6J mice with three AChE inhibitors that have been implicated as causative agents for GWI: pyridostigmine bromide (PB), the anti-sarin prophylactic; chlorpyrifos (CPF), an organophosphate insecticide; and *N*,*N*-diethyl-*m*-toluamide (DEET), a common insect repellent. We are measuring outcomes with behavioral tests including evaluation of activity and resting periods with a PhenoMaster home cage monitoring system. Previously, we reported acute hippocampal gene expression changes following 10 days of toxicant exposure, including significant downregulation of several neuronal immediate early genes (IEGs) such as *Arc*, *Egr1*, and *Nr4a1*, as well as hippocampal-dependent memory impairment in a Y-maze task. Here, we quantified protein expression of Arc, a synaptic plasticity factor, in granule cells of the dentate gyrus at 4 hours post-exposure and evaluated the effects of toxicant exposure on Arc expression in response to a novel enriched environment. We also assessed dendritic arborization in granule cells with Golgi staining and examined the effects of treatment with a neuroprotective Nrf2 activator, tert-butylhydroquinone (tBHQ), at 14 weeks post-exposure. GWI mice displayed fewer Arc^+ granule cells in the dentate gyrus and reductions in both basal and induced Arc expression at 4 hours post-exposure. GWI mice also presented chronic dendritic arbor reduction loss of spines in granule cells at 14 weeks post-exposure which was reversed by the neuroprotective treatment. Our results suggest that IEGs may only be dysregulated immediately following exposure but could contribute to long-term detrimental effects on hippocampal neuroplasticity, which may be improved with induction of neuroprotective factors.

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Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 EY026576
R01 EY029796

Title: Photopic visual function is preserved in the face of massive retinal remodeling: temporal sequence of pathophysiology in a mouse model of retinitis pigmentosa

Authors: *C. ELEFTHERIOU[1], N. M. ALAM[1], C. CORONA[1], E. IVANOVA[2], B. T. SAGDULLAEV[2], G. T. PRUSKY[1];
[1]Burke Neurolog. Inst., White Plains, NY; [2]Regeneron Pharmaceuticals, Tarrytown, NY

Abstract: Purpose: Retinitis Pigmentosa (RP) originates with a mutation in the rhodopsin gene, leading to photoreceptor cell death, retinal remodeling and a complete loss of visual function. Rhodopsin is expressed in rod photoreceptors, the first cells to succumb, followed by cones via a

"bystander-effect" derived from the strong structural and functional processes linking these two populations. Remodeling is characterized by the emergence of anomalies such as reactive Müller glia, vascular network degeneration, synaptic rewiring, and aberrant spontaneous firing in retinal ganglion cells (RGCs). How these processes are related, contribute to disease progression, and can be manipulated for therapeutic benefit, is not known. **Methods:** We investigated the behavioral, anatomical and physiological progression of pathophysiology in mice with the P23H mutation on the rhodopsin gene, the most common form of autosomal dominant RP in humans. We studied photopic and scotopic visual acuity tracking with unprecedented temporal granularity, establishing trajectories for vision loss in this progressive degeneration model. Using immunohistochemical staining, we mapped photoreceptor array loss and structural remodeling onto those functions. In parallel, we evaluated spontaneous and light-driven intracellular calcium dynamics in the neuronal, glial, and vascular populations of explanted retinas using multiphoton microscopy. **Results:** In the face of dramatic and progressive photoreceptor loss with age, photopic measures of visual acuity and contrast sensitivity remained largely intact up to post-natal day (PND) 300, but then declined fast until complete loss of function by PND 360. Conversely, scotopic measures of vision remained intact until PND 200 before complete loss of function by PND 250. These were preceded by an age-dependent increase in the hyperactive bursting of retinal ganglion cells paralleled by an age-dependent decrease in the synchronicity of glio-vascular signaling. These culminated in a near-complete loss of function after PND 400, with retinas displaying no dynamic calcium activity except for a small population of melanopsin-expressing retinal ganglion cells. **Conclusions:** We generated a multi-dimensional map of pathophysiology for the P23H mouse model, identifying novel stages of dysfunction and physiological biomarkers. Among those, we found that hyperactive neuronal activity emerges before aberrant glial and vascular changes, and before the dramatic decline of photopic spatial visual function. Future experiments may find which circuits are compromised early by aberrant noise, and if blocking it may delay onset of loss of function.

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Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: AG061251

Title: Cofactor heparan sulfate modulates the scaffolding, spread, and clearance of misfolded prion aggregates in the brain

Authors: *P. AGUILAR CALVO[1], A. MALIK[1], D. SANDOVAL[4], C. BARBACK[4], C. ORRU[5], A. L. KRAUS[6], D. PIZZO[1], P. NILSSON[7], B. CAUGHEY[8], D. VERA[2], J. ESKO[3], C.

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[1]Pathology and Med., [2]Radiology, [3]Univ. of California San Diego, La Jolla, CA; [4]UCSD, La Jolla, CA; [5]Natl. Inst. of Allergy and Infectious Dis., Rocky Mountains, CO; [6]Case Western Reserve Univ., Cleveland, OH; [7]KTH - Royal Inst. of Technol., Stockholm, Sweden; [8]NIAID/NIH Rocky Mountain Labs, Hamilton, MT; [9]Pathology and Med., UC San Diego, La Jolla, CA

Abstract: The extracellular molecules (cofactors) that modulate the spread, scaffolding and clearance of protein aggregates in neurodegenerative disease are poorly understood. Heparan sulfate proteoglycans (HSPGs) are major components of the cellular glycocalyx, vascular basement membrane and brain extracellular matrix, where they bind tau, amyloid-β, prion protein (PrP), and α-synuclein aggregates, promoting their cellular uptake and/or fibril formation. Here we used *Ndst1$^{f/f}$SynCre^{+}* mice, which produce poorly sulfated *neuronal* HS due to the deletion of the *N-deacetylase, N-sulfotransferase1* (*Ndst1)* gene, to address how manipulating HS sulfation levels impacts prion deposition and clearance in the brain. Prion-infected *Ndst1$^{f/f}$SynCre^{+}* mice displayed a 40% longer survival and showed a 60% reduction in prion levels in brain compared to littermate controls. Prion plaques were significantly smaller in *Ndst1$^{f/f}$SynCre^{+}* brains and were present at sites of cerebrospinal fluid (CSF) - interstitial fluid exchange, including meningeal vasculature and perivascular areas. Prion aggregates (PrP^{Sc}) in *Ndst1$^{f/f}$SynCre^{+}* brains were more soluble, but their conformation was not altered as the electrophoretic mobility, the glycoprofile, the stability in chaotropes, and the fluorescent life-time decay (FLIM) of prion-bound fluorescent probes were similar for prions in *Ndst1$^{f/f}$SynCre^{+}* and *Ndst1$^{f/f}$SynCre^{-}* brains. Using radiolabeled PrP^{C} and positron emission tomography (PET) scan, we detected higher levels of radioactivity in the *Ndst1$^{f/f}$SynCre+* spinal cords 24 hours after the stereotaxic injection in brain parenchyma (caudate-putamen), suggesting that reduced HS sulfation may accelerate the clearance of PrP^{Sc} into the CSF. Importantly, the prion-infected *Ndst1$^{f/f}$SynCre^{+}* mice did not show higher levels of prion accumulation in the spinal cord as compared to *Ndst1$^{f/f}$SynCre^{-}* mice, which suggests that increasing PrP clearance into the CSF would not lead to higher PrP^{Sc} deposition in spinal cord. Our results provide evidence for the first host cofactor that modulates the scaffolding and clearance of PrP^{Sc} aggregates through the central nervous system, and offer a new target for the rational design of protective therapies based on manipulating HS sulfation to disrupt the HS interaction with misfolded proteins.

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Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

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Program #/Poster #: 282.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AG18031
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The Brown Foundation, Joe and Dorothy Dorsett Innovation in Science Healthy Aging Award
Translational Research Initiative Grants from LSUHSC

Title: Down-regulation of NF-L expression in human neuronal-glial (HNG) primary cell cultures by a microbiome-derived LPS-induced miRNA-30b-5p

Authors: Y. ZHAO[1], V. R. JABER[2], A. I. POGUE[3], N. M. SHARFMAN[4], ***W. J. LUKIW**[5]; [1]Cell Biol. and Anat., LSUHSC-NO Neurosci., New Orleans, LA; [2]LSU Neurosci. Ctr., LSU Hlth. Sci. Ctr., New Orleans, LA; [3]Alchem Biotek Res., Toronto, ON, Canada; [4]LSU Neurosci. Ctr., [5]LSU Neurosci. Center, Departments of Neurol. and Ophthalmology, Louisiana State Univ. Hlth. Sci. Ctr., New Orleans, LA

Abstract: Microbiome-derived Gram-negative bacterial lipopolysaccharide (LPS) has been shown by multiple laboratories to reside within AD-affected brain neocortical and hippocampal neurons. LPS and other pro-inflammatory stressors strongly induce a defined set of NF-kB (p50/p65)-sensitive human microRNAs including a brain-enriched *Homo sapien* microRNA-30b-5p (hsa-miRNA-30b-5p; miRNA-30b). Here we provide evidence that this neuropathology-associated miRNA, known to be up-regulated in Alzheimer's disease (AD) brain and LPS-stressed human neuronal-glial (HNG) cells in primary co-culture targets the neurofilament light (NF-L) chain mRNA 3'-untranslated region (3'-UTR) conducive to the post-transcriptional down-regulation of NF-L expression observed within LPS-stressed HNG cells. A deficiency of NF-L is associated with a consequent atrophy of the neuronal cytoskeleton and disruption of synaptic architecture. Interestingly miRNA-30b has previously been shown to be highly expressed in amyloid-beta (Aβ) peptide-treated animal and cell models and Aβ peptides promote LPS entry into neurons. Increased miRNA-30b expression induces neuronal injury, neuron loss, neuronal inflammation, an impairment of synaptic transmission and synaptic failure in neurodegenerative disease and in transgenic murine models of AD. This gut microbiota-derived LPS-NF-kB-miRNA-30b-NF-L pathological signaling network underscores a positive pathological link between the LPS of gastrointestinal (GI)-tract resident microbes and the inflammatory neuropathology, disordered cytoskeleton and disrupted synaptic signaling of the AD brain and stressed brain cells in culture. The experimental findings in this paper also represent the first example of a microbiome-derived neurotoxic glycolipid having significant detrimental miRNA-30b-mediated actions on the expression of NF-L, an abundant neuron-specific filament protein known to be important in the maintenance of neuronal cytoarchitecture, axonal caliber and synaptic organization.

Disclosures: Y. Zhao: None. **V.R. Jaber:** None. **A.I. Pogue:** None. **N.M. Sharfman:** None. **W.J. Lukiw:** None.

Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Natural Sciences and Engineering Research Council of Canada, Grant 121795.

Title: Comprehensive comparison of various neurotoxins' impact on dopaminergic health and mitochondria using a zebrafish model

Authors: *M. EKKER[1], **M. KALYN**[2], K. HUA[2], C. DAVID WONG[3];
[1]Univ. of Ottawa, Ottawa, ON, Canada; [2]Univ. of Ottawa, OTTAWA, ON, Canada; [3]Univ. of Malaya, Kuala Lumpur, Malaysia

Abstract: Mitochondrial dysfunction is a prominent feature within Parkinson's disease (PD) where chemo-ablative models commonly adopt neurotoxins that specifically target the mitochondria of dopaminergic neurons to mimic a disease state. The zebrafish has recently emerged as a model to recapitulate Parkinsonian symptoms due to the possession of a functional analogue to the human nigrostriatal pathway affected in PD. To date, there have been conflicting studies that show variability in zebrafish vulnerability to common toxins and pesticides that have shown to impact dopaminergic health. Here, have exposed Tg(*dat:eGFP*) larval zebrafish and found, through a direct comparison of the LC50 dose for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA),1-methyl-4-phenylpyridinium (MPP+), paraquat and rotenone, that MPTP elicits that largest degenerative effect on ventral diencephalic dopaminergic neurons (n=9-15) and on locomotion (n=20-25). Progressing into an adult model, cerebroventricular microinjections of MPTP were performed on Tg(*dat:eGFP*) and Tg(*dat:tom20 MLS:mCherry*) zebrafish to investigate the mitochondrial consequences *in vivo*. MPTP injections resulted in 40-55% reductions in dopaminergic neurons residing in the olfactory bulbs and telencephalon of the forebrain (n=6) which translated into transient sensorimotor phenotypes through impaired olfaction and motor performance (n=10). Within the olfactory bulbs of the Tg(*dat:tom20 MLS:mCherry*) zebrafish, MPTP injections resulted in a drastic increase in fragmented mitochondria relative to the controls. To delineate between apoptosis or mitophagy driving this effect, whole brain gene expression analyses of MPTP-injected zebrafish showed increases in *pink1* and *parkin* transcript levels that strongly suggest mitophagy activation (n=3). No apparent sex differences were observed in any part. Taken together, these data can contribute to optimizing chemo-ablative zebrafish models to maximize dopaminergic cell death for regeneration studies and to a better understanding of the mitochondrial impact on dopaminergic survivability. This work was funded and supported by the Natural Science and Engineering Research Council of Canada.

Disclosures: **M. Ekker:** None. **M. Kalyn:** None. **K. Hua:** None. **C. David Wong:** None.

Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.08

Topic: C.01. Brain Wellness and Aging

Title: Epitranscriptomic Regulation in the hippocampus of Normal Ageing

Authors: *H. HUANG[1], R. SONG[2], J.-L. WONG[2], V. ANGGONO[1], J. WIDAGDO[1];
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Abstract: Brain aging is associated with a general decline in cognitive function and an increase in vulnerability to neurodegenerative disorders. Gene expression changes underlie several molecular and cellular hallmarks associated with age-related processes in the brain affecting neurons and non-neuronal cells. *N*6-methyladenosine (m6A), the most abundant internal modification in eukaryotic mRNAs, plays important roles in regulating RNA metabolisms and thereby, the cellular gene expression profiles. Importantly, m6A signaling plays critical roles in regulating many brain functions, including learning and memory. To gain an understanding of the m6A-RNA landscape during normal aging, we used C57Bl/6 mice (n=3) to assess the m6A-changes in the young (3-month-old) versus aged (20-month-old) hippocampal RNA. Total RNA sequencing revealed differential regulation of genes from several biological pathways known to be modulated during brain aging, namely, those in the immune response pathways and the extracellular matrix organization. Using methylated RNA immunoprecipitation-sequencing (MeRIP-Seq), we identified a total of 8,084 and 7,527 putative m6A sites in the young and aged hippocampus, which correspond to 4,834 and 4,585 genes, respectively. Using fold-change as a measurement, 426 genes were significantly hypermethylated, and 102 genes were significantly hypomethylated by at least 1.5-fold in aged compared to the young hippocampus. Importantly, we uncovered a positive correlation in the changes of m6A modification and the total RNA expression during aging. These parallel changes of total transcript levels and their m6A-modification could be due to the co-transcriptional regulation of m6A and/or the stabilising effect of m6A on the modified transcripts. This study reveals the m6A dynamics during normal brain aging and warrants further investigation in determining the causal role(s) of m6A in the age-dependent transcriptomic changes.

Disclosures: H. Huang: None. **R. Song:** None. **J. Wong:** None. **V. Anggono:** None. **J. Widagdo:** None.

Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Interrelated environmental, poverty and food insecurity factors in COVID-19 and neurodegeneration: Elucidating mechanisms and forewarning

Authors: *E. M. EDROSA[1], E. L. OHAYON[1,2];
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Abstract: The COVID-19 pandemic has brought to the forefront multiple disparity-related risk factors including poverty and food insecurity. Similarly, environmental factors have been implicated in the initiation, outcome, and effects of the pandemic. In this study, we specifically examine the interrelation between environmental, poverty-related and food insecurity risk factors as they relate to neural health and neurodegeneration in the context of the pandemic. We began by performing a review and meta-analysis of literature findings relating to the poverty and environmental factors as seen in both pre-pandemic and COVID-impacted communities. Stress and nutritional deficiencies were two of several interacting factors that increase the risk of various forms of neurodegeneration. Conversely, at the environmental level, Normalized Difference Vegetation Index (NDVI) and decreased exposure to neighborhood greenness similarly show a correlation with conditions that increase the risk for neuroinflammation (e.g., heart disease, high blood pressure, obesity, and diabetes). To make matters worse, the same disadvantaged communities that already bore an alarmingly-unequal burden of neurodegenerative disease have been the most impacted by the COVID-19 pandemic. As a way of further elucidating these relationships, we applied a multi-scale framework to examine and model how factors ranging from the molecular to environmental interact to initiate, cause, and influence events at the brain, individual, and population levels. By applying a multi-scale approach, we illustrate how these factors might be simultaneously increasing vulnerability to neurodegenerative disease and decreasing the ability to avoid, delay onset, and/or slow the progression of these conditions. The multi-scale perspective thus helps highlight how the conditions may be related as well as forewarns of a possible alarming rise in neurodegeneration cases as the long-term effects of the COVID-19 pandemic propagate. The resulting framework also suggests potential health initiatives by which we might address these factors at the individual and population-levels.

Disclosures: E.M. Edrosa: None. **E.L. Ohayon:** None.

Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant EY030747
NIH Grant EY031248
NIH Grant RR027093

Title: N-acylethanolamide metabolizing enzymes are upregulated in human neural progenitor derived neurons exposed to sub-lethal oxidative stress

Authors: *P. KOULEN, R. S. DUNCAN;
Univ. of Missouri, Kansas City, Kansas City, MO

Abstract: N-acyl amides (NAAs) are a class of lipids that consist of an acyl group N-linked to an amino acid, neurotransmitter, taurine or ethanolamide group and include some endocannabinoids. These lipids are synthesized in a wide variety of organisms and in multiple cell types, including neurons. N-acylethanolamides (NAEs) are involved in numerous cellular and physiological processes and their concentrations are elevated in response to ischemia and physical trauma to play a role in neuroprotection. Human neural progenitor cell-derived cells, such as ReN cells, have become more utilized for the study of human neuronal development and neurodegenerative diseases as they can be easily differentiated into neurons. In this study, we determined whether ReN cells, as an in vitro model system for studying development, differentiation and protection of neuronal cell, express proteins involved in canonical NAE signaling and whether oxidative stress can influence their expression. We determined that sub-lethal oxidative stress upregulates the expression of all NAE signaling proteins tested - cannabinoid receptor type 1 (CB1), cannabinoid receptor type 2 (CB2), fatty acid amidohydrolase (FAAH), NAE hydrolyzing acid amidase (NAAA) and N-acyl phosphatidylethanolamine specific PLD (NAPE-PLD). In addition, we determined that oxidative stress increases the nuclear localization of FAAH, and to a lesser extent, NAAA and NAPE-PLD. This study is a first step toward determining how oxidative stress affects the expression of these proteins and their potential roles in the neuronal defense against oxidative stress.

Disclosures: P. Koulen: None. **R.S. Duncan:** None.

Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS R01 NS086981
NINDS R21 NS121806
NICHD R21 HD090637
NINDS F31 NS105387
Conrad N. Hilton Foundation #20150232

Title: Worse neurodegeneration in male compared to female mice with experimental autoimmune encephalomyelitis

Authors: C. E. MEYER, A. W. SMITH, Q.-A. NGUYEN, N. ITOH, R. VOSKUHL, ***A. MACKENZIE-GRAHAM**;
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Abstract: Women with multiple sclerosis (MS) show increased susceptibility to disease and increased inflammatory activity, whereas men demonstrate more severe disability progression and more GM atrophy. To better understand the mechanisms underlying this sexual dimorphism in neurodegeneration, we investigated whether the most commonly used mouse model for multiple sclerosis, chronic experimental autoimmune encephalomyelitis (EAE), would also demonstrate sex differences in GM atrophy and neuropathology. We used voxel-based morphometry to identify localized GM atrophy by comparing males and females to their respective healthy controls. Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging-compatible Tissue-hYdrogel (CLARITY) and immunohistochemistry were used to identify sex differences in cerebral cortex and spinal cord pathology. We observed more extensive GM atrophy in EAE males compared to healthy males as compared to that in EAE females compared to healthy females. We further identified a sex-specific relationship between worse walking disability and worse GM atrophy in the sensorimotor cortex in males. Males with EAE showed significantly more neuronal loss in the cerebral cortex and greater axonal transection in the spinal cord than females with EAE. Together, these results demonstrate increased neurodegeneration in male mice with chronic EAE, thereby modeling worse neurodegeneration in males with MS.

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Poster

282. Mechanisms and Modulators of Neurodegeneration and Aging

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 282.12

Topic: C.01. Brain Wellness and Aging

Support: Good Samaritan 750360599
Oregon Partnership for Alzheimer's Research

Title: Long-term obesity produces sex-specific changes to hippocampal autophagy/lysosomal proteins and cognitive behavior

Authors: *D. M. OSBORNE, S. B. BAER, A. D. DORN;
R.S. Dow Neurobio., Legacy Res. Inst., Portland, OR

Abstract: Obesity negatively affects the brain, precipitating the development of neurodegenerative diseases, like Alzheimer’s Disease (AD). Brain regions like the hippocampus are particularly vulnerable to obesity-related insults and develop early pathological changes related to AD. Autophagic and lysosomal changes are among the earliest measurable changes that occur prior to the appearance of traditional AD pathological markers like amyloid-beta and neurofibrillary tangles. However, little is known about how obesity affects central autophagic/lysosomal function, nor have sex differences been examined. We examined how obesity changes key markers of autophagic/lysosomal function in the hippocampus, whether sex

differences exist in these markers, and whether they were amenable to treatment interventions known to affect autophagic/lysosomal activity. Female and male mice were placed on an obesogenic diet for 10 months, at which point a third were put on 40% caloric restriction (CR) and another third received CR+chloroquine (CQ), a known autophagy inhibitor. Females and males differed greatly in their behavioral and physiological responses to obesity and treatment interventions. Generally male cognitive behavior didn't change much due to diet, but obese males did benefit slightly from CR. Obese females however, performed worse than controls, and benefitted from CR, in stark contrast to control females who performed worse following CR; both behaviors were reversed by CQ. Cognitive behavior in females or males did not correlate to blood glucose regulation, which was drastically affected by treatments. Obesity changed hippocampal levels of Atg5, Atg12, LAMP1, folliculin, and Slc38a9 in males only. In females cathepsin B was decreased due to obesity. Obesity also prevented CR- or CR+CQ-induced changes in LAMP1, TFE3, and FNIP2 in females only. Many of these molecular changes mirror those observed in AD patients and mouse models. These results suggest that obesity may predispose the brain to neurodegeneration via severe perturbation in autophagic/lysosomal functioning, and that sex heavily influences how obesity affects cellular waste management in the hippocampus.

Disclosures: D.M. Osborne: None. **S.B. Baer:** None. **A.D. Dorn:** None.

Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effect of frequency-specific electromagnetic field on redox and its electrophysiological correlates

Authors: H. H. C. LEE[1], **S. REZNIK BALTER**[2], N. W. HODGSON[1], B. S. WEISINGER[2], E. SHOHAMI[3], *G. M. DONIGER[2], A. PARABUCKI[2], Y. SEGAL[2], T. K. HENSCH[4,1]; [1]Boston Children's Hosp., Boston, MA; [2]BrainQ Technologies Ltd., Jerusalem, Israel; [3]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [4]Harvard Univ., Cambridge, MA

Abstract: Cellular oxidative stress in the brain is a key factor underlying a variety of neurodegenerative conditions. Pulsed extremely low frequency and low intensity electromagnetic field (ELF-EMF) treatment has been shown to reduce oxidative stress in stroke patients and improve the rehabilitation process as measured by functional and clinical measures like the Fugl-Meyer Assessment (FMA) and modified Rankin Scale (mRS). Still, inadequate understanding of this effect may limit its application in clinical practice. Notably, it is unknown how stimulation parameters such as frequency and duration of exposure impact the effect, and whether it is global or limited to specific brain regions. Here, ELF-EMF was applied to adult male C57Bl/6J mice to investigate its effects on oxidative stress and neuronal oscillatory activity. Mice were exposed to

40 Hz ELF-EMF (3 hours daily) across several periods (acute: single exposure, subacute: 5 days, chronic: 4 weeks). Regional brain content of glutathione in its reduced (GSH) or oxidized form (GSSG) were measured by HPLC as a readout of cellular oxidative stress. Acute ELF-EMF treatment (3 hours) initially decreased the GSH/GSSG ratio compared to sham-exposed mice, while continued exposure (4 weeks) elevated it globally across multiple brain regions (prefrontal cortex, primary visual cortex, thalamus, hippocampus, cerebellum). The biphasic oxidative stress dynamics were found to be frequency-specific (strongest at 40 Hz, moderate for 7.6 Hz and absent at 60 Hz). The acute oxidative stress response and its recovery were replicated in an additional cohort using urinary 8-isoprostane (8-IsoP), a peripheral marker of oxidative stress. Acute 8-IsoP level predicted the magnitude of increase in relative beta and gamma EEG power four weeks after exposure began, suggesting that 8-IsoP is an early biomarker for EEG response to ELF-EMF. The trajectory of spectral changes in EEG following EMF exposure in mice is consistent with that observed in a human stroke recovery cohort, in which beta enhancement correlated with functional recovery as measured by FMA (to be explored further in future clinical trials). Our study identifies a dynamic redox regulation process underlying the efficacy of ELF-EMF as a global neuromodulatory intervention supporting a tailored EMF stimulation protocol in terms of duration and frequency.

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Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: U.S. Dept. of Veterans Affairs; # 1IO1 BX004884

Title: Dendrite degeneration is induced by the innate inflammatory response via cofilin/actin rod formation

Authors: *G. URUK[1,2], S. WON[1,2], E. MOCANU[1,2], R. A. SWANSON[1,2];
[1]Neurol., U.C.S.F., San Francisco, CA; [2]Neurol., S.F.V.A.M.C., San Francisco, CA

Abstract: The innate immune response has been well-established as contributing to neuronal loss in stroke and in other conditions. By contrast, little is known about the effect of inflammation on neurites (axons and dendrites) of neurons that survive the initial insult. To address this issue, we first employed a model of inflammation alone, without prior brain injury. The innate immune response was induced in cell culture by addition of S100B to the medium, and in vivo by intracortical injections of the alarmin S100B into mice, The cell cultures were comprised of primary mouse neurons plated onto mixed glia (astrocytes plus microglia). Neurons in cell cultures exposed to S100B exhibited a time- and dose-dependent loss of neurites that far exceeded loss of neuronal cell bodies. Importantly, neurite loss was preceeded by formation of formation of cofilin/actin rods. Both rod formation and neurite degeneration were prevented in cultures containing either cofilin-1 deficient neurons or microglia that were deficient in NADPH-oxidase deficient microglia (and thus unable to generate superoxide). Studies performed in vivo showed parallel results: S100B caused cofilin/actin rod formation followed by neurite degeneration that far exceeded neuronal death. Both rod formation and neurite degeneration were attenuated in mice deficient in NADPH oxidase or cofilin-1. These findings demonstrate that (1) inflammation can cause neurite degeneration far out of proportion to neuronal death, and (2) neurite degeneration is caused by cofilin/actin rod formation in response to microglial superoxide production. This study demonstrates a mechanism by which microglial activation is sufficient to cause the dendritic loss in the near absence of associated neuronal cell death. The results are relevant to post-stroke injury and other conditions in which the innate immune response is activated.

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Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

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Program #/Poster #: 283.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MH081917
AG056815

Title: A peptide blocking the ADORA1-neurabin interaction is anticonvulsant and inhibits epilepsy in an Alzheimer's model

Authors: S. SAGGU[1], Y. CHEN[2], L. CHEN[2], D. PIZZARO[2], S. PATI[2], L. L. MCMAHON[3], K. JIAO[1], *Q. WANG[4];
[1]MCG, Augusta, GA; [2]Dept. of Physiol. and Biophysics, Univ. of Alabama At Birmingham, Birmingham, AL; [3]Dept. of Neurosci., Med. Univ. of South Carolina, Charleston, SC; [4]Med. Col. of Georgia, Augusta, GA

Abstract: Epileptic seizures are common sequelae of stroke, acute brain injury and chronic neurodegenerative diseases, including Alzheimer's disease (AD), and cannot be effectively controlled in approximately 40% of patients, thereby necessitating the development of novel therapeutic agents. Activation of the A1 receptor (A1R) by endogenous adenosine is an intrinsic mechanism to self-terminate seizures and protect neurons from excitotoxicity. However, targeting A1R for neurological disorders has been hindered by side effects associated with its broad expression outside the nervous system. Here we aim to target the neural-specific A1R/neurabin/RGS4 complex that dictates A1R signaling strength and response outcome in the brain. We developed a peptide that blocks the A1R-neurabin interaction to enhance A1R activity. Intracerebroventricular or intranasal administration of this peptide shows marked protection against kainate-induced seizures and neuronal death. Furthermore, in an AD mouse model with spontaneous seizures, nasal delivery of this blocking peptide reduces epileptic spike frequency. Significantly, the anticonvulsant and neuroprotective effects of this peptide are achieved through enhanced A1R function in response to endogenous adenosine in the brain, thus avoiding side effects associated with A1R activation in peripheral tissues and organs. Our study informs new anti-seizure therapy applicable to epilepsy and other neurological illness with comorbid seizures.

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Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant NS096687
NIH Grant NS116028

Title: Creb mediated neuroprotection in nematode excitotoxicity emphasizes non-canonical signaling and reveals transcriptional regulation of inhibitory ion channel expression

Authors: Z. Z. MENDELOWITZ[1,2], *I. MANO[1,2];
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Abstract: Excitotoxic neurodegeneration is seen in acute conditions like brain ischemia and in a rage of progressive neurodegenerative diseases. While it is well known that excitotoxicity is

triggered by excessive accumulation of Glutamate and hyper-activation of Glutamate Receptors (GluRs), our understanding of the process downstream from GluR hyperactivation that leads to necrotic cell death remains unclear. The lack of consensus and the incomplete understanding of the events that follow the onset of excitotoxicity result in great unmet need for patients in the clinic. Notably, the degenerative mechanisms are also partially mitigated by neuroprotective mechanisms that are dependent on GluR-activated transcriptional programs. We use a *C. elegans* model to investigate excitotoxic necrosis and identify evolutionarily conserved transcriptional programs in excitoprotection. We find that non-canonical activation of CRH-1/CREB mediates neuroprotection from excitotoxicity. We provide, to our knowledge, the first transcriptome-wide data-set that includes both coding and non-coding transcripts from neurons at risk of and experiencing excitotoxicity-induced necrotic cell death. Analyzing transcriptome-wide changes in degenerating and at-risk neurons, we identify several groups of genes whose expression changes with excitotoxicity and CRH-1/CREB availability. We specifically find that evolutionarily conserved inhibitory ion channel expression is modulated by CREB-dependent neuroprotection from excitotoxic necrosis in nematodes. The role of the inhibitory ion channels in neuroprotection under excitotoxic conditions is further validated through both genetic and pharmacological manipulations. Our characterization of CREB-dependent neuroprotective transcriptional targets in at-risk neurons suggests novel and evolutionarily conserved avenues for neuroprotection from excitotoxicity.

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Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: SetPoint Medical

Title: Vagus nerve stimulation ameliorates clinical symptoms and pathology in a rat EAE model of multiple sclerosis

Authors: C. NATARAJAN[1], M. GUNASEKARAN[1], D. CHERNOFF[1], *Y. LEVINE[1,2,3]; [1]Setpoint Med. Corp., Valencia, CA; [2]Hofstra-Northwell Sch. of Med., Hempstead, NY; [3]Karolinska Institutet, Stockholm, Sweden

Abstract: Background: Despite multiple approved therapies available for treating multiple sclerosis (MS), there remains a need for new therapeutic options. Vagus nerve stimulation (VNS) can activate neuro-immune reflexes that attenuate inflammation, increase T_{reg} populations, and is neuroprotective in the central nervous system (Immunol Rev 2012; 248(1):188). We hypothesized that VNS would be ameliorative in an experimental autoimmune encephalomyelitis (EAE) model of MS. Aims: To investigate the effect of VNS on modulating the clinical

symptoms and pathology in a rodent model of MS. Methods: VNS devices (SetPoint Medical, CA) were fully implanted in 6-week-old female Lewis rats. Following recovery, EAE was induced with myelin basic protein (0.1 mg/rat) and complete Freund's adjuvant (Hooke Laboratories, CT). Conscious rats were treated with VNS or Sham VNS from day 7, the day prior to the typical onset of clinical symptoms, to day 21 post-induction. As a positive control, separate groups of rats were treated QD with teriflunomide (3 mg/kg or 1 mg/kg and vehicle) by oral gavage. EAE clinical scores (0-5 scale) were recorded once daily in a treatment-blinded manner and plotted against day post first observed symptom (DPS). H&E staining was performed in spinal cord (SC) sections to witness infiltrating immune cells. Results: VNS decreased disease manifestation and burden compared to the sham treatment. Total AUC of clinical score vs. DPS was significantly reduced (30 %, $p < 0.01$). H&E staining of VNS SC demonstrated reduction in the infiltration of inflammatory cells during the onset, peak and remitting stages of EAE. In addition, there was no statistical difference in clinical burden between the VNS group and the high dose teriflunomide group, nor between the sham VNS group and the vehicle group. Conclusions: These data demonstrate that daily VNS significantly ameliorated the disease burden and number of symptomatic days in rat EAE, similar in degree to high dose teriflunomide. Further, reduced infiltration of immune cells into the SC reveals the neuroprotective effects of VNS, and the underlying mechanisms of these neuroprotective effects in glial cells and immunocytes are being investigated.

Disclosures: C. Natarajan: A. Employment/Salary (full or part-time):; SetPoint Medical Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SetPoint Medical Corporation. **M. Gunasekaran:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); gunamanoj@gmail.com. **D. Chernoff:** A. Employment/Salary (full or part-time):; SetPoint Medical Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SetPoint Medical Corporation. **Y. Levine:** A. Employment/Salary (full or part-time):; SetPoint Medical Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SetPoint Medical Corporation.

Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01CA227064-04

Title: HDAC6 inhibition reverses chemobrain by altering glutamate signaling to restore neuronal function

Authors: *V. K. MARTINEZ[1,2], C. CRONKITE[2], A. K. SINGH[1], D. ZHANG[1], M. ZAMORANO[1], J. DUMAN[2], D. GROSSHANS[1], A. KAVELAARS[1], C. HEIJNEN[1], A. J. SHEPHERD[1];
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Abstract: There are no FDA approved treatments for chemobrain (chemotherapy-induced cognitive impairment), one of the most debilitating sequalae of chemotherapy. Our findings suggest that HDAC6 inhibitor ACY-1083 may alter glutamate signaling to restore cognitive function in aged female mice treated with chemotherapy doxorubicin, a step towards an effective therapeutic for chemobrain. Synaptic glutamate was increased in the hippocampus of doxorubicin-treated mice. Doxorubicin increases TNFα levels, a cause of glutamate toxicity. HDAC6 inhibition blocked glutamate signaling in an acute stress mouse model and Parkinson's disease models. *We hypothesized that ACY-1083 blocks glutamate signaling in the hippocampus of aged female mice treated with doxorubicin.* To test this hypothesis, we used the puzzle box test (PBT) and novel object placement recognition test (NOPRT) to assess cognitive function; immunofluorescent staining of postsynaptic density 95 (PSD95) to asses synaptic integrity; in vitro multielectrode array (MEA) to assess neuronal function; and SEP-tagged receptors to assess changes in receptor surface expression levels. All studies were blinded, include control groups saline/vehicle and saline/ACY-1083, and replicated unless stated as preliminary. Because (1) cancer is an age-related disease; (2) breast cancer is the most common cancer worldwide; and (3) doxorubicin is the most commonly used chemotherapy for breast cancer, we conducted this study in aged female mice and mature primary rat hippocampal neurons treated with doxorubicin and ACY-1083. Analysis was conducted in Prism as Two-way and One-way ANOVA with Tukey's posthoc correction. We confirmed that ACY-1083 reverses doxorubicin-induced chemobrain in aged female mice with a restoration in time to solve the PBT and discrimination index in the NOPRT. Closely linked synaptic integrity in the CA1 region of the same mice was also preliminarily restored. After doxorubicin treatment, mean firing rate, number of network bursts, and network burst frequency were restored with ACY-1083 in vitro, with altered responses to GluR1 agonist and antagonist. Furthermore, doxorubicin-induced increases in GluR1 SEP-tagged fluorescent surface expression levels were restored with ACY-1083. Together, these data suggest that ACY-1083 influences glutamate signaling as a contributing mechanism to restore cognitive function in chemobrain. Understanding the effect of HDAC6 inhibition on glutamate signaling in the context of chemobrain is not only novel, but it will provide insight for diseases and injuries with a common pathway in glutamate toxicity.

Disclosures: V.K. Martinez: None. **C. Cronkite:** None. **A.K. Singh:** None. **D. Zhang:** None. **M. Zamorano:** None. **J. Duman:** None. **D. Grosshans:** None. **A. Kavelaars:** None. **C. Heijnen:** None. **A.J. Shepherd:** None.

Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Giving Back Fund
Stand By Eli Foundation

Title: Identification of Mechanisms and Potential Gene Therapy Treatment for IRF2BPL-related Neurological Diseases

Authors: *S. SINHA RAY[1], F. ROUSSEL[2], C. N. DENNYS[4], S. B. LIKHITE[5], J. CAPORALE[3], A. SIERRA DELGADO[3], M. BAIRD[2], N. WEIN[2], K. MEYER[2];
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Abstract: NEDAMSS (Neurodevelopmental Disorder with Regression, Abnormal Movements, Loss of Speech, and Seizures) is a recently discovered neurological disorder caused by heterozygous truncations in the intron-less transcriptional regulator, *IRF2BPL*. The gene is ubiquitously expressed, however the function of the protein is currently unknown. Recent case reports indicate that nonsense variants in the *IRF2BPL* gene lead to severe neurodevelopmental regression. To study mechanisms involved in the newly described NEDAMSS disease, we developed the first *in vitro* model for the disorder by directly reprogramming patient skin fibroblasts to astrocytes and neurons. We found that full-length wild-type IRF2BPL is primarily localized to the nucleus, while truncated patient variants sequester the wild-type protein to the cytoplasm and cause aggregation. Additionally, patient astrocytes failed to support neuronal survival in co-culture assays and exhibited aberrant mitochondria and respiratory dysfunction. Moreover, overexpressing patient related truncated IRF2BPL *in vivo* in wildtype mice, caused severe motor defect in cage hanging tests. To ameliorate the disease phenotype, we established AAV gene therapy vector candidates to modulate IRF2BPL protein expression. Importantly, treatment of patient astrocytes with the AAV classical gene replacement vector significantly rescued neuronal viability in coculture assays and improved mitochondrial function. Our findings provide insights into mechanisms involved in NEDAMSS and reveal a potentially promising treatment for this severe disorder.

Disclosures: S. Sinha Ray: None. **F. Roussel:** None. **C.N. Dennys:** None. **S.B. Likhite:** None. **J. Caporale:** None. **A. Sierra Delgado:** None. **M. Baird:** None. **N. Wein:** 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 Therpeutics, Audentes/Astellas. **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 Therpeutics.

Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Jax Scholar 2019

Title: Cerebral organoids reveal genetic differences associated with resilience in an in vitro stroke model

Authors: *D. CORTES, K. CHARLAND, M. PERA;
The Jackson Lab., Bar Harbor, ME

Abstract: Stroke is one of the leading causes of death. Although stroke is a multifactorial disease, the genetic differences between patients contribute significantly to variation in risk, survival, and recovery. Progress in establishing the genetic underpinnings of stroke has been slow, in part because human genetic studies require large patient cohorts and sampling brain tissue for mechanistic studies is not feasible. Furthermore, the genetic and mechanistic differences that distinguish between susceptibility (death or severe sequelae) and resilience (recovery) in stroke patients are not well understood and this limits our ability to identify targets that can be translated into new therapeutics. Thus, there is a critical need for an efficient approach to elucidate the genetic mechanisms that drive resilience to stroke so that novel therapeutic targets can be identified and exploited. Here, we use a stem cell (SC)-based approach to rapidly analyze phenotypical differences among genetically diverse populations. Previously we developed a protocol to differentiate neurons and cerebral organoids from genetically diverse mouse and human SCs, making readouts between mouse and human more comparable. For this study, we developed an *in vitro* stroke-like paradigm which we used to test for phenotypical differences among genetically diverse neurons using SCs from the founders of the collaborative cross mice, and human SCs from the iPSCORE collection. After measuring oxidative stress, DNA fragmentation, LDH release, neuronal survival, mitochondrial, electrophysiological, and caspase activity; we found a varied response to the insult associated with the genetic diversity. We conducted RNA-seq analysis and found that Mgarp and Ptn, genes important for cell survival and mitochondrial activity were differentially expressed between the most susceptible and resilient strains. Transcriptional regulatory network analysis revealed that differences in resilience are linked to *Vdr*, *Tfap2c*, and *Irf7* transcriptional factors (TFs). To validate the effect of these TFs using our *in vitro* stroke model, we incubated human cerebral organoids with commercially available molecules that activate these TFs. We found that a combination of these molecules can increase the survival of neurons after the insult. After generating CRISPRa human SCs to activate the endogenous TFs, we were able to corroborate the pro-survival effects of these TFs in cerebral organoids. The present model demonstrates how complex genetic factors modify differential responses to stroke. The transcriptional adaptation associated with a favorable response to stroke can lead to much-needed new therapeutics.

Disclosures: D. Cortes: None. **K. Charland:** None. **M. Pera:** None.

Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS R01NS100007
Dana-Farber Cancer Institute microgrant #120205

Title: Beta-2 adrenergic receptor (ADRB2) signaling as a modulator for ER-mitochondria contacts

Authors: *Y. LIM[1,2], I.-T. CHO[1,3], H. G. RENNKE[1], G. CHO[1,2];
[1]Brigham and Women's Hospital/Harvard Med. Sch., Boston, MA; [2]Cedars-Sinai Med. Ctr., Los Angeles, CA; [3]Bristol Myers Squibb, Redwood City, CA

Abstract: Membrane contacts between the endoplasmic reticulum (ER) and mitochondria (Mito) are crucial for many cellular functions, and abnormal levels of these contacts are detrimental to normal cellular physiology. Despite the increasing evidence implicating ER-Mito contacts in the pathogenesis of many neurodegenerative diseases, little is known about how the interactions between these organelles are regulated within the cell. Here we screened a compound library to identify chemical modulators for ER-Mito contacts in HEK293T cells. Multiple agonists of G-protein coupled receptors (GPCRs), beta-adrenergic receptors (β-ARs) in particular, scored in this screen. Analyses in multiple orthogonal assays validated that β2-AR activation promotes physical and functional interactions between the two organelles. Furthermore, we have elucidated potential downstream effectors mediating β2-AR-induced ER-Mito contacts. Together our study identifies β2-AR signaling as an important regulatory pathway for ER-Mito coupling and highlights the potential role of this pathway in therapeutic approaches to multiple neurodegenerative disorders.

Disclosures: Y. Lim: None. **I. Cho:** None. **H.G. Rennke:** None. **G. Cho:** None.

Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Brockman Foundation
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The G.R. Lincoln Family Foundation
Gordon & Evie Safran Foundation
Training Program in Free Radical and Radiation Biology from the University of Iowa (T32 CA078586)
The Oberley Seed Grant

Title: Neuroprotective treatment with P7C3-A20 prevents chronic brain damage and neuropsychiatric impairment after whole brain radiotherapy.

Authors: *E. VAZQUEZ-ROSA[1,4,5,6,7], M.-K. SHIN*[1,4,5,6], K. CHAUBEY[1,4,5,6], S. BARKER[1,4,5,6,2], C. J. CINTRON-PEREZ[1,4,5,6], Z. BUD[1,4,5,6,8], M. DHAR[1,4,5,6], E. MILLER[1,4,5,6,3], Y. KOH[1,4,5,6,2], Y. YU[1,4,5,6], K. P. LINDLEY[1,4,5,6], A. VYAS[1,4,5,6], K. A. MAPSUKAR[7], J. D. SHOENFELD[7], H. FUJIOKA[9], B. M. WILSON[5], D. R. SPITZ[7], B. G. ALLEN[7], A. A. PIEPER[1,4,5,6];
[1]Dept. of Psychiatry, [2]Dept. of Pathology, [3]Dept. of Neurosci., Case Western Reserve Univ., Cleveland, OH; [4]Harrington Discovery Institute, Univ. Hosp. Cleveland Med. Ctr., Cleveland, OH; [5]Geriatric Res. Educ. and Clin. Ctr. (GRECC), Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH; [6]Inst. for Transformative Mol. Medicine, Sch. of Medicine, Case Western Reserve Univ., Cleveland, OH; [7]Free Radical and Radiation Biology, Dept. of Radiation Oncology, Univ. of Iowa Hosp. and Clinics, Iowa City, IA; [8]Frances Payne Bolton Sch. of Nursing, Case Western Reserve Univ., Cleveland, OH; [9]Cryo-Electron Microscopy Core, Case Western Reserve University, Sch. of Med., Cleveland, OH

Abstract: Whole-brain radiotherapy (WBRT), the most common treatment for patients suffering from metastatic brain tumors, is associated with life-altering neuropsychiatric impairment. In fact, 50-90% of patients experience a significant decline in neurocognitive function one year after WBRT. The neuroprotective aminopropyl carbazole P7C3-A20 has demonstrated significant cognitive protection in multiple pre-clinical models of neurodegeneration, including age-related cognitive decline, stress-induced depression, traumatic brain injury, stroke, Parkinson's disease, and Alzheimer's disease. To investigate whether P7C3-A20 might also provide a new therapeutic approach to protecting patients from neurodegeneration after WBRT, we evaluated the efficacy of P7C3-A20 in mitigating brain damage and neuropsychiatric impairment after exposing mice to 10 Gy whole-brain radiation. We observed that daily treatment of mice with P7C3-A20 after WBRT attenuated both cognitive deficits and depression-like behavior, reduced reactive oxygen species damage and neurodegeneration, protected the blood-brain barrier, decreased neuroinflammation, and increased the magnitude of hippocampal neurogenesis. Importantly, P7C3-A20 treatment did not impair radiation-induced tumor cell killing. Our results indicate that treatment with P7C3-A20 or related compounds might provide therapeutic benefits for WBRT patients by reducing brain damage and preventing neuropsychiatric impairment.

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Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Cure Parkinson's UK RM2017002125
Michael J Fox Foundation Therapeutic Pipeline Program (MJFF021205)
Advance Queensland Mid-Career Fellowship (AQRF03116-17RD2)

Title: Bruton's tyrosine kinase BTK drives inflammasome activation and synuclein pathology in Parkinson's disease

Authors: N. JAYABALAN[1], N. J. GROVES[2], N. BIRCH[2], K. Z. HANTON[2], S. JEWELL[3], S. JOSE[3], J. D. O'SULLIVAN[3], R. J. ADAM[2], D. OFENGEIM[4], N. A. HAGAN[5], ***R. GORDON**[3]; [1]Ctr. for Clin. Res., Univ. of Queensland, Herston, Australia; [2]Fac. of Med., [3]The Univ. of Queensland, The Univ. of Queensland, Brisbane, Australia; [4]Rare and Neurologic Dis. Res. TA, [5]Neurosci., Sanofi, Framingham, MA

Abstract: Parkinson's disease (PD) manifests as a debilitating spectrum of motor and non-motor deficits and there are currently no effective treatments to slow or halt PD progression. The alarming rise in prevalence of PD, together with its complex multifactorial aetiology, highlights the urgent unmet need to develop more effective disease-modifying therapies. Chronic immune and inflammasome activation underlies PD pathology and is an attractive target for disease modification. Inhibition of the NLRP3 inflammasome has been shown to prevent α-synuclein pathology and dopaminergic neurodegeneration, demonstrating its potential as a disease-modifying therapeutic strategy for PD (Gordon et al., Science Translational Medicine 2018). The mechanisms by which the NLRP3 inflammasome is triggered and drives disease progression in PD have not been defined and could enable more effective treatment strategies to be developed. Herein, we demonstrate that Bruton's Tyrosine Kinase (BTK), a member of the TEC family, is highly upregulated in the nigrostriatal system of human PD patients. Our mechanistic studies in pre-clinical models of PD uncovered that BTK upregulation and activation occurs at the peak of NLRP3 inflammasome activation in the nigrostriatal system and is triggered by pathological synuclein aggregates in immune cells. Pharmacological inhibition of BTK blocked immune and inflammasome activation markers in primary microglia and prevented dopaminergic neuronal death *in vitro*. Most importantly, once daily dosing with an orally active BTK inhibitor in PD models, ameliorates multiple neuropathological processes linked to PD in the nigrostriatal system, including inflammasome activation, synuclein pathology, CNS infiltration of immune cells, motor deficits, striatal dopamine loss and neuropathology. Together, our results identify BTK as a novel and clinically relevant target for neuroprotection in PD. CNS-targeted BTK inhibitors in clinical development could be effective therapeutic agents for disease-modification by inhibiting multiple pathological processes linked to PD progression.

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Poster

283. Neurodegeneration, Mechanisms, and Therapeutics

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 283.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Positive feedback of apoptosis and autophagy pathways by sodium propionate to control glioblastoma progression through PPARγ signaling: correlatingin vitroandin vivo observations

Authors: *A. FILIPPONE[1], M. LANZA[1], G. CASILI[4], M. CAMPOLO[2], I. PATERNITI[1], S. CUZZOCREA[1], E. ESPOSITO[3];
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Abstract: There is an imperative need for new therapeutic approaches to improve the outcome of patients with glioblastoma multiforme (GBM). Several studies have suggested sodium propionate (SP), belonging to short-chain fatty acids (SCFAs), has a potent antiproliferative effect on various cell types of breast and colon cancers. Moreover, peroxisome proliferator-activated receptors (PPARs) ligands, have been proposed to possess anticancer properties. Here, we aimed to investigate the PPAR-γ/SCFAs binding way of acting in *in vitro* and *in vivo* models of GBM. GBM cells (U87 cell line) were used in *in vitro* study and treated with different concentrations of SP (50 and 100 mM). U87 cells were also used for PPAR-γ knockdown by using PPAR-γ siRNA or Ctr siRNA. After 24 h, cells were lysed and used for analysis. In *in vivo* study, BALB/c mice were inoculated in the right flank with 3×10^6 U-87 cells. SP (doses of 30 and 100 mg/kg) and GW9662 (1 mg/kg) were administered orally and intraperitoneally, respectively. *In vitro* exposure of U87 cell line to SP (50 and 100 mM) resulted in prominent apoptosis activation through increased p53 and caspase-3 protein expression levels. Also, the autophagy pathway was promoted by SP treatments by influencing Beclin 1 and autophagy-related protein 5 (ATG-5) expressions and initiated autophagosome formation and accumulation identified in the conversion of LC3I to LC3II. Furthermore, knockdown of PPARγ by RNA silencing, sensitized GBM cells by showing that SP possesses an agonist profile to PPAR-γ. Once we demonstrated *in vitro* the molecular mechanism of SP to induce apoptosis and autophagy through PPAR-γ, we next investigated the role of the PPAR-γ/SCFAs binding to suppress, by administrations of SP (30 and 100 mg/kg) and PPAR-γ inhibitor GW9662 (1 mg/kg) in counteracting tumor progression in an *in vivo* model of GBM. Here, we reported that SP was able to decrease tumor growth, resolve GBM tissue features, and invasion of tumor cells in adjacent parenchyma. Our results provide a line of evidence indicating that SP promotes

apoptosis and autophagy pathways through a PPAR-γ-dependent mechanism, causing a deceleration of tumor progression and cell cycle in GBM cells. Moreover, the PPAR-γ/SCFAs binding is also involved in the mechanism of tumor growth *in vivo* by attenuation of GBM histopathological hallmarks. Therefore, the PPAR-γ/SCFAs binding is a potential target and candidate for the management of GBM through the powerful SP abilities to counteract tumorigenesis.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Comparison of Human Primary microglia and Human iPSC derived microglia cells as in vitro models for microglia activation

Authors: M. BSIBSI[1], K. LO[1], J. DEGROOT[2], ***D. F. FISCHER**[3], M. VLAMING[1]; [1]Charles River, [2]Charles River, Leiden, Netherlands; [3]Charles River, Charles River, Saffron Walden, United Kingdom

Abstract: Microglia are resident immune effector cells in the CNS and play an essential role in neuroinflammation, ischemic and neurodegenerative disease. Therefore, microglia cells are considered a potential therapeutic target for neurodegenerative diseases. To fully understand the role of microglia, the preferred strategy would be to study primary human microglia isolated from post-mortem human brain tissue. Microglia can be isolated from both control and diseased human brain tissue with confirmed neuropathology. However, the obvious limitation on brain collection and yield of isolated cells restricts the ability to perform screening studies. Induced pluripotent stem cells (iPSCs)-derived microglia, may provide a suitable alternative for screening studies and large-scale compound validation. Yet, to effectively use iPSC-derived microglia, one must characterize the extent to which these cells faithfully represent biological processes in primary brain tissue.
Here, we compared the gene expression and cytokine release from primary human microglia cells obtained from tissue provided by the Netherlands Brain Bank and iPSC-derived microglia. Exposure of primary and iPSC-derived microglia to LPS resulted in increased TNF-α secretion in a concentration and time dependent manner. LPS-mediated TNF-α secretion was strongly inhibited by dexamethasone. Priming of primary and iPSC-derived microglia primed with LPS and treatment with nigericin, a potent inflammasome activator, resulted in robust secretion of IL-1β and IL-18. Furthermore, nigericin induced IL-1β and IL-18 release was blocked by the inflammasome inhibitor MCC950 in both cell types. Taken together, we successfully demonstrated that primary and iPSC-derived microglia respond similarly to LPS and nigericin

treatment. In addition, both cell types can serve as a reliable tool for evaluating the potency and efficacy of prospective drugs for multiple neurological diseases associated with microglia activation, such as Alzheimer's and Parkinson's Disease.

Disclosures: **M. Bsibsi:** A. Employment/Salary (full or part-time):; Charles River. **K. Lo:** A. Employment/Salary (full or part-time):; Charles River. **J. Degroot:** A. Employment/Salary (full or part-time):; Charles River. **D.F. Fischer:** A. Employment/Salary (full or part-time):; Charles River. **M. Vlaming:** A. Employment/Salary (full or part-time):; Charles River.

Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Human iPSC microglia for assessing new treatments in neurological diseases.

Authors: *K. R. WALKER, J. BELLEC-DYEVRE, C. CANCIO, M. PAQUET, E. ESNEAULT, F. SIMON;
Porsolt S.A.S., Le Genest Saint Isle, France

Abstract: Microglial activation and the ensuing inflammatory response in the central nervous system, plays an important role in neurodegenerative diseases such as Alzheimer's and Parkinson's. Activated Microglia, in addition to their role in phagocytosis, may induce the secretion of proinflammatory cytokines, including TNF alpha, and IL-6, as well as, Capsase-1 activation via the NLRP3 inflammasome, which controls the release of IL-beta and IL-18. The aim of the present study was to asses functional and physiological properties of microglia derived from human induced pluripotent stem cells (human iPSC) by measuring the expression of proinflammatory cytokines secreted following LPS stimulation and their ability to phagocytose bacterial particles.Expression levels of IL-16, IL-8, IL-10 and TNF alpha were clearly increased in the supernatants at 6 and 24hrs following LPS stimulation demonstrating microglial activation. These effects were partially inhibited by Dexamethasone. The addition of an adenosine triphosphate pulse following LPS was required to induce IL-1 beta secretion, confirming activation of the inflammasome pathway. Microglial functionality was assessed via kinetic monitoring of the phagocytosis of bacterial particles, coupled with pH-Rodo fluorochrome. An increase of fluorescent signal was observed over time, an activated microglia exhibited functional phagocytosis. This effect was fully inhibited in the presence of Cytochalasin D, an inhibitor of actin polymerization.These results suggest that human iPSC-derived microglia exhibit functional characteristics similar to human microglia, including phagocytosis and cytokine-mediated inflammatory responses. This translational model may be used to study neuroinflammation, a crucial hallmark of neurodegenerative disorders in humans, and to assess the efficacy of early-stage therapeutic targets.

Disclosures: **K.R. Walker:** A. Employment/Salary (full or part-time):; Porsolt S.A.S. **J. Bellec-Dyevre:** A. Employment/Salary (full or part-time):; Porsolt S.A.S. **C. Cancio:** A. Employment/Salary (full or part-time):; Porsolt S.A.S. **M. Paquet:** A. Employment/Salary (full or part-time):; Porsolt S.A.S. **E. Esneault:** A. Employment/Salary (full or part-time):; Porsolt S.A.S. **F. Simon:** A. Employment/Salary (full or part-time):; Porsolt S.A.S..

Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Institute of Neurological Disorders and Stroke (NS104160)
National Institute on Aging (AG067781, AG045571, AG073153)
National Institute on Aging Alzheimer Disease Center (AG072977)

Title: Production of Reactive Oxygen Species by Human Microglia in Response to Soluble Oligomeric and Fibrillar Amyloid-β Peptide

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Abstract: Human microglia are responsible for the first line of immune defense in the central nervous system and have been found to have a central role in the pathobiology of cognitive decline. Among various factors associated to cognitive decline is the deleterious effect of the amyloid-β (Aβ) peptide that can lead to Alzheimer's disease (AD). When Aβ accumulates in the brain, an inflammatory immune response is triggered in activated microglia. Activated microglia produce reactive oxygen species (ROS) to foreign or abnormal substances. Prolonged activation of microglia in chronic conditions such as AD can have adverse effects on neurons. Most studies of microglia response to Aβ have utilized rodent models. The purpose of this study was to investigate this response in microglia isolated and cultured from postmortem human brains. Microglia from the gray matter of fresh autopsied prefrontal cortical tissue of two elderly participants with above average cognitive performance were extracted and cultured. Cells from passage 3 - 5 were seeded in 8 chamber slides (3x10 cell/well). Microglial cells were cultured in the presence of microglia medium (SienCell, Inc), supplemented with 5% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 ml/500ml primocin, 1% microglia growth supplement (ScienCell), and 10 ng/ml GM-CSF (Sigma-Aldrich). After the cells reached 70%

confluence, the media was removed and replaced by 100 µl of 1mg BSA/RPMI. Various concentrations of fibrillar or soluble oligomeric Aβ (2.5ug, 5ug, and 10ug), prepared using specific aging protocols, or vehicle were added to the wells. After a 30-minute incubation at 37°C, 20 µl of 6 mg/ml Nitroblue Tetrazolium (NBT) was added to each well and incubated for an additional 90 minutes. The media was then removed, and 100µl/well methanol was added (5 minutes) to fix the cells. The slide was rinsed 3 times in xylene and coversliped with SubX mounting media. Optical density of the blue ROS reaction product (formazan salt) in individual microglia was measured using the Image J software. Dose-dependent increases in ROS production by microglia were observed in response to stimulation by both oligomeric and fibrillar Aβ. Oligomeric Aβ had a greater effect on ROS production by human microglia when compared with fibrillar Aβ in the 5ug (1.54x difference) and 10µg (2.2x difference) doses. These findings demonstrate that human microglia are activated in the presence of both oligomeric and fibrillar Aβ, but more so after stimulation by oligomeric Aβ. Cultured primary human microglia will be useful in studies of microglia function and inflammation.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Rapid and consistent generation of functional microglia from reprogrammed hiPSCs to study mechanisms in neurodegeneration and neuroinflammation

Authors: *M. RAMAN, C. FAIRBAIRN, E. YATES, P. BARTON, R. HICKMAN, P. PARAC, B. KLAPHOLZ, S. MILDE, L. TURNER, A. KNIGHTS, V. WINFIELD, R. O'REILLY, H. GARNETT, G. BELLI VALLETTA, C. ARUTHAN, E. GALIMBERTI, C. UGURLU, M. BYRNE, Z. JAWAD, K. FIRTH, R. HOPKINS, F. PATELL-SOCHA, T. MOREAU, W. BERNARD, M. KOTTER;
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Abstract: Microglia are the tissue resident macrophages of the brain, accounting for 75-80% of leukocytes and 10-15% of total cells within the central nervous system (CNS). These cells survey neuronal function, play roles in neurogenesis, synaptic remodeling and are the first responders to infection, and are thereby implicated in various CNS diseases. Currently the life sciences sector relies predominantly on rodent models to recapitulate disease states for research and drug discovery. However, animal models do not always recapitulate human cell and disease phenotypes. To bridge this translational gap, several *in vitro* human models have been developed for the study of microglia, most typically primary microglia extracted directly from either

embryonic, neonatal or adult tissue. However, primary cells are limited in supply, difficult to source, and typically show donor-to-donor and user variability. There is a pressing need for functional, consistent, and scalable disease-relevant human microglia models for both research and therapy development. We have developed a proprietary gene-targeting strategy, named optimised inducible overexpression (opti-ox), that enables highly controlled expression of transcription factors to rapidly reprogram human iPSCs (hiPSCs) into somatic cell types with high precision and in a scalable manner. To this end, we have generated hiPSC-derived microglia, termed ioMicroglia, using opti-ox precision reprogramming. Indeed, transcription factor-driven reprogramming using opti-ox™ technology, converts hiPSCs within days to functional microglia. ioMicroglia, 10 days post-revival, display typical microglia morphology and express key microglia phenotypic markers (TMEM119, P2RY12, IBA1, CX3CR1, CD11b, CD45, and CD14). RNA sequencing demonstrates that ioMicroglia have a transcriptomic signature that is similar to primary adult and foetal microglia. ioMicroglia also display typical microglial functions including phagocytosis (Amyloid Beta and bacterial particles), and proinflammatory cytokine secretion (IL-6 and TNFα), upon stimulation. In addition, ioMicroglia can be co-cultured with glutamatergic (ioGlutamatergic) neurons, whilst maintaining their function. In summary, using gene engineering and precision cellular reprogramming, we have developed ioMicroglia, providing a functional, scalable, fast, and easy-to-use hiPSC-based model system for basic research and drug discovery applications in the CNS and immune fields.

Disclosures: **M. Raman:** A. Employment/Salary (full or part-time):; bit.bio. **C. Fairbairn:** A. Employment/Salary (full or part-time):; bit.bio. **E. Yates:** A. Employment/Salary (full or part-time):; bit.bio. **P. Barton:** A. Employment/Salary (full or part-time):; bit.bio. **R. Hickman:** A. Employment/Salary (full or part-time):; bit.bio. **P. Parac:** A. Employment/Salary (full or part-time):; bit.bio. **B. Klapholz:** A. Employment/Salary (full or part-time):; bit.bio. **S. Milde:** A. Employment/Salary (full or part-time):; bit.bio. **L. Turner:** A. Employment/Salary (full or part-time):; bit.bio. **A. Knights:** A. Employment/Salary (full or part-time):; bit.bio. **V. Winfield:** A. Employment/Salary (full or part-time):; bit.bio. **R. O'Reilly:** A. Employment/Salary (full or part-time):; bit.bio. **H. Garnett:** A. Employment/Salary (full or part-time):; bit.bio. **G. Belli Valletta:** A. Employment/Salary (full or part-time):; bit.bio. **C. Aruthan:** A. Employment/Salary (full or part-time):; bit.bio. **E. Galimberti:** A. Employment/Salary (full or part-time):; bit.bio. **C. Ugurlu:** A. Employment/Salary (full or part-time):; bit.bio. **M. Byrne:** A. Employment/Salary (full or part-time):; bit.bio. **Z. Jawad:** A. Employment/Salary (full or part-time):; bit.bio. **K. Firth:** A. Employment/Salary (full or part-time):; bit.bio. **R. Hopkins:** A. Employment/Salary (full or part-time):; bit.bio. **F. Patell-Socha:** A. Employment/Salary (full or part-time):; bit.bio. **T. Moreau:** A. Employment/Salary (full or part-time):; bit.bio. **W. Bernard:** A. Employment/Salary (full or part-time):; bit.bio. **M. Kotter:** A. Employment/Salary (full or part-time):; bit.bio.

Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Evaluation of microglial activation, stress response and axonal neurodegeneration in patients with NeuroCOVID hospitalized at the New Civil Hospital of Guadalajara Dr. Juan I. Menchaca

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Abstract: Although the nervous system is highly protected from most viral threats, through epithelial barriers, the blood-brain barrier, and immune responses, SARS-CoV-2 can invade directly or through the axonal transport system to access the CNS. Mainly is accompanied by a neuroinflammatory state that can evolve from acute to chronic, depending on the state of microglial activation. Therefore, NeuroCOVID patients are more susceptible to long-term neuropsychiatric and neurocognitive disorders, including depression, obsessive-compulsive disorder, loss memory, psychosis, and increased risk of Parkinson's and Alzheimer's diseases. The aim of this work is identifying the neuronal mechanisms underlying neuronal infection through blood biomarkers of microglial activation, stress response, and cognitive neurodegeneration by using ELISA immunoassays. We observed that biomarkers of microglial activation, stress, and neurodegeneration associated with SARS CoV2 infection in patients with NeuroCOVID were altered.

Disclosures: D. Fernández-Quezada: None. **A. Herra-Cortez:** None. **C. Méndez-Valle:** None. **D. Martínez-Fernández:** None. **J. Hernández-Bello:** None. **J. García-Estrada:** None. **Á. Ontiveros:** None. **H. Velázquez-Santana:** None. **S. Luquin:** None.

Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH NIAAA 2R01AA023797-06A1
NIH NCATS 1TL1TR003019
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Title: Unraveling ethanol-induced neuroinflammation and microglial activation in a human induced pluripotent stem cell-derived neural tri-culture system

Authors: *A. J. BORELAND[1], Y. ABBO[1], A. C. STILLITANO[1], R. G. GABRIEL[1], Y. HAPIAK[1], R. P. HART[2], Z. P. PANG[1];

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Abstract: Alcohol use disorders (AUDs) affect increasingly large numbers of people with limited therapeutic success. People with AUDs are at risk for developing alcohol-associated dementia and cognitive impairment. While the cellular mechanisms underlying this dementia-related pathology remain enigmatic, there is an increasing focus on neuroinflammation as a hallmark of AUD-related dementia. Dysregulated neuroimmune interactions between neurons and glial cells, namely astrocytes and microglia, present a potential candidate for understanding how alcohol may cause neuropathology. Here we aim to investigate ethanol-induced neuroinflammation in a human context. We generated a human induced pluripotent stem cell (hiPSC)-derived triculture model of human neurons, astrocytes, and microglia. We found that a one-week chronic intermittent ethanol exposure upregulated the expression of inflammation-related genes. Specifically, ethanol was found to robustly stimulate the NLR family pyrin domain containing 3 (NLRP3) inflammasome innate immune pathway, including downstream Interleukin-1β (IL-1β), in a dose-dependent manner. Meanwhile, ethanol exposure increased microglial activation. Canonical microglia activation genes ionized calcium-binding adaptor molecule-1 (IBA1), macrophage colony-stimulating factor (M-CSF), and CD68 were significantly upregulated in addition to lysosomal-associated membrane protein (LAMP1) suggesting upregulation of lysosome production. Ethanol exposure was found to stimulate the type-I interferon response with significant upregulation of interferon-stimulated gene 15 (ISG15). Finally, ethanol was found to cause subtle changes in microglia morphology using fractal analysis. Ongoing work focuses on how ethanol-induced neuroinflammation, including microglial activation, affects neuronal function, such as intrinsic physiological properties and synaptic transmission. Unraveling the molecular and cellular mechanisms underlying ethanol-related neuroimmune dysregulation in a human context may further understanding the underlying mechanism of AUD and AUD-related dementia.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH/NINDS R01 NS112212
NIH/NINDS R01 NS122777

Title: Human microglia-like cells show impaired mitochondrial respiration in response to the neurodegenerative disease-relevant pro-inflammatory mediators HMGB1 and IL-1β

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Abstract: Suppression of mitochondrial oxygen consumption is thought to promote pro-inflammatory, neurotoxic microglia disease phenotypes seen in many neurodegenerative diseases. The Toll-Like Receptor 4 (TLR4) agonist lipopolysaccharide (LPS) combined with the cytokine interferon-γ (IFN-γ) is used to model neurotoxic microglial activation in vitro. However, in neurodegeneration, TLR4-immune signaling is largely propagated by endogenous molecules released by damaged cells rather than by the bacterial endotoxin LPS, which has species-specific effects and can also directly activate caspase-11. High Mobility Group Box 1 (HMGB1) is an endogenous TLR4 agonist believed to contribute to the cognitive deficits seen in Alzheimer's Disease and traumatic or ischemic brain injury. It may act in part by triggering interleukin-1β (IL-1β) release through NLRP3 inflammasome activation. The goal of this exploratory study was to test the prediction that HMGB1 and IL-1β can drive the suppression of mitochondrial oxygen consumption in human male induced microglia-like (iMG) cells, similarly to observations with LPS/IFN-γ in rodent models. Human iMGs were generated from peripheral blood mononuclear cells of healthy male adults using an established differentiation protocol and validated by staining for microglial markers P2RY12 and CX3CR1. Using a Seahorse XF24 to measure respiration, we found that treatment of iMGs with either HMGB1 (500 ng/ml) or IL-1β (50 ng/ml) for 18 hours decreased both basal and maximal mitochondrial oxygen consumption rate, with the latter measured after induction by uncoupler and excess pyruvate (10 mM) to remove substrate limitations. No toxicity was observed and IFN-γ (20 ng/ml) failed to exacerbate either response. We then compared the effects of HMGB1 in the human iMG model to those seen in the HAPI mouse male microglial cell line following classical LPS/IFN-γ stimulation (10 ng/ml each) or when substituting HMGB1 for LPS. Both LPS/IFN-γ and HMGB1/IFN-γ treatments resulted in near-complete suppression of basal and maximal HAPI cell respiration, but LPS/IFN-γ also caused moderate cell death. HMGB1/IFN-γ induced less nitric oxide production and different cell morphology relative to the LPS/IFN-γ treatment. Overall, data support the prediction that human male microglia-like cells suppress mitochondrial oxygen consumption in response to disease-relevant pro-inflammatory stimuli. Results also suggest that stimulation by HMGB1 and LPS is not equivalent. Future experiments will determine if TLR4 binding and/or IL-1β secretion is required for the effects of HMGB1 in human or rodent cells and test whether the responses are sex-dependent.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Interferon-induced transmembrane protein 3 and Tau in Alzheimer's Disease

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Abstract: Interferon-induced transmembrane protein 3 and Tau in Alzheimer's Disease Lauren Jonas[1, 2], Georgia Frost[2], Yueming Li[1, 21]Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medicine, New York, NY, 10021, USA, [2]Chemical Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021, USA,
Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular amyloid plaques and intracellular hyperphosphorylated tau. Recent work highlights Interferon-induced transmembrane protein 3 (IFITM3), an innate immunity protein, as being a key contributor to amyloid-associated AD pathology. Upregulated in post-mortem AD patients and by AD-associated inflammatory cytokines, in vitro and *in vivo* work has shown IFITM3 to play a role in amyloid processing, as well as in the disease-associated immune response. While much remains to be understood on IFITM3's role in amyloid-related pathology, little is known of the relationship between IFITM3 and tau. Current behavioral and biochemical studies indicate that an IFITM3 deficit in the tau (PS19) mouse model contributes to preserved cognitive function, altered hyperphosphorylated tau deposition, and reduced neuronal degeneration. This project aims to elucidate the pathways through which IFITM3 functions, as current studies highlight IFITM3's critical role in AD, as well as its potential as a novel target for pharmacological intervention.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Radiological Society of North America Research Scholar Grant
Mallinckrodt Institute of Radiology Summer Research Program

Title: Childhood obesity is linked to putative neuroinflammation in brain white matter, hypothalamus, and striatum

Authors: *Z. LI[1], A. SAMARA[2], M. RAY[3], J. RUTLIN[3], C. A. RAJI[4], J. S. SHIMONY[5], P. SUN[7], S.-K. SONG[5], T. HERSHEY[6], S. A. EISENSTEIN[4];
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Abstract: Introduction
Childhood obesity is increasingly prevalent and confers elevated risk of developing medical complications across the lifespan. Given the brain's prominent involvement in homeostatic and hedonic eating, it is crucial to understand brain health in obesity. Recent studies using quantitative T2-weighted MRI and diffusion-weighted MRI showed obesity-related putative neuroinflammation in human brain. To assess and extend these findings, this study characterized tissue microstructure of white matter (WM) tracts and energy regulation and reward processing brain regions in children across levels of adiposity, using diffusion basis spectrum imaging (DBSI).
Methods
Using data from the Adolescent Brain Cognitive Development[SM] Study (ABCD Study®), DBSI metrics indicative of putative neuroinflammation were computed for WM tracts, hypothalamus, nucleus accumbens, caudate nucleus, and putamen. DBSI metrics were compared between children with normal-weight vs. obesity. DBSI metrics were also correlated to baseline and one-year change in continuous adiposity measures (waist circumference, BMI, and BMI *z*-scores). Striatal DBSI findings were compared to those of restriction spectrum imaging (RSI).
Results
A total of 265 nine- and ten-year old children from the ABCD Study® 2.0.1 data release (5 underweight; 127 with normal-weight; 64 with over-weight; 79 with obesity) who met inclusion and exclusion criteria were randomly selected. Relative to children with normal-weight, children with obesity had lower DBSI fiber fraction (FF; reflects apparent axonal/dendritic density) and higher DBSI restricted fraction (RF; reflects cellularity) in WM tracts throughout the brain (voxel-wise FWE-corrected $p < 0.05$), as well as higher DBSI-RF in the hypothalamus, nucleus accumbens, and caudate nucleus (Cohen's d's ≥ 0.37, p's ≤ 0.0087). Across all children, greater baseline waist circumference was related to higher DBSI-RF in the hypothalamus (standardized $\beta = 0.17$, $p = 0.0033$), nucleus accumbens (standardized $\beta = 0.21$, $p = 0.0001$), and caudate nucleus (standardized $\beta = 0.14$, $p = 0.013$). Results were consistent for BMI and BMI *z*-scores. Although not passing multiple comparison correction, greater DBSI-RF in the nucleus accumbens predicted one-year gain in BMI (standardized $\beta = 0.14$, $p = 0.022$). Similar results were observed using RSI metrics.
Conclusion
These findings demonstrate that childhood obesity is associated with putative neuroinflammation in WM tracts, hypothalamus, and striatum, contributing to a growing understanding of the role of adiposity in brain health across the lifespan.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

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Program #/Poster #: 284.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 1K22NS118975
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Title: Disrupted Interferon type I response in plasma and peripheral monocytes from cognitive impaired patients

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Abstract: Human immunodeficiency virus (HIV) targets CD4+ immune cells, including monocytes. Despite the effectiveness of antiretroviral therapy controlling viral load and improving quality of life, patients with HIV (PWH) develop neurocognitive disorders associated to the infection, driven by monocytes infiltrating into the brain, neuroinflammation, and neuronal dysfunction. Type I interferon signaling is one of the most potent antiviral responses, but in the central nervous system (CNS) performs functions in important processes such as microglia activation, synaptic plasticity, and cognitive function. We hypothesize that disrupted IFN-I signaling triggers monocyte infiltration and cognitive decline. We measured Interferon alpha (IFNa1) in the plasma of HIV-negative, HIV-positive and Alzheimer's disease (AD) patients by ELISA. Then we compared these results with a cytokine array of plasma and cerebrospinal fluid (CSF) of PWH. Finally, we selected peripheral blood mononuclear cells (PBMCs) from a subgroup of patients and measured interferon alpha/beta receptor 1 (IFNAR1) in CD14+ monocytes by flow cytometry. Although not statistically significant, IFNa1 levels are slightly higher in the plasma of PWH cognitive impaired and significantly higher Alzheimer's disease patients ($p=0.035$), compared to HIV-negative participants. In a cytokine array of plasma from PWH ($n=48$), higher levels of IFNa2 were detected in the plasma of cognitive impaired ($n=21$) patients, compared to normal ($n=27$) patients ($p = 0.022$). IFNa2 levels were similar in the CSF. Flow cytometry of PBMCs from a subgroup of patients, revealed that the percentage of IFNAR1+ CD14+ monocytes and IFNAR1 levels are decreased in PBMCS from HIV-positive

and AD patients (p=0.025) compared to HIV-negative controls. Moreover, monocyte activation, measured by CD163 expression levels, was also slightly decreased in PBMCs from PWH, and significantly decreased in AD patients (p=0.031). Phosphorylation of Interferon Regulatory Factor 3 (IRF3) induces its translocation to the nucleus, activation of the interferon-stimulated response element promoter and further activation of IFN-alpha and IFN-beta promoters. Western blotting of PBMCs revealed that IRF3 phosphorylation in Ser386 is active and increased in PBMCs from PWH compared to HIV-negative (p=0.036). Human brain organoids co-cultured with these monocytes in vitro also demonstrated significant activation and dysregulation of IFN-I signaling. Thus, disrupted IFN-I signaling and receptor levels in monocytes may contribute to their infiltration to the CNS and cognitive decline in both PWH and Alzheimer's disease patients.

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Poster

284. Microglia, Neuroinflammation, and Immune Function: Human

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 284.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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FCT: UIDP/04539/2020
FCT: LA/P/0058/2020
COMPETE: POCI-01-0145-FEDER-007440

Title: Are IDO1^{+}Dendritic Cells a druggable target in Parkinson's Disease?

Authors: *M. GONÇALVES[1,2,3], A. MORGADINHO[7], P. RODRIGUES-SANTOS[1,4,5], J. S. ALMEIDA[5], V. ALVES[5], M. SANTOS-ROSA[5], C. A. FONTES-RIBEIRO[1,2,3], C. JANUÁRIO[6], M. COSENTINO[8], F. C. PEREIRA[1,2,3];
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Abstract: Motivation: Dysfunctions in the immune system have been pointed as a critical component of susceptibility and progression of Parkinson's Disease (PD). The contribution of dendritic cells (DC) and monocytes (Mo) to peripheral inflammation and neuroinflammation is

unexplored in PD. These myeloid cells may express indoleamine 2,3-dioxygenase 1 (IDO1), which is involved in immunosuppression. Also, these cells are sensitive to dopamine (DA). However, its immune modulatory function is scarcely known. **Aim:** herein we immunophenotyped PD patients circulating myeloid cells, including their capacity to induce IDO1. **Methods:** Peripheral blood samples were collected, and subsets of DC and Mo were analyzed by flow cytometry. The study groups consisted of 31 PD patients [67±9 years, 55% females] selected from Movement Unit of Coimbra Hospital and University Centre with a Unified Parkinson's Disease Rate Scale (UPDRS)III score mean of 19±9 and 26 healthy age matched controls [69±9 years, 48% females]. For the *in vitro* studies, 15 samples of each group were selected. Total blood was incubated with lipopolysaccharide (LPS [1 ng/mL], aiming to induce IDO1) in the presence or absence of DA (100 µM) during 6h. Samples were then acquired using FACSDiva software in a FACSCanto II flow cytometer. Data was presented as mean±standard deviation. Statistical analysis was performed using Unpaired t-test, Mann-Whitney, two-way ANOVA followed by Sidak's multiple comparisons test and Pearson r correlation. Differences were significant at $p<0.05$. **Results:** PD patients showed an increased relative frequency (RF, in %) of classical DC type 1 (cDC1) ($p<0,0421$). Accordingly, our PD patients also showed increased RF of Natural Killer cells ($p<0,0224$) and Mo expressing the adhesion molecule CD49d ($p<0,0141$). Additionally, patients had decreased RF of intermediate and non-classical Mo ($p<0,0206$ and $p<0,0107$), respectively). Importantly, RF of $IDO1^{+}$DC is increased ($p<0,0228$) in these patients, which is not correlated with UPDRSIII. Moreover, DA decreased LPS-induced IDO1 in DC and monocytes, in both groups, similarly. **Conclusion:** Our data suggest that there are changes in the landscape of circulating myeloid cells in PD. Importantly, increased $IDO1^{+}$DC seem to be part of the repertoire of immune alterations in PD. Furthermore, IDO1 may be a target to attenuate peripheral/central inflammation. *In vitro* studies highlight that DA reduces the IDO1 inducibility of circulating myeloid cells. This suggest that dopaminergic therapy may have immune implications and therefore affecting the disease progression. Overall, this study might have diagnostic and therapeutic impact in PD.

Disclosures: M. Gonçalves: None. **A. Morgadinho:** None. **P. Rodrigues-Santos:** None. **J.S. Almeida:** None. **V. Alves:** None. **M. Santos-Rosa:** None. **C.A. Fontes-Ribeiro:** None. **C. Januário:** None. **M. Cosentino:** None. **F.C. Pereira:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.01

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Identification of a gene in the genomic vicinity of apolipoprotein E regulated by a DNA motif created by the high-risk variant APOE4 and validation as the key mediator of early Alzheimer's disease pathological events

Authors: *R. URFER, A. URFER-BUCHWALDER;
Selonterra, Inc., San Mateo, CA

Abstract: The most clinically significant prevalent genetic risk factor for late onset Alzheimer's disease (AD) is APOE4, a variant of the apolipoprotein E gene. We have previously established that the SNP that determines APOE4 (rs429358) creates a *de novo* binding motif for the transcription factor NRF1. This site is located within a short sequence motif repeated several times along exon 4, and part of a homotypic cluster of NRF1 binding motifs, structural features typically observed in transcriptional enhancers. Aside from APOE4, several of those repeat elements carry variants associated with AD, namely APOE3-Christchurch (rs121918393), APOE2 (rs7412), APOE*2 (rs199768005) and the protective variant R251G (rs267606661). Hence, we hypothesized that APOE4 may not cause AD due to the resulting change in the protein sequence but instead acts at the DNA level by dysregulating the expression of genes located nearby on human chromosome 19. This dysregulation lies at the root of APOE4-dependent neurodegeneration. We measured the expression levels of a panel of genes within 2Mb of APOE4 in iPS-derived isogenic APOE4 and APOE3 neurons as they have been established as a suitable AD model. We identified a small set of statistically significantly regulated genes, one of which has been described as an AD-associated gene in the literature. We subsequently confirmed that these genes are similarly dysregulated in an AD-patient derived neuronal line. In order to identify which of these genes is the key mediator of AD dysfunction, we assembled a panel of markers for biological functions known to be affected early in AD (e.g., dendritic spines and synaptic loss, neuronal plasticity impairment and cell-cycle re-entry). Using the Accel siRNA technology from Dharmacon we knocked down the expression of each of the potential target genes individually in APOE3 neurons and measured the ensuing effects of gene silencing. We found that one of the five candidate genes not only strongly regulated the expression of key downstream AD marker genes but also the expression of the neighboring AD-associated gene. We further validated this new target and linked its activity to major pathways dysfunctional in AD by treating APOE3 or alternatively APOE4 neuronal cultures with either a selective antagonist or an agonist tool compound directed against the key target and measuring the effects on AD marker genes. We are confident that this new, unexploited target is the key mediator of early pathological AD events. Our next step will be to initiate a small molecule discovery/medicinal chemistry program to identify a potent and selective small molecule agonist suitable for a Phase 1 proof-of-concept trial.

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

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.02

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Intramural Research Training Program

Title: Apoe modification by reactive aldehydes in brains of individuals with alzheimer's disease, mild cognitive impairment, and non-ad controls

Authors: *E. KANG, E. CALZADA, G. KEYES, C. RAMSDEN;
NIH, Natl. Inst. on Aging, Baltimore, MD

Abstract: Alzheimer's disease (AD) is a progressive neurologic disorder that affects 5.8 million individuals in the United States age 65 and older. ApoE is the major apolipoprotein that supplies neurons with lipids that are required to synthesize and remodel synaptic membranes. *APOE* variants are the strongest genetic risk factor for sporadic AD, and ApoE is enriched in the central core of neuritic plaques. However, molecular mechanisms linking ApoE to the etiology of AD are incompletely understood. Using custom-designed antibodies targeting aldehyde-modified ApoE, our group recently showed that a portion of the ApoE in the core of neuritic plaques is modified by reactive lipid aldehydes. In the present work, we sought to characterize ApoE in human brain lysates by using custom-generated antibodies targeting aldehyde-modified ApoE to perform western blot and immunoprecipitation. Temporal cortex brain lysates were generated from postmortem specimens from 12 individuals who were categorized as non-AD controls (n=4), mild cognitive impairment (MCI, n=4), and AD (n=4). Ages ranged from 71 to 92; each group included two females and two males with mixed *APOE3*/*APOE4* homozygous and heterozygous genotypes. Using protein extraction, quantitation, and western blot, we found varying detection of higher molecular weight (~80kDa) migrating forms of aldehyde modified-ApoE between samples. Of note, low molecular weight forms (15-20kDa range) were more prevalent in MCI lysates when detected using two separate antibodies. To determine the identity of these prominent bands, immunoprecipitation of lysates using antibodies targeting unmodified ApoE and modified ApoE is in progress for mass spectrometry characterization of eluates. In summary, we seek to understand the nature of ApoE modification by reactive aldehydes in human brain and determine its potential role in the pathogenesis of AD.

Disclosures: E. Kang: None. **E. Calzada:** None. **G. Keyes:** None. **C. Ramsden:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.03

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant NS090934
NIH Grant AG58518

JBP Foundation
NSF Grant DGE-2139839
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Chan Zuckerberg Initiative Imaging Scientist Award 2020-225726
CDI-CORE-2015-505

Title: Structure of Human Lipidated Apolipoprotein E

Authors: *M. R. STRICKLAND, Y. CHEN, M. J. RAU, B. T. SUMMERS, R. KOSSINA, H. JIANG, R. ZHANG, J. ULRICH, J. A. J. FITZPATRICK, D. M. HOLTZMAN; Washington Univ. in St. Louis, Saint Louis, MO

Abstract: The Apolipoprotein E (APOE) gene is the most important genetic risk factor for late-onset Alzheimer's Disease (AD). ApoE is a lipid associated protein that plays a vital role in intercellular cholesterol and lipid transport, especially in the central nervous system (CNS) where it is the primary apolipoprotein. ApoE has three major isoforms (ApoE2, ApoE3, and ApoE4) of which ApoE3 is the most common. A single copy of the ApoE4 allele increases AD risk approximately 4-fold and two copies increase disease risk approximately 12-fold relative to the ApoE3 allele. The ApoE2 allele decreases risk for AD relative to the ApoE3 allele. Despite resulting in a dramatic increase in disease risk, ApoE4 only differs from ApoE3 by a single amino acid. To better understand the significance of this change, we aim to study the structure of lipidated ApoE; its physiological conformation. Previous studies investigating the structure of ApoE have been hampered by both the lipid environment of the physiological forms of ApoE and the tendency of the non-lipidated form to rapidly aggregate. We aim to address these issues by utilizing single particle cryo-electron microscopy (cryoEM) to determine the structure of lipidated ApoE. Advances in direct electron detectors and automated data acquisition strategies now routinely allow the determination of protein structures to near-atomic resolution. Using this method, we plan to determine the structure of lipidated ApoE2, ApoE3, and ApoE4 in order to understand conformational differences between the isoforms to reveal mechanistic insights into the role of ApoE for AD risk. In this study, we utilize both astrocyte-produced ApoE particles and artificially-lipidated recombinant ApoE. We have characterized these particles using size-exclusion chromatography and nondenaturing gradient gel electrophoresis. We have shown in our study that the artificial particles recapitulate the features of astrocyte produced ApoE particles and thus serve as a suitable initial model for understanding ApoE structure. In this study, we have also utilized monoclonal Fab and Fab_2 fragments to further characterize the ApoE particles and assist in alignment of the ApoE particles during 2D classification. Our current results have revealed that there are two ApoE proteins present in each lipoparticle which adopt an antiparallel orientation. Future research will pursue obtaining a high-resolution structure of the three isoforms to better understand any conformational differences that exist between the ApoE isoforms.

Disclosures: M.R. Strickland: None. **Y. Chen:** None. **M.J. Rau:** None. **B.T. Summers:** None. **R. Kossina:** None. **H. Jiang:** None. **R. Zhang:** None. **J. Ulrich:** None. **J.A.J. Fitzpatrick:** None. **D.M. Holtzman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health, Cure Alzheimer's Fund, Tau Consortium, JPB Foundation, Good Ventures, Ludwig Foundation, C2N Diagnostics, Novartis, NextCure, Ionis.

E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N Diagnostics LLC., Eli Lilly. F. Consulting Fees (e.g., advisory boards); Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Alector.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.04

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH AG054104
NIH AG064231

Title: Human ApoE2 and ApoE3 Slow Amyloid Accumulation in the Amyloid Precursor Protein Knock-in Mouse NLF

Authors: *P. FENIK[1], Y. ZHU[2], A.-H. CHOU[1], S. C. VEASEY[1];
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Abstract: Apolipoprotein E (ApoE) is a cholesterol carrier with an important role in amyloid clearance. The lipoprotein exists in humans as three major isoforms, ApoE2, ApoE3 and ApoE4. Relative to ApoE3, ApoE2 in an allelic dependent fashion reduces the risk of Alzheimer's disease and is associated with slower hippocampalatrophy in older adults. Interestingly, ApoE2 and ApoE3 targeted replacement (TR) in mice increase longevity and motor performance with aging, relative to ApoE4 TR or ApoE-/-, but whether replacement of mouse ApoE with human ApoE2 or ApoE3 would confer benefit in the APP knock-in (KI) NLF model of Alzheimer's is not known. Here, we generated APPKI NLF+/-/ApoE2+/- mice and APPKI NLF+/-/ApoE WT mice. Mice were aged to 20 months and then transcardially perfused. Brains were cryopreserved, sectioned and examined for alterations in amyloid deposition and glial responses. Ab42 and 6E10 percent area in CA1 were higher in the mice with mouse ApoE exclusively (APPKI NLF/ApoE WT) than in either humanized ApoE knock-in, $q=5.4$, $p<0.01$ and $q=4.8$, $p<0.5$, respectively. There was no difference in hippocampal Ab42 or 6E10 immunoreactive percent area between ApoE2 and 3. Remarkably while Thioflavin-T revealed plaques in APPKI NLF/ApoE WT mice (1.3±0.4% area), no plaques were observed in either the ApoE2 or ApoE3 mice. In addition, Iba-1 percent area in CA1 was higher in APPKI NLF ApoE WT than in APPKI NLF/ApoE2 or APPKI NLF/ApoE3, $q=7.3$, $p<0.001$. ApoE2 and ApoE3 gene therapy trials are now planned to determine whether therapy with ApoE2 or ApoE3 in young adult APPKI NLF mice can slow the progression of neurobehavioral impairments and amyloid plaque depostion in APPKI NLF mice. These findings provide support that human ApoE2 and ApoE3 are superior, relative to murine ApoE in maintaining amyloid homeostasis and support the concept that increased ApoE2 or 3 may improve amyloid homeostasisin at least some variants of familial Alzheimer's disease.

Disclosures: **P. Fenik:** None. **Y. Zhu:** None. **A. Chou:** None. **S.C. Veasey:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.05

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Elucidating mechanisms of familial Alzheimer's disease resistance through ApoE3 Christchurch using patient derived iPS cell cerebral organoids

Authors: *R. C. MAZZARINO[1], G. N. VACANO[3], J. SANCHEZ[4], M. N. O'HARE[1], Y. T. QUIROZ[5], F. LOPERA[6], J. ARBOLEDA[2];
[1]Schepens Eye Res. Inst., [2]Harvard Inst. of Med., Boston, MA; [3]Vacano Informatics LLC, Arvada, CO; [5]Psychiatry, [4]Harvard Med. Sch., Charlestown, MA; [6]Neurosci. Group of Antioquia, Medellín, Colombia

Abstract: Alzheimer's disease is a progressive neurodegenerative condition that leads to dementia. We recently reported that the ApoE3 Christchurch mutation may be protected against Alzheimer's disease (AD) in individuals carrying *PSEN1 E280A* mutation, yet the mechanism of AD resistance remains unknown. Thus, we used patient blood cells from two patients from the Colombian kindred with PSEN1 E280A mutation and reprogrammed them to generate induced pluripotent stem cells. We chose the female ApoE3 Christchurch protected patient as well as a non-protected female patient who developed mild cognitive impairment due to *PSEN1 E280A* at the typical expected age of clinical onset (45yo). We have performed genetic editing for both patient derived iPS cells targeting the PSEN1 and ApoE3 mutations and also selected for non-edited isogenic controls thus creating eight iPS cell lines, four derived from each patient. Within this, we are now able to identify possible mechanisms of action of the ApoE3 Christchurch with and without the *PSEN1 E280A* background and confirm the mechanisms in a control patient cell line. We have successfully generated cerebral organoids using these cell lines and performed single cell RNAseq. Preliminary QC analysis of the scRNAseq data indicates metrics with low percent mitochondrial mapping and high RNA feature mapping with correlation coefficients near -0.05 and 0.82 respectively for all samples. Differential gene expression between comparison groups revealed approximately 2000 genes with log2fold change greater than 1 or less than -1, depending on compared lines. We have also identified 21 cluster subpopulations within our datasets.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.06

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: MRC Grant MR/S026495/1

Title: Is beta-amyloid accumulation in Alzheimer's disease accelerated by APOE4-mediated neurovascular dysfunction?

Authors: *J. J. HENDERSON, S. ANDERLE, K. SHAW, H. TREWHITT, C. N. HALL; Univ. Of Sussex, Brighton, United Kingdom

Abstract: Blood flow is key to neuronal health. When the vasculature is impaired, insufficient oxygen and nutrients are supplied to neurons, leading to tissue hypoxia and neuronal damage. APOE4 is the most common genetic risk factor for the development of late onset Alzheimer's Disease (AD). Preclinical findings from our lab suggest that APOE4 confers an impairment in the ability of the brain's blood supply to match demand, and that the brain regions first affected by AD-related pathology have vascular properties which make them particularly vulnerable to hypoxic damage. This points to the possibility that APOE4 increases the risk of developing AD by impairing neurovascular function. Because, in vitro, beta-amyloid (Aβ) accumulation can be promoted by hypoxia, it is possible that neurovascular dysfunction leads to hypoxic damage in vulnerable regions, promoting the aggregation Aβ and further AD pathology. However, no prior experiment has been able to separate the independent and interacting effects of vascular dysfunction and Aβ production, largely due to lack of appropriate models. This study utilises a novel mouse model that allows for the characterisation of the neurovascular deficits incurred due to APOE4 expression before activation of Aβ production, and then the tracking of Aβ accumulation and its effects on cognition and the neurovasculature. The study utilises a three-pronged approach with behavioural testing, in vivo and ex vivo imaging. This poster will describe the ex vivo aspect of the study. APOE3/3 and APOE4/4 mice with fluorescent pericytes were bred with a tetO-APPSwe/Ind line and a BCaMKIIα-tTA line, to enable control of Aβ production via doxycycline. At 0, 8 or 16 weeks after onset of Aβ production, mice were injected with methoxy-X04 to label Aβ plaques and pimonidazole to enable labelling of hypoxic tissue, and perfused with FITC to visualise blood vessels. Immunohistochemistry was conducted for soluble Aβ, pimonidazole (hypoxyprobe), GFAP and Iba1. Confocal imaging was subsequently performed and analysis carried out using ImageJ and MATLAB to compare neurovascular integrity, Aβ aggregation and degradation, hypoxia and inflammation between genotypes. We have successfully created a strain of mice expressing the human APOE3 or APOE4 genotype, in which we can control Aβ production. We have stained and imaged forebrain slices at three timepoints after the onset of Aβ production. Analysis of these images is ongoing. Preliminary data suggest an increase in soluble but not aggregated Aβ over this timescale. The novel mouse model we have created will allow us to understand how neurovascular dysfunction contributes to the increased AD risk conferred by APOE4.

Disclosures: J.J. Henderson: None. **S. Anderle:** None. **K. Shaw:** None. **H. Trewhitt:** None. **C.N. Hall:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 285.07

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Grants from the Alzheimer's Association. to J.F.A.-V
UH3 NS100121 to J.F.A.-V.
RF1 NS110048 to J.F.A.-V.

Title: Anti-apoe-glycosaminoglycan antibodies to model possible mechanisms of protection against alzheimer's disease

Authors: *C. MARINO[1,4], M. O'HARE[1,4], T. H. DOAN[1,4], P. PEREZ-CORREDOR[1,4], S. AREVALO-ALQUICHIRE[4,1], Y. T. QUIROZ[2,3], J. F. ARBOLEDA-VELASQUEZ[4]; [1]Ophthalmology, Harvard Med. Sch., Boston, MA; [2]Psychiatry, [3]Neurol., Harvard Med. Sch., Charlestown, MA; [4]Ophthalmology, Schepens Eye Res. Inst. at Mass Eye and Ear, Boston, MA

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease leading to dementia affecting more than 6 million individuals in the US. Although the hallmarks and the clinical aspects leading to AD are well characterized, the multitude of risk factors contributing to this disease made so far challenging the discovery of effective therapeutic targets. One of the main risk factors known to increase the risk of sporadic AD is the presence of the *APOE4* allele, a variant of Apolipoprotein E (*APOE*). Conversely, we discovered that homozygosity for the *APOE Christchurch* (*APOEch* or R136S) variant delays the age of onset of autosomal dominant AD by almost three decades. Our previous studies supported the hypothesis that the possible mechanism of protection is via a loss of interaction with glycosaminoglycans (GAGs). Here, we aim to characterize a small library of antibodies that compete with the ApoE-GAGs interaction using enzyme-linked immunosorbent assay (ELISA), surface plasmon resonance (SPR), affinity chromatography, *in vitro* cytotoxicity, and immunohistochemistry. Preliminary results showed that our top-candidate anti-ApoE antibody has a femtomolar affinity for ApoE4 ($Kd<1*e^{-11}$), that is not cytotoxic and that reduces ApoE4-derived cytotoxicity *in vitro*. Overall, our study proposes ApoE-GAGs protein-protein interaction as a novel disease-modifying target relevant for AD.

Disclosures: C. Marino: None. **M. O'Hare:** None. **T.H. Doan:** None. **P. Perez-Corredor:** None. **S. Arevalo-Alquichire:** None. **Y.T. Quiroz:** None. **J.F. Arboleda-Velasquez:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.08

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: R01AG061114
RO1AG061114-01S1
R61/33NS114353

Title: Evaluating the Interaction of APOE Genotype and the Angiotensin Type 1 Receptor in Modulating Hippocampal Neuron Function

Authors: *S. SCHEINMAN, L. M. TAI;
Univ. of Illinois at Chicago Dept. of Anat. and Cell Biol., Chicago, IL

Abstract: Recent evidence suggests that the Angiotensin Type 1 (AT1) receptor plays a role in Alzheimer's disease (AD), the leading cause of dementia worldwide. For example, studies in human AD patients and preclinical rodent models indicate that pharmacological inhibition of the AT1 receptor with Angiotensin Receptor Blockers (ARBs) has beneficial effects on memory and cognition. Additionally, we have demonstrated that brain AT1 receptor signaling may interact with *APOE4*, the greatest genetic risk factor for AD, to modulate memory-relevant behavior and synaptic function in the hippocampus. However, fundamental/mechanistic research is lacking on how the AT1 receptor impacts neurons in the hippocampus and there is no data in the context of *APOE*. Therefore, it is important to evaluate the extent to which *APOE* genotype modulates the role of the AT1 receptor on hippocampal neuron function. Initially, we examined levels of the AT1 receptor by APOE genotype in the hippocampus as receptor levels often impact function. Using western blot analysis of male and female mice that express either the human *APOE3* or *APOE4* gene in the absence (EFAD- mice) of presence (EFAD+ mice) of Aβ overproduction, we found that hippocampal levels of the AT1 receptor were ~20-25% higher with *APOE4* as compared to *APOE3* at 6 months of age, an effect that was not seen at 3 months of age. Confirming this result, immunohistochemical analysis revealed that the AT1 receptor staining intensity was ~16-20% higher with *APOE4* as compared to *APOE3* and was localized primarily to neurons in the CA3 and CA1 regions of the hippocampus. These data suggest that higher levels of the AT1 receptor with *APOE4* may have functional consequences on the neurons in the Schaffer collateral pathway. Thus, due to the significance of the Schaffer collateral pathway in learning and memory, we next evaluated the extent that *APOE* modulates the role of the AT1 receptor on long-term potentiation (LTP), a form of synaptic plasticity that is the cellular basis for memory formation and storage. Ongoing studies indicate altered induction and maintenance of hippocampal LTP with *APOE4* in the presence of the AT1 receptor ligand, Angiotensin II. These results suggest that altered levels of AT1 receptor in the hippocampus with *APOE4* has functional effects on neuronal signaling and connectivity that could contribute to AD risk and development. Developing therapeutic strategies targeting the AT1 receptor may be particularly efficacious for *APOE4*-associated neuron dysfunction, however further mechanistic and preclinical studies are warranted.

Disclosures: S. Scheinman: None. **L.M. Tai:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.09

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: RO1 AG061879
P01AG066591 P-3

Title: Opposing effects on transcriptional activation induced by APOE alleles disrupt cellular homeostasis

Authors: *C. GERONIMO-OLVERA, C. GALICIA-AGUIRRE, J. BONS, L. MCFARLIN, S. SCHEELER, J. SIMONS, S. MELOV, B. SCHILLING, L. M. ELLERBY;
Buck Inst. for Age Res., Novato, CA

Abstract: Alzheimer's disease (AD) is the leading cause of dementia and mortality in the elderly. However, theunderlying pathological mechanisms remain unclear. Although several genetic risk factors for late-onsetAD have been identified, the apolipoprotein E4 (APOE4) is the most significant susceptibility factor knownto date. Compared to APOE3, the predominant isoform in the population, APOE2, is less common and isprotective against AD and associated with longer lifespan. Conversely, APOE4 markedly increases therisk of AD. Postmortem transcriptomics of brains carrying APOE genetic polymorphisms showed thatAPOE-ε4 is associated with alterations in processes associated with aging, such as inflammation,oxidative stress, and metabolism, while APOE-ε2 genes are associated with increased expression ofpathways related to proteostasis and DNA signaling. Recent findings demonstrated the age-associatedaccumulation of nuclear APOE results in the destabilization of heterochromatin, leading to cellularsenescence. Cellular senescence occurs in response to endogenous and exogenous stresses, includingepigenetic alterations, and has been linked to aging. The development of a pro-inflammatory senescence-associated secretory phenotype (SASP) is a key feature of senescence. APOE may play a crucial role inaging, and its expression increases with disease (i.e. AD) and cellular senescence. We hypothesize thatAPOE-ε2 and APOE-ε4 are associated with opposing effects on the regulation of the epigenetic landscape,leading to abnormal transcriptional activation of genes related to cellular senescence, which acceleratesthe pathological process promoted by aging. We generated neurons form isogenic iPSCs (APOE ε2/ε2,ε3/ε3 and ε4/ε4 genotypes) by inducible expressing Neurogenin-2 (NGN-2). iPSCs-derived neurons werecultured during 28 DIV to perform RNA sequencing and proteomics. Transcriptional profiling identifiedseveral differentially expressed genes (DEGs) in each genotype and preliminary Gene ontology analysisreveal that DEGs in APOE4 are related with synaptic function. In agreement, proteomic analysis revealthat among the proteins differentially regulated in APOE2 compared to APOE3 and APOE4 are proteinsassociated with DNA repair. These preliminary data suggest that APOE4 alters transcriptomic andproteomic patterns, which in turn may accelerate pathological processes induced by aging.

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Poster

285. ApoE and Associated Pathways

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Program #/Poster #: 285.10

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: College of Pharmacy and Office of Academic Clinical Affairs (FRD #19.29), University of Minnesota
NIH RF1 AG058081
NIH RF1 AG056976
NIH R21 AG056025

Title: Ameliorating APOE4-related deficits with a clinically tested HDL mimetic peptide in human induced pluripotent stem cell (iPSC)-derived models

Authors: *K. FREDRIKSEN[1], A. VEGOE[2], T. D. O'BRIEN[2], S. YUAN[3], L. LI[4];
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Abstract: The greatest genetic risk factor for developing late onset Alzheimer's disease (AD) is the APOE gene, which in humans has 3 alleles; APOE2 (E2) is protective against AD, APOE3 (E3) is neutral, and APOE4 (E4) increases AD risk. APOE is the most abundant apolipoprotein in the brain, where it is mainly produced and secreted by astrocytes. APOE plays a key role in lipid metabolism and transport by binding lipids (primarily phospholipids and cholesterol) to form high- density lipoprotein (HDL)-like particles in the interstitial and cerebrospinal fluid. E2 and E3 are efficiently lipidated, whereas E4 is poorly lipidated. The degree of lipidation is important for APOE function, including maintaining lipid homeostasis, regulating Aβ aggregation and clearance, and modulating neuroinflammation. Emerging therapeutic strategies for AD include targeting APOE lipidation and altering E4 properties to resemble that of E3 or E2. Previous studies in our lab have shown that the small clinically tested HDL-mimetic peptide 4F can increase APOE secretion and lipidation in primary mouse astrocytes and microglia, and primary human astrocytes. Although aggregated Aβ inhibits APOE secretion and lipidation, we have shown that 4F rescues this inhibition. Our current studies aim to corroborate these findings in additional human models and determine the functional effects of 4F on E4-related phenotypes using human induced pluripotent stem cell (iPSC)-derived 3D cerebral organoids and astrocytes. Human iPSCs from E2, E3, or E4 homozygous individuals were differentiated into cerebral organoids and astrocytes, followed by treatment with 4F. Preliminary results suggest that 4F treatment promotes secretion and lipidation of all three APOE isoforms in iPSC-derived organoids and astrocytes. Furthermore, the 4F-induced increase in lipidation is the most robust in

E4 organoids, and treatment with 4F restores secretion and lipidation levels of E4 to that of E2 or E3. Studies are underway to investigate the efficacy of 4F treatment to reverse E4-associated pathogenic effects and mitigate neuroinflammatory responses upon treatment with aggregated Aβ or pro-inflammatory cytokines in iPSC-derived models. The results are expected to highlight the therapeutic potential of HDL-mimetic peptides for sporadic AD.

Disclosures: K. Fredriksen: None. **A. Vegoe:** None. **T.D. O'Brien:** None. **S. Yuan:** None. **L. Li:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.11

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG058778

Title: Connecting PERK Signaling to Cholesterol Biosynthesis and Neurodegeneration

Authors: *R. VISWANATHAN, B. STOVEKEN, S. PARDO, D. MOLLEUR, H. GUDLAVALLETI, S. WEINTRAUB, J. LECHLEITER;
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Abstract: The Unfolded protein response (UPR) is activated when misfolded proteins accumulate in the endoplasmic reticulum (ER), providing valuable time for the cell's attempt to restore cellular homeostasis. Misfolded proteins are a common phenotype of many neurodegenerative diseases including Alzheimer's, frontotemporal dementia, and progressive supranuclear palsy. Interestingly, the UPR stress-sensor, PERK, is activated in the brains of patients with neurodegenerative tauopathies. However, both positive and negative modulation of PERK activity can be therapeutically beneficial. The specific mechanisms by which PERK signalling contributes to neurodegenerative diseases need to be more clearly defined. We performed global proteomics to identify pathways that were differentially regulated by the presence or absence of PERK (PERK knockout (KO)) in mouse embryonic fibroblasts (MEFs) using data-independent acquisition mass spectrometry (DIA-MS). Protein identification and relative quantification were accomplished with Scaffold DIA (Proteome Software). The results for comparisons among experimental groups were submitted to Reactome for pathway analysis. DIA-MS analysis of wild-type and PERK-KO MEFs identified greater than 5900 proteins. Cholesterol biosynthesis was the top over-represented pathway for proteins with greater than or equal to 1.5x downregulated relative abundance. Of note were lanosterol 14-alpha demethylase (greater than 4x down) and 24-dehydrocholesterol reductase (DHCR-24)(greater than 2x down). Cholesterol dysregulation has been linked to a variety of diseases, including cardiovascular disease, diabetes, neurodegenerative disorders and cancer. An elevation of the level of cholesterol has previously been shown to correlate with ER stress and activation of the stress

response pathway. Preliminary analysis suggest PERK regulates key enzymes in the cholesterol biosynthesis pathway. Through western blot analysis, we show that PERK-KO MEFs have lower levels of DHCR-24, an enzyme which catalyzes desmosterol to cholesterol in the final step of cholesterol biosynthesis pathway. DHCR-24 is also known as Seladin-1 (Selective Alzheimer's disease indicator 1) and has been implicated in Alzheimer's disease as having a neuroprotective role. Experiments are underway to examine the mechanism by which PERK is influencing the cholesterol biosynthesis pathway and how this impacts pathology in a neurodegenerative disease model. This study suggests an important role for PERK in regulating cholesterol biosynthesis, potentially revealing a mechanistic link to various neurodegenerative diseases. Funded by NIH RF1 AG058778 (JL).

Disclosures: R. Viswanathan: None. **B. Stoveken:** None. **S. Pardo:** None. **D. Molleur:** None. **H. Gudlavalleti:** None. **S. Weintraub:** None. **J. Lechleiter:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.12

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG062166

Title: Transcriptional regulation of ApoE and ABCA1 in the aging brain

Authors: *K. DENNEY[1], Y. LEI[2], X.-Y. LU[2];
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Abstract: The ApoE gene coding for the apolipoprotein E protein is a strong risk factor for late-onset Alzheimer's Disease (AD). Human ApoE exists in three major isoforms (ApoE2, ApoE3 and ApoE4) that confer different risk for AD. While most research has focused on the specific ApoE isoforms, transcriptional regulation of ApoE and its expression levels could also contribute to the risk of developing AD. In contrast to humans, mice express only one form of ApoE that behaves like human ApoE3. The goal of this study was to investigate transcriptional regulation of ApoE during aging in mice. We have demonstrated that expression levels of ApoE in the hippocampus and prefrontal cortex increased with aging and further characterized ApoE protein expression in microglia and neurons at different ages. On-going experiments compare age-related changes in ApoE expression in astrocytes, microglia and neurons. Brain sections from young (6-8 week), middle-age (11-12 month) and aged (20-22 month) C57/BL mice were used for immunostaining and RNAscope assay for ApoE and specific markers for astrocytes, microglia and neurons. The ApoE function depends on its lipidation status, which is controlled by ABCA1, an ATP-binding cassette protein. Therefore, in parallel with ApoE studies,

expression of ABCA1 and its transcriptional regulation are also being investigated in the aging brain. We propose that age-related transcriptional alterations of ApoE and ABCA1 and improper ApoE-ABCA1 interactions lead to abnormal lipid accumulation, contributing to brain aging and underlying AD pathogenesis. This work was supported by NIH Grant AG062166.

Disclosures: K. Denney: None. **Y. Lei:** None. **X. Lu:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 285.13

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH R01AG067228

Title: Investigating long-term, progressive cerebral microvascular alterations in late-onset Alzheimer's disease using APOE4 transgenic mouse model

Authors: *J.-H. LEE, S. STEFAN, K. WALEK, A. KIM, J. LEE;
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Abstract: Apolipoprotein e4 (APOE4) is the strongest genetic risk factor in late-onset Alzheimer's disease (LOAD) with its contributing roles not only for neuropathological changes such as amyloid deposits and formation of neurofibrillary tangles but also for vascular dysfunctions such as blood-brain barrier breakdown, reduced regional cerebral blood flow, and lower glucose metabolism. However, much is still unknown how APOE4 gene affects both structural and functional properties of cerebral vasculature in relation to aging and LOAD. In this study, we investigated long-term, progressive cerebral microvascular alterations *in vivo* using APOE4 transgenic mouse model with imaging modalities such as a label-free spectral-domain optical coherence tomography (OCT), a laser speckle contrast imaging (LSCI) and an intrinsic optical signal imaging (IOS). We acquired OCT angiogram and Doppler OCT datasets to quantify relative changes in diameter and blood flows from all vessel types: pial, penetrating arterioles/venules, capillaries. With IOS/LSCI, we tested functional reactivity using whisker stimulation (2-s duration, air-puff) to detect hemodynamics changes in somatosensory cortex over time. All imaging experiments (OCT, IOS, LSCI) started at 13 weeks of age (WOA) then repeated every 4 weeks until the mice groups (APOE4-TR and APOE3-TR, both male and female, n=9-10 per group) reach 57 WOA. The large datasets from 3D OCT imaging are still under analysis currently, but the statistical analysis for the IOS/LSCI datasets is complete. As a result, the peak of the stimulus-evoked oxygenated hemoglobin (HbO) concentration change showed upward trend over time in APOE4-TR mice with increasing slopes present in both female and male (0.072 and 0.212 μM/month). In contrast, decreasing slopes were found in APOE3-TR mice (female and male, -0.053, -0.519 μM/month). As for group comparisons of these fitted slopes, a statistically significant difference was found only in male mice (p=0.011)

between APOE4-TR and APOE3-TR, suggesting APOE gene may have sex-dependent effect on hemodynamics change in relation to aging. We also quantified the time for reaching these peaks of HbO concentration change and the slopes of APOE4-TR and APOE3-TR female and male mice were -0.136, -0.119, 0.091, and -0.036 second/month, respectively. Interestingly, for time to peak, only female mice groups showed a statistically significant difference ($p=0.002$), supporting the notion of possible sex-dependent effect of APOE gene in hemodynamics change of aging brain. Overall, we hope our longitudinal measurements of vascular metrics could serve as a basis for better understanding of LOAD and aging brain.

Disclosures: **J. Lee:** None. **S. Stefan:** None. **K. Walek:** None. **A. Kim:** None. **J. Lee:** None.

Poster

285. ApoE and Associated Pathways

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Program #/Poster #: 285.14

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH RF1 AG058081
NIH RF1 AG056976
University of Minnesota Office of Academic Clinical Affairs FRD #19.29

Title: Treatment with an HDL-mimetic peptide reverses APOE4-induced lipidomic/metabolomic alterations in the brain of humanized APOE mice

Authors: *A. CHANG[1], W. QUI[1], M. GLITTENBERG[2], D. LI[2], L. LI[1];
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Abstract: APOE4 is the primary genetic risk factor for late onset Alzheimer's disease (AD), the most common cause of dementia worldwide. APOE functions as the main lipid carrier in the brain, with additional roles in neuronal growth and repair, synaptogenesis, immunomodulation, and proteostasis including Aβ clearance/degradation and tau aggregation/toxicity. In both humans and animal models of AD, APOE4 is hypolipidated compared to APOE2 and APOE3, which drives earlier and more abundant Aβ pathology. Recent work in our lab has shown the ability of high-density lipoprotein (HDL) mimetic peptide D-4F to increase ApoE lipidation and secretion and counteract the detrimental effects of Aβ on APOE function *in vitro*. The goal of this study is to investigate the effect of D-4F treatment on brain lipid profiles of humanized APOE3/3 (E3/3) and APOE4/4 (E4/4) knock-in mice. We performed a pilot lipidomic and metabolomic analysis of cortical tissue extracts from middle-aged (12 months) female E3/3 and E4/4 mice that received daily intraperitoneal injections of either D-4F or vehicle (PBS) at 9 months of age ($n = 3$ mice per group), using the MxP® Quant 500 Kit (Biocrates Life Sciences). Snap-frozen cerebral cortical tissue samples from the mice were processed following the manufacturer instructions and analyzed on a triple-quadrupole mass spectrometer (AB Sciex

QTRAP 5500). Our results revealed major APOE4-associated lipid changes in the brain. Compared with E3/3 mice, many lipid levels were decreased in the brain of E4/4 mice. Among the major lipid classes, the decrease in acylcarnitines (ACs) and phosphatidylcholines (PCs) was most prominent. ACs are important transporters of long-chain fatty acids into mitochondria for β-oxidation and play neuroprotective roles. PCs are the most abundant phospholipids and are critical components of cell membranes including mitochondrial membranes. These results are consistent with mitochondrial structure and function deficits associated with APOE4. Remarkably, D-4F treatment increased the levels of those ACs and PCs in the brain of E4/4 mice, demonstrating a reversal in the major lipidomic alterations associated with APOE4. These findings highlight the dysregulation of lipid homeostasis in E4/4 brains, contributing to the pathogenic process of AD, and provide additional evidence that D-4F treatment counteracts the lipid changes associated with increased AD risk. Follow-up studies are underway to elucidate the mechanisms underlying lipid and mitochondrial changes associated with APOE4 to uncover the protective mechanisms of D-4F in E4/4 mice and other AD models.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.15

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimer's Association UH3 NS100121
RF1 NS110048

Title: Characterization of novel anti-ApoE-glycosaminoglycanantibodiesin human brain tissue from a kindred with autosomal dominant Alzheimer's disease

Authors: *P. PEREZ[1,2], C. MARINO[1,2], D. SEPULVEDA-FALLA[4], J. P. MEJÍA-CUPAJITA[5], A. VILLEGAS[5], Y. T. QUIROZ[3], F. LOPERA[5], J. ARBOLEDA-VELASQUEZ[1,2];
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Abstract: In Colombia, there is a population with a rare mutation in the Presenilin 1 (PSEN1) gene that is causative of early onset Alzheimer's disease (AD). Unlike sporadic late-onset AD, people with PSEN1 E280A mutation develop mild cognitive impairment at the mean age of 44. Genetic variants of Apolipoprotein E (APOE) can be either protective or risk factors for sporadic AD. However, the role of APOE in PSEN1 carriers is not clear. In 2019, we discovered an individual among the Colombian *PSEN1* E280A population with a second rare mutation in *APOE* (R136S, APOE3ch), that was associated with her protection against cognitive decline for

almost three decades. This protective mutation is located in the binding site of ApoE with heparan sulfate proteoglycans (HSPGs). Previous research suggests that ApoE3Ch decreases the binding interaction with HSPGs. To investigate the protective role of this mutation, we designed a library of antibodies that target the HSPGs binding region (HsbR) of ApoE. Here, we aimed to test the specificity of these novel antibodies to target ApoE wild type and ApoE3ch R136S in the human temporal cortex from *PSEN1* E280A Colombian population of females, including the *APOEch* carrier. We performed immunohistochemical staining using commercial anti-ApoE antibody (E6D7) as the positive control, along with the antibodies designed against ApoE HsbR wild type: 1H4 and 7C11, and the antibody 19G designed for the ApoE3ch HsbR R136S. We also included in our analysis PSEN1 *E280A* individuals with different *APOE* alleles: *APOE* 2/3, *APOE* 3/4, *APOE* 4/4, *APOE* 3/3ch and *APOE* 3ch/3ch. We captured images at different magnifications and analyzed them by signal densitometry using ImageJ. Using 1H4 antibody, we found high levels of ApoE-like amyloid plaques staining in all *APOE* haplotypes carriers. 7C11 staining showed an intracellular pattern and low staining compared to 1H4, and reduced staining in homozygous *APOE* 3ch/3ch tissue. The localization of the 19G staining was intracellular, and it was reduced in *APOE* 2/3, *APOE* 3/3, *APOE* 4/4 and heterozygous *APOE* 3/3ch carriers, compared to the homozygous *APOE* 3ch/3ch. In this work, we confirmed the specificity for the intracellular localization of both 7C11 and 19G antibodies to target ApoE-HSPG binding site of ApoE wild type and ApoE Christchurch, respectively. Those antibodies can serve as a novel therapeutic tool to mimic the protective effect of the Christchurch mutation.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.16

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NS126467
NS097805

Title: Apolipoprotein E4 impairs cerebrovascular regulation through perivascular macrophages and Nox2-derived radicals

Authors: *A. ANFRAY, Y. HATTORI, J. SEO, N. CASEY, M. M. SANTISTEBAN, S.-J. AHN, G. WANG, J. ANRATHER, L. PARK, C. IADECOLA;
Feil Family Brain and Mind Res. Inst., Weill Cornell Med., New York, NY

Abstract: Apolipoprotein E4 (ApoE4), a leading risk factor for Alzheimer's disease and vascular cognitive impairment, is associated with marked alterations in cerebral blood flow

(CBF) and its regulation. Using ApoE4 targeted replacement mice (ApoE4-TR), which express human ApoE4 under the control of the ApoE mouse promoter, we found that the neurovascular dysfunction is mediated by vascular oxidative stress (Nat Comm. 9:3816, 2018). However, the cellular bases of the deleterious effects of ApoE4 on neurovascular function remain undetermined. Perivascular macrophages (PVM), brain resident myeloid cells located in the perivascular space and closely associated with pial and penetrating vessels, mediate vascular oxidative stress in models of hypertension and amyloid accumulation through reactive oxygen species (ROS) produced by Nox2 (JCI 126:4674, 2016; Circ Res. 121:258, 2017). Therefore, we tested the hypothesis that PVM are responsible for the vascular oxidative stress and cerebrovascular dysfunction induced by ApoE4. CBF was recorded by laser-Doppler flowmetry in the somatosensory cortex of urethane-chloralose anesthetized mice (both sexes; age 3-4 months; n=5/group). We found that in C57Bl/6 mice neocortical superfusion with recombinant ApoE4 (rApoE4; 0.5-10 µg/ml), but not rApoE3, attenuates the increase in CBF produced by whisker stimulation (WS) or by topical application of the endothelium-dependent vasodilator acetylcholine (ACh, 50 µM) (WS, -62±3%; ACh, -48±8%; $p<0.05$), as observed in ApoE4-TR mice. PVM depletion using i.c.v. administration of clodronate completely prevented the neurovascular dysfunction induced by rApoE4 superfusion ($p>0.05$) and ameliorated cerebrovascular responses in ApoE4-TR mice. In brain cells isolated from ApoE4-TR mice, ROS production, assessed by flow cytometry using dihydroethidine as a marker, was increased in PVM (+64±11%; $p<0.05$), but not in microglia ($p>0.05$) or endothelial cells ($p>0.05$). Furthermore, neocortical superfusion of the Nox2 peptide inhibitor gp91ds-tat (1µM), but not its scrambled control peptide, counteracted the neurovascular dysfunction produced by rApoE4 ($p<0.05$). These findings, collectively, indicate that the neurovascular dysfunction induced by ApoE4 is mediated by PVM through Nox2-derived ROS and raise the possibility that targeting Nox2 in PVM may be therapeutic targets to counteract the deleterious neurovascular alterations associated with the ApoE4 genotype.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.17

Topic: C.02. Alzheimer’s Disease and Other Dementias

Title: Freestyle 293-f cells as a system to generate recombinant apolipoprotein E to evaluate effects on neuritogenesis using PC12 cells as a model system

Authors: M. CUNNINGHAM, J. ELSHAAR, R. DURANT, M. MONROE, *K. ADAMS; Biol. Sci., Bridgewater State Univ., Bridgewater, MA

Abstract: The *APOE* gene is the greatest genetic risk factor for Alzheimer's disease (AD) and encodes apolipoprotein E (apoE), which functions as the major lipid transporter between cells of the central nervous system. Three major *APOE* alleles exist in human population—*APOE*ε2, ε3, and ε4. ApoE is produced predominantly by astrocytes in the brain, from which it is secreted as a 299 amino acid protein. The isoforms encoded by the three alleles differ at only two positions: 112 and 158. ApoE3 (the most common isoform) has cysteine and aspartic acid at 112 and 158, respectively, whereas apoE2 has cysteine at both sites and apoE4 has arginine at both. Importantly, individuals who inherit one or two alleles encoding apoE4 have a 5 or 10 times greater risk, respectively, of developing AD compared to individuals with apoE3, whereas inheritance of apoE2 confers a modest protection against AD. However, while the effects of apoE isoforms on AD susceptibility have been known for approximately 30 years, the mechanism(s) through which they modulate susceptibility remains a very active area of research. Many *in vitro* studies have evaluated effects of recombinant apoE on various events related to AD pathogenesis, including amyloid-beta oligomerization, amyloid plaque and tau deposition, neuronal development and integrity, and synaptic density. One issue of consideration in such studies, however, is the source of apoE. While recombinant apoE derived from *E. coli* has been commonly used, differences in its structure, posttranslational modifications, and bioactivity relative to mammalian cell-derived apoE must be considered when interpreting data in the context of AD pathogenesis. With these concerns in mind, we have recently employed and characterized FreeStyle 293-F cells™ (ThermoFisher Scientific) as a system to produce abundant quantities of human cell-derived recombinant apoE for use in *in vitro* experiments. Our data identify transfection parameters—including transfection reagent to plasmid ratio, cell density, and expression duration—that optimized recovery of apoE isoforms. In addition, experiments are underway using the 293-F-derived apoE to evaluate isoform specific effects on neuritogenesis using PC12 cells, which are a well-established model for neuronal development.

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Poster

285. ApoE and Associated Pathways

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Program #/Poster #: 285.18

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH Grant AG062618

Title: Establishing an iPSC-derived platform to study apolipoprotein E genotype in brain cell types and its effect on extracellular vesicle cargo and bioactivity

Authors: *A. VERDUZCO ESPINOZA[1], L. CAMPANATI[1], K. BROWN[1], N. NA[1], K. K. BALDWIN[2], H. CLINE[1];
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Abstract: Intercellular communication mediates glial regulation of neuronal homeostasis and synaptic dynamics. The fact that in early asymptomatic Alzheimer's disease (AD) dysregulation of astrocyte and microglia metabolism accompanies a dysregulation of synaptic function suggests an important role for intercellular communication in the onset and progression of neurodegenerative diseases like AD (Johnson et al., 2020). We are interested in studying the effects of apolipoprotein E ε4 (APOE4), a genetic risk factor for AD, on extracellular vesicles (EVs), an avenue of communication that may be implicated in regulating synaptic function. Using human induced pluripotent stem cells (iPSCs) from healthy 80+ year-old (Wellderly) donors with homozygous APOE3 genotypes and isogenic CRISPR-edited APOE4 lines, we are generating induced neurons (iNs) and microglia (iMGLs) to study cell type specific EV cargos and bioactivity. We generated iNs from iPSCs of both APOE genotypes by forced expression of transcription factor NGN2 and confirmed iN expression of neuronal and synaptic proteins by immunocytochemistry (Zhang et al., 2013). We also generated iMGLs by differentiating iPSCs first into hematopoietic progenitor cells (HPCs) and then into microglia using astrocytic and neuronal factors that mimic the environment of the developing brain (McQuade et al., 2018). By day 28 of differentiation from HPCs, iMGLs of both genotypes express microglia markers like TREM2 and IBA1 by both RT-qPCR and immunocytochemistry. Functional characterization also revealed that more than 95% of iMGLs of both APOE genotypes have phagocytic activity after a 16hr incubation with pre-opsonized fluorescent latex beads. To identify the potential bioactivity of EVs on synapse density, we treated APOE3 iNs with EV-containing iMGL conditioned media (CM). While CM from APOE4 iMGLs had no effect, APOE3 iMGL CM increased the density of post-synaptic Homer-1 puncta, suggesting potential APOE genotype-specific bioactivity of microglia derived EVs on neuronal synapses. Furthermore, we have isolated and characterized EVs from iMGL conditioned media, confirming the size and distinct cup-like morphology of isolated exosomes by transmission electron microscopy, as well as the expression of exosome markers like Alix and Flotillin-1 by western blot. In the future, we will assess isolated EV bioactivity on synaptic dynamics. This work will serve as a platform on which to investigate how APOE genotype alters cell-type specific EV cargo and bioactivity.

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Poster

285. ApoE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Study on Aβ phagocytosis function of ApoE4 genotype blood monocytes

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[1]Dept. of Neurol. and Ctr. for Clin. Neurosci., Daping Hosp., Chongqing, China; [2]Key Lab. of Aging and Brain Dis., Chong Qing, China; [3]Inst. of Brain and Intelligence, [4]State Key Lab. of Trauma, Burns and Combined Injury, Third Military Med. Univ., Chongqing, China; [5]Ctr. for Excellence in Brain Sci. and Intelligence Technol., Chinese Acad. of Sci., Shanghai, China

Abstract: ApoE4 is the strongest genetic risk factor for sporadic Alzheimer's disease (AD). Previous studies have shown that innate immune dysfunction induced by ApoE4 plays an important role in brain Aβ deposition and AD. GWAS studies showed that mutations in genes related to phagocytosis function of monocytes significantly increased the risk of AD. We previously found that decreased Aβ uptake ability of blood monocytes in AD patients was involved in the occurrence and development of AD, but the mechanism was unclear. This study aimed to analyze the correlation between ApoE4 genotype and Aβ phagocytosis function of monocyte, which may give a new insight on the mechanism of monocyte Aβ uptake dysfunction in AD. In this study, blood monocytes of ApoE4 gene carriers (N=30) and non-carriers (N=32) from cognitively normal population, and blood monocytes of ApoE4/4 transgenic mice and ApoE3/3 transgenic mice were separated by Ficoll density gradient centrifugation. The uptake amount of $A\beta_{42}$ was detected by flow cytometry after co-culture of monocytes with $A\beta_{42}$-FITC.Compared with none-ApoE4 gene carriers,$A\beta_{42}$ uptake by total monocytes ($CD14^{dim}$&$CD14^{+}$) (p=0.0117), classical monocytes ($CD14^{+}CD16^{-}$)(p=0.0083), intermediate monocytes ($CD14^{+}CD16^{+}$) (p=0.0457) and non-classical monocytes ($CD14^{dim}CD16^{+}$) (p=0.0230) were significantly decreased in ApoE4 gene carriers. Univariate analysis showed that the $A\beta_{42}$ phagocytosis function of three subtypes of monocytes is that $CD14^{+}CD16^{+}$> $CD14^{dim}CD16^{+}$ > $CD14^{+}CD16^{-}$ ($P < 0.0001$). In ApoE4/4 transgenic mice, the $A\beta_{42}$ uptake ability of monocytes ($CD115^{+}CD11b^{+}$) was significantly decreased compared with that in ApoE3/3 transgenic mice (P=0.0234).Taken together, this study identified Aβ uptake ability of monocytes in cognitively normal subjects and mice carrying ApoE4 gene is decreased than that without ApoE4 gene, which indicates that ApoE4 gene may be an important cause of monocyte Aβ phagocytosis dysfunction. We speculate that Aβ uptake dysfunction of ApoE4 genotype monocyte may lead to excessive Aβ deposition in the brain and participate in the occurrence and development of AD, which needs to be further investigated in the future.

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Poster

285. ApoE and Associated Pathways

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Support: NIH grant AG061776
Cure Alzheimer's Fund

Title: *APOE-ε4* but not *APOE-ε3* genotype synergizes with chronic sleep disruption to accelerate Aβ plaque deposition and Aβ associated tau seeding and spreading in APPPS1-21 mice

Authors: *C. WANG, A. NAMBIAR, C. LEE, S. PARHIZKAR, M. R. STRICKLAND, C. E. WALLACE, A. C. MOORE, J. R. CIRRITO, D. M. HOLTZMAN;
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Abstract: Alzheimer's Disease (AD) is the most common cause of dementia and a major public health problem. Currently, around 47 million people worldwide are living with dementia. Thus, identifying modifiable risk factors for AD is critical. The apolipoprotein E *(APOE)* gene is the strongest genetic risk factor for late-onset AD. *APOE*-ε4 genotype is strongly associated with increased AD risk and amyloid-β (Aβ) plaque accumulation in humans, as compared to the other common *APOE* genotypes such as ε3. In relation to sleep, recent human studies suggest that *APOE* genotype modulates the effect of sleep disruption on AD risk, suggesting a possible link between apoE and sleep in AD pathogenesis which is relatively unexplored. To test our hypothesis that *APOE* genotype modifies Aβ deposition and formation of Aβ plaque-associated tau seeding and spreading in the form of neuritic plaque (NP)-tau pathology in response to chronic sleep deprivation (SD), we used APPPS1-21 mice with targeted replacement of the murine Apoe locus with human *APOE*-ε3, or -ε4. At four months of age, experimental mice were aseptically and unilaterally injected with tau-enriched human AD brain lysates in the dentate gyrus and overlying cortex and were placed in automated SD chambers set at a 30 sec interval for 6 hours per day (8 am to 2 pm) for 8 weeks or in normal cages with normal sleep (NS) conditions. After treating mice with chronic SD or NS, we quantified total and fibrillar Aβ plaques and NP-tau pathology. Interestingly, chronic SD in *APPPS1-21* mice significantly increased Aβ deposition in the presence of apoE4 but not apoE3. Furthermore, *APOE-ε4* genotype synergized with chronic SD and Aβ to drive tau seeding and spreading in the form of NP-tau pathology. To have a better understanding why *APPPS1-21:ε4/4* mice are more significantly affected by SD, we are currently using several approaches, including assessing whether SD influences interstitial fluid (ISF) Aβ clearance and by Aquaporin 4 (*AQP4*), a major driver of the glymphatic clearance system, in an isoform-dependent fashion.

Disclosures: C. Wang: None. **A. Nambiar:** None. **C. Lee:** None. **S. Parhizkar:** None. **M.R. Strickland:** None. **C.E. Wallace:** None. **A.C. Moore:** None. **J.R. Cirrito:** None. **D.M. Holtzman:** 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.; Holtzman lab receives research grants from the National Institutes of Health, Cure Alzheimer's Fund, Tau Consortium, the JPB Foundation, Good Ventures, the Ludwig Foundation, C2N Diagnostics, Novartis, NextCure, and Ionis. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Co-founder, C2N Diagnostics LLC. F. Consulting Fees (e.g., advisory boards); Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Alector.. Other; a patent licensed by Washington University to C2N Diagnostics on the therapeutic use of anti-tau antibodies. This anti-tau antibody program has now been licensed to Abbvie;, 2) a patent licensed by Washington University to Eli Lilly on a humanized anti-Aβ antibody.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.21

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: ADRC P30 AG066507

Title: Infection of SARS-COV-2 in 3D human-induced pluripotent stem cell brain assembloids model of Alzheimer's Disease with APOE variants

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Abstract: The long-term effects of SARS-COV-2 (SCV2) infection are not understood. SCV2 infection has demonstrated increased severity in those with apolipoprotein-E (APOE) variants, a genetic risk factor for Alzheimer's Disease (AD). Following SCV2 infection, there have been reported cognitive and neurological implications in individuals. SCV2 has been reported to cause AD-like pathology in post- mortem brain via dysregulation of the calcium signaling pathway. However, the potential effects of SCV2 on advancing AD pathology requires further understanding. To investigate the synergistic effects of genetic predisposition to AD and SCV-2 infection, human induced pluripotent stem cell (hiPSC) assembloids derived from APOE-ε alleles, including APOE-ε4/4, APOE-ε4/3 and APOE-ε3/3 cell lines were developed. The assembloids created were novel in the types of brain cells and connections formed consisting of excitatory and inhibitory cortical neurons, astrocytes, oligodendrocytes, microglia, vascular endothelial cells and pericytes. The hiPSCs assembloids developed provide an important development in investigating the implications of infection on the brain and will be an insightful avenue of research regarding the SCV2 infection on neuronal function. In our study, we used these models to understand the mechanism of SCV2 infection on the brain. First, hiPSCs were infected with SCV2 after 3 months of culture. Then, samples were analyzed post infection using biochemical and molecular techniques to measure AD markers including amyloid-beta (Aβ) and tau in insoluble and soluble fractions, and markers of SCV2 infection including SCV2 nucleocapsid and spike protein. In our results, we found that post-infection of SCV2, there was a significant increase in Aβ and tau proteins in the insoluble and soluble fractionations of the APOE variants, specifically in the APOE-ε4/4 and APOE-ε4/3 insoluble fractionations. Overall, our data shows an increase in AD biomarkers in an isoform dependent manner following SCV2 infection. Further research will investigate downstream markers of the calcium signaling pathway as a possible mechanism of synergistic interaction in progressing AD pathology. Understanding the biochemical mechanisms that may be implicated by SCV2 infection in the

brain, specifically for those predisposed to AD via APOE variants, can provide insight into AD pathology of the brain and avenues of treatment options for those with SCV2.

Disclosures: **J. Condoleo:** None. **H. Wang:** None. **C.K. Bullen:** None. **X. Rong:** None. **H. Fang:** None. **S.S. Karuppagounder:** None. **Y. Gao:** None. **T.M. Dawson:** None. **V.L. Dawson:** None. **J. Xu:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.22

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH grant NS090934
Cure Alzheimer's Fund
JPB Foundation (DMH)
Washington University Center for Cellular Imaging (WUCCI) supported by Washington University School of Medicine

Title: Characterization of Apolipoprotein E3 Christchurch mutation - a potential protective variant for autosomal dominant Alzheimer's disease

Authors: *Y. CHEN[1,2], J. LORD[3], S. PARHIZKAR[2], M. R. STRICKLAND[2], R. KOSSINA[4], J. FITZPATRICK[4], J. ULRICH[2], M. COLONNA[1], D. M. HOLTZMAN[2];
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Abstract: Autosomal-dominant Alzheimer's disease (ADAD) is caused by mutations in one of three genes - amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*) or presenilin 2 (*PSEN2*) - or duplication of APP. However, a recent case of a homozygous mutation inn ApoE3 - R136S - named as ApoE3 Christchurch mutation was found in a PSEN1 mutation carrier. The cognitive impairment in this patient was extremely delayed. The patient still developed severe amyloid pathology due to the PSEN1 mutation; however, intriguingly that her tau PET scan showed minimal tauopathy with an atypical pattern. The precuneus/posterior cingulate region which is preferentially affected by Alzheimer's disease (AD) pathology was also preserved in this case according to the cerebral metabolic rate for glucose (CMRgl) images. Preliminary analyses in this report suggested that ApoE3ch partially loses the binding ability to heparin sulfate and reduces the accelerating effect of ApoE3 on Aβ oligomerization and fibrillization. To fully understand the mechanism of this putatively protective variant (ApoE3ch) in ADAD, we have generated a novel human APOE knockin (KI) mouse with the human ApoE3ch (R136S) present in the homozygous state and characterized the physiological changes caused by this single mutation comparing with ApoE3 KImice. We have found a significant increase of body weight

specifically in males at both young (3month) and aged (9.5month) mice and a slight increase in aged females. Consistent with the case report, we observed an increase of total cholesterol (CL), VLDL, and LDL particles in plasma both in male and female mice. By size-exclusion chromatography (SEC) and paired WB, we showed that the increased level of VLDL and LDL particles is associated with ApoB and ApoE in the mouse plasma. In CSF, the total CL and ApoE level was not different between the ApoE3ch vs. ApoE3 in young mice but accumulated in the aged mice measured by ELISA. SEC separation of pooled CSF samples indicated a unique size distribution of ApoEch lipoprotein particles in female specifically. The WB data of different lipoprotein-associated receptors indicated a change in the lipid environment in the brain. Experiments with AD-Tau injections into the ApoE3ch vs ApoE3 mice are ongoing to investigate the spreading of tau pathology. Our conclusion is that the presence of the ApoE3ch mutation in the homozygous state leads to a hyperlipidemia-like phenotype in the peripheral system and alters cholesterol and lipid metabolism in the in the CNS with sex differences. These changes may ultimately impact AD pathology. In ongoing experiments the ApoE3ch mice are being crossed to amyloid and tauopathy mouse models to fully assessing the mutation.

Disclosures: Y. Chen: None. **J. Lord:** None. **S. Parhizkar:** None. **M.R. Strickland:** None. **R. Kossina:** None. **J. Fitzpatrick:** None. **J. Ulrich:** None. **M. Colonna:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH, Alector, Amgen, Ono, Pfizer. F. Consulting Fees (e.g., advisory boards); Vigil, NGMBio, Cell Signaling Technologies. **D.M. Holtzman:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; NIH grant NS090934, Cure Alzheimer's Fund, JPB Foundation (DMH), Washington University Center for Cellular Imaging (WUCCI) supported by Washington University School of Medicine.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C2N Diagnostics LLC.. F. Consulting Fees (e.g., advisory boards); Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Alector.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.23

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Bone morphogenic protein (BMP) and Apolipoprotein E (APOE) interaction in Alzheimer's patient IPSC-derived astrocytes.

Authors: *A. K. LINDEN, A. AFFANEH, C.-Y. PENG, J. A. KESSLER;
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Abstract: Individual risk for Alzheimer's disease (AD) increases dramatically with the presence of an apolipoprotein E (APOE) 4 allele. Within the brain, astrocytes express the majority of APOE, a protein that traffics lipid and cholesterol species intercellularly and helps clear debris such as amyloid beta from the extracellular milieu. In addition to genetic risk factors, aging is the strongest risk factor for AD, implicating biochemical changes that occur throughout the aging process that enhance predisposition to pathophysiological cascades. While bone morphogenic protein (BMP) signaling is typically implicated in astrogliogenesis, it has been shown to increase in both mouse and human brains over the course of aging. BMP4 protein has been shown to interact with APOE in a variety of organs throughout the body; however, it remains unknown if enhanced BMP4 signaling in the brain changes astrocyte physiology in an APOE-dependent manner. Using three Alzheimer's patient-derived IPSC's with APOE E4/E3 genotypes as well as CRISPR/Cas9-corrected APOE E3/E3 isogenic controls, we investigated APOE and BMP4 interactions in induced astrocytes (iA). iA cultures were functionally characterized using glutamate uptake and verified to express a variety of mature astrocyte markers. Baseline cytokine release and lineage commitment did not change based on APOE genotype. Upon BMP4 treatment, mature APOE4 iA expressed significantly higher levels of APOE protein, suggesting that downstream signaling cascades of BMP4 influence APOE expression in an isoform-dependent manner. Using this paradigm, BMP4 signaling was activated for 72 hours followed by single-cell RNA sequencing of BMP4 treated or vehicle-treated astrocytes. The transcriptomics revealed changes in a variety of biological processes in an APOE-dependent manner upon BMP4 activation.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.24

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Research Grant Council GRF16124916
Food and Health Bureau HMRF05163736
PolyU Start-up Fund P0030307

Title: Apolipoprotein E4 directly disrupts lipid profile and myelination in the aging mouse brain

Authors: *G. W. Y. CHENG[1], J. HUANG[2], M. H. Y. YEUNG[1], Z. CHEN[2], K. K. S. MOK[1], S. H. S. YEUNG[1], K. HERRUP[3], H. K. F. MAK[4], K. W. Y. CHAN[2], K.-H. TSE[1];
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Abstract: Apolipoprotein E allele ε4 (*APOE4*) is the strongest genetic risk factor for sporadic Alzheimer's disease (sAD). White matter abnormality is common in *APOE4* carriers, and such MRI-identified pathology is likely mediated by the defective functions of APOE4 in lipid transport. Recently, we reported an amyloid-independent oligodendrocyte (OL) loss in the postmortem sAD brain tissues carrying *APOE4* without major changes in myelin protein expression. Here, we hypothesize that *APOE4* compromises myelin integrity *per se* by disrupting the lipid metabolism in myelinated regions of the aging brain. To investigate, *Apoe*-knockout (*Apoe*$^{-/-}$, n = 22) and humanized *APOE* knock-in mice (hAPOE3, n = 24, and hAPOE4, n = 30) were examined at 6, 10 and 16 months old by a battery of phenotype test (metabolic cage) and neuroimaging (3T-magnetic resonance (MR) imaging with diffusion kurtosis (DKI) & MR spectroscopy (MRS)), followed by postmortem histological and lipidomic analysis. All strains employed were independent of amyloid-related transgene. First, the aging hAPOE4 colony exhibited a significantly higher weight among all strains starting from 10 months old (mean = 39.8 g, $P < 0.0001$) which returned to baseline at 16 months. Such increased weight in hAPOE4 coincided with a significantly lower RER (respiratory exchange ration; V_{CO2}/V_{O2}) and metabolic rate (energy expenditure, Kcal) at 10 months versus hAPOE3. This phenotypic data confirmed that hAPOE4 exerts a robust effect on metabolism. The potential change in the lipid profile of the brain was further investigated by neuroimaging. DKI and MRS analysis of a voxel (3x3x3 mm^3) encompassing the neocortex, external capsule, and hippocampus (Bregma -1.35 to -3.08) revealed an opposing trend of mean diffusivity and fractional anisotropy between hAPOE3 and hAPOE4 in aging. Intriguingly, MRS detected a significant increase in Lipid/Creatine ratio (at 1.3 ppm, $P = 0.044$) at this voxel in the aging hAPOE4 animals without any effects on neurotransmitters. The lipid elevation was confirmed by histology data where a significantly elevated lipophilic dye signal (FluoroMyelin) was found in the hAPOE4 white matter. This radio-pathological correlation is suggestive of potential myelin breakdown or turnover in the region. To pin down the chemical pathology underlying the myelin breakdown, a digital pathology-based analysis of the OL lineage coupled with MALDI-based mass spectroscopy on brain tissues is being conducted to identify the lipidomic changes in the myelinated regions. Together, *APOE4* may lead to premature myelin breakdown by directly disrupting lipid metabolism in the aging brain.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.25

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: Alzheimersfonden (AF-968408)
Swedish Research Council grant (2019-01125)

Title: The effect of ApoE isoforms on the endolysosomal system and lipid metabolism in primary neurons

Authors: *E. NYBERG, S. KONINGS, O. KLEMENTIEVA, I. K. MARTINSSON, G. K. GOURAS;
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Abstract: Most Alzheimer disease (AD) cases are classified as late onset, a multifactorial disorder influenced by different genes, environmental factors and lifestyle. APOE genotype is known as the most important genetic risk factor for developing AD. Carrying one or two copies of APOE4 increases one's risk of developing AD by 3-12 times. Understanding the cellular differences between the ApoE isoforms will be crucial for understanding the fundamental pathways that are disrupted in AD and potential pathways that can be therapeutically targeted. Accumulating evidence is suggesting that ApoE isoforms influence the endosomal system, one of the earliest sites affected in AD and its capacity seems to be reduced with ageing. However, our understanding how the isoforms affect the endolysosomal pathway in neurons is still limited. To study cellular changes in neurons we are using mixed primary neuronal cultures (5-10% astrocytes), harvested from ApoE KO mice and targeted replacement mice, expressing human ApoE3 or ApoE4. Neurons in culture have accelerated maturation and with time in culture gradually show signs of age-like stress. We have started to study two different time points in culture, 18 days in vitro (DIV18) and 25 days in vitro (DIV25), to explore whether isoform differences occur early on or develop as the cellular stress increases. To explore the endolysosomal system we are analysing protein levels of relevant markers as well as immunofluorescence to gain deeper understanding of potential differences between ApoE isoforms. Our preliminary data suggests that endo-lysosomal markers, such as LAMP1, are changed in ApoE4 neurons. When studying the trafficking of fluorescently tagged EGF (which has an established endolysosomal route), preliminary results are pointing toward potential differences between both DIV18 and DIV25 but also between ApoE isoforms, suggesting that both time in culture and ApoE isoforms influence the endolysosomal pathway. Due to the key role of ApoE as a lipid carrier, we are further interested in the ApoE isoforms effect on lipid homeostasis in neurons. By staining neutral lipids, we see that primary neurons contain lipid inclusions along their processes, and preliminary data indicate differences between ApoE isoforms. We are further interested in whether the endosomal system and lipid droplet homeostasis are connected to each other. Understanding the effect of ApoE-isoforms on these two systems will aid us in providing potential new insights into therapy for AD.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.26

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIA Grant R15AG055043

Title: Differential susceptibilities of Apolipoprotein E isoforms to proteolytic degradation by brain enzymes

Authors: M. M. KEMEH, K. HAYASHI, *N. LAZO;
Clark Univ., Worcester, MA

Abstract: Apolipoprotein E is primarily expressed in three isoforms: ApoE2, ApoE3 and ApoE4. ApoE2 is protective against Alzheimer's disease (AD), ApoE3 is neutral, and ApoE4 is the most influential genetic risk factor for late-onset AD. The susceptibility of these isoforms to proteolytic degradation in the absence and presence of lipid assemblies is not well understood. Here, we performed limited proteolysis (LP) of ApoE by plasmin and human neutrophil elastase (HNE). To monitor LP, we used circular dichroism spectroscopy to detect structural changes in APOE, and high resolution mass spectrometry to identify cleavage sites and degradation products. Our results show that ApoE4 is the most susceptible isoform to HNE-dependent degradation. ApoE3 was not proteolyzed by plasmin, but ApoE2 and ApoE4 were degraded. The implications of these results on the biochemical and biophysical properties of ApoE are discussed.

Disclosures: M.M. Kemeh: None. **K. Hayashi:** None. **N. Lazo:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.27

Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Role of age-related bone morphogenic protein signaling and APOE genotype in Alzheimer's Disease

Authors: *A. AFFANEH, A. K. LINDEN, J. A. KESSLER;
Neurol., Northwestern Univ., Chicago, IL

Abstract: The apolipoprotein E (*APOE*) E4 isoform is the strongest reported genetic risk factor for sporadic Alzheimer's disease (AD), though the mechanism of the effects has not been well defined. *APOE E4* genotype is correlated with earlier onset of AD as well as increased progression of neurotoxic proteins such as phosphorylated tau (p-tau) spread. Along with genetic risks, it is clear that age-related changes play a role in the onset of disease. Aging is correlated with biochemical and physical changes in brain, such as an increase in born morphogenic protein 4 (BMP4). APOE-BMP regulation has been shown in many organs outside the central nervous system. Therefore, we sought to investigate the relationship between *APOE* genotype and tau

phosphorylation upon activation of BMP signaling using induced pluripotent cell derived neurons from AD patients. We differentiated *APOE* E4/E3 and CRISPR/Cas9 corrected isogenic *APOE* E3/E3 iPSCs into excitatory forebrain neurons and activated or inhibited BMP signaling. We found that activation of BMP signaling increased the intracellular levels of apoE and p-tau/tau ratio, with exacerbated effects in *APOE*4 neurons. Specifically, through non-canonical BMP signaling, activated kinase activity is shown to influence *APOE*4 mediated effects. Future experiments will investigate specific mechanisms in which age-related changes in BMP signaling lead to increased neurotoxic protein accumulation in an *APOE E*4 independent manner.

Disclosures: A. Affaneh: None. **A.K. Linden:** None. **J.A. Kessler:** None.

Poster

285. ApoE and Associated Pathways

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Topic: C.02. Alzheimer's Disease and Other Dementias

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Title: Apolipoprotein E expression and secretion are modulated by copper-dependent and -independent mitochondrial dysfunction

Authors: *M. WYNNE[1], O. OGUNBONA[1], A. R. LANE[1], A. GOKHALE[1], S. A. ZLATIC[1], C. XU[1], Z. WEN[1], D. DUONG[1], S. RAYAPROLU[1], A. IVANOVA[1], E. A. ORTLUND[1], N. T. SEYFRIED[1], A. CROCKER[2], V. SHANBHAG[3], M. PETRIS[3], N. SENOO[4], S. KANDASAMY[4], S. M. CLAYPOOL[4], A. P. WINGO[1], T. S. WINGO[1], S. RANGARAJU[1], A. I. LEVEY[1], E. WERNER[1], V. FAUNDEZ[1];
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Abstract: Mitochondria are dynamic organelles that influence cellular function through both cell-autonomous and non-cell autonomous mechanisms, such as production of paracrine and endocrine factors. Here, we demonstrate that mitochondrial regulation of the secretome is more extensive than previously appreciated, as both genetic and pharmacological disruption of the inner mitochondrial membrane caused upregulation of the Alzheimers disease risk factor apolipoprotein E (APOE) and other secretome components. This upregulation of secretory proteins was of a similar extent as modifications to the mitochondrial annotated proteome. Gene

editing of SLC25A family inner mitochondrial membrane transporters, as well as genetic and pharmacological disruption of copper-dependent and independent steps of electron transport chain assembly and function, caused upregulation of APOE transcript, protein, and secretion, up to 16-fold. These APOE phenotypes were robustly expressed in diverse cell types and iPSC-derived human astrocytes as part of an inflammatory gene expression program. Moreover, in the 5xFAD mouse model of Alzheimer's disease, age-dependent increased expression of APOE correlated with low expression of respiratory chain complexes in brain. We propose that mitochondria act as novel upstream regulators of APOE-dependent cellular processes in health and disease.

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Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 285.29

Topic: C.02. Alzheimer’s Disease and Other Dementias

Support: NIH Grant R01 AG047644
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Title: Aav delivery of apoe2 can counteract some effects of apoe4 expression in amyloid deposition and neuroinflammation in mouse model of amyloidosis

Authors: *R. J. JACKSON[1], M. S. KEISER[2], J. C. MELTZER[1], D. P. FYKSTRA[1], L. TECEDOR[2], P. RANUM[2], E. CARRELL[2], Y. CHEN[2], D. M. HOLTZMAN[3], B. L. DAVIDSON[2], B. T. HYMAN[1];
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Abstract: Dementia and more specifically Alzheimer’s Disease (AD) currently pose a global health crisis with more than 6 million cases of AD in the US alone. Inheritance of the ε4 allele of apolipoprotein E (ApoE) is the strongest genetic risk factor associated with the sporadic form of AD, whereas the less common ApoE ε2 allele has the opposite effect. However, the mechanisms whereby ApoE confers risk and protection remain uncertain. ApoE4 genotype has been associated with worsening plaque deposition, increased speed of cognitive decline as well as

increased neuroinflammatory phenotype in microglia. We had previously shown that the introduction of ApoE4 or ApoE2 into the ependymal lining via AAV in amyloid plaque-bearing transgenic mice (expressing wild type murine apoE) exacerbated or reduced amyloid plaque burden respectively (Hudry 2013 Sci Trans Med). Here we used a similar AAV based gene transfer approach to introduce ApoE2 into a plaque-bearing mouse model that expresses human ApoE4 in place of mouse *apoe* (APP/PS1/ApoE4) to investigate if the positive effects of ApoE2 are sufficient to counteract the negative effects of ApoE4, and to test whether the effects of ApoE4 on neuroinflammatory phenotype of microglia was cell autonomous or cell nonautonomous.
We found that the introduction of ApoE2 via ependymal expression in this APP/PS1/ApoE4 mouse model reduced the size and density of cortical amyloid plaques and also reduced the concentration of oligomeric Aβ in the brains of these animals.
In addition, we observed that the introduction of ApoE2 in this APP/PS1/ApoE4 mouse model dramatically reduces the activation of microglia near plaques in the cortex. This indicates that ApoE4 might prime the microglia towards an inflammatory phenotype and that exogenous ApoE2 is able to interrupt or reduce this aberrant activation. These results suggest that viral delivery of secreted ApoE2 into the ependyma of ApoE4 carriers might be an effective therapeutic strategy to impact both the classical lesions of AD (e.g. plaque deposition) and the increased neuroinflammatory profile observed in sporadic AD.

Disclosures: R.J. Jackson: None. **M.S. Keiser:** None. **J.C. Meltzer:** None. **D.P. Fykstra:** None. **L. Tecedor:** None. **P. Ranum:** None. **E. Carrell:** None. **Y. Chen:** None. **D.M. Holtzman:** None. **B.L. Davidson:** None. **B.T. Hyman:** None.

Poster

285. ApoE and Associated Pathways

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 285.30

Topic: C.02. Alzheimer's Disease and Other Dementias

Support: NIH/NIA: P01AG062817

Title: Apoe4 disrupts insulin signaling and glucose metabolism in n2a cells and the ketogenic diet mediator beta hydroxybutyrate rescues apoe4 survival

Authors: *C. GARCIA, A. TOMILOV, J. SANDOVAL, G. CORTOPASSI;
Dep. of Mol. Biosci., UC Davis, Davis, CA

Abstract: Background. *APOE4* is the single biggest genetic risk factor for sporadic Alzheimer's Disease (**AD**). *APOE4* inheritance confers deficits in cerebral glucose metabolism and insulin sensitivity that are present years before AD signs and symptoms. It was recently shown that ApoE4 binds the insulin receptor (**IR**) and impairs downstream signaling. Of many theories for ***how*** ApoE4 triggers AD pathophysiology, the ApoE4 Neuronal Insulin Resistance

(**NIR**) hypothesis posits that ApoE4 drives AD pathophysiology through disrupting neuronal insulin signaling and glucose metabolism, and the experiments described below test this hypothesis.
Objective. 1) How do *APOE* isoforms impact insulin signaling and metabolism in neuronal N2a neuroblastoma cells? 2) Can an alternate fuel source, beta hydroxybutyrate (**BHB**), positively impact neuronal survival in the metabolically-stressed *APOE4* context?
Methods. ApoE-IR binding kinetics were measured by co-immunoprecipitation (**co-IP**) of transiently-transfected HEK cells and biolayer interferometry (**BLI**) of purified IR and ApoE protein. N2a cells were stably transfected with GFP (no APOE control), -E3 (wild-type), or -E4 (risk allele) vectors. Cells were dosed with 0.2nM-400nM insulin and p-Akt levels were measured. Mitochondrial metabolism was measured by Agilent Seahorse. Cell survival was assayed by CellTitre-Glo. All experiments were done $\geq$ biological triplicate. ANOVAs with multiple-comparisons corrections and student's T-tests tested significance.
Results. 1) Both ApoE3 and -E4 isoforms bind the insulin receptor via co-IP and BLI in ~200nM range, but there is no significant difference in their affinities. 2) Both ApoEs increase the rate and maximal insulin-dependent p-Akt response above no-ApoE. Also, ApoE3 has a higher basal insulin response, higher slope and higher maximal insulin response than ApoE4: E3>E4>no-ApoE. 3) In the same cells, we observe mitochondrial glucose oxidation is APOE-dependent: E3>E4>no-ApoE. Result 2 and 3 suggest that ApoE3's significantly higher glucose oxidation rate is the result of its higher insulin response. 4) We also find that the Ketogenic substrate BHB improves neuronal survival in metabolically-stressed *APOE4* cells.
Conclusions. These data support the hypothesis that *APOE4* drives NIR and could disrupt downstream glucose oxidation and contribute to ApoE4-dependent neuropathophysiology. These results could help explain the cerebral hypometabolism of glucose in human ApoE4 carriers that have been known for many years. The ability of BHB to rescue survival in *APOE4* neurons may suggest Ketogenic strategies *in vivo* to ameliorate ApoE4-dependent pathophysiology.

Disclosures: C. Garcia: None. **A. Tomilov:** None. **J. Sandoval:** None. **G. Cortopassi:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: JSPS KAKENHI 20K07958

Title: Inhibitory effect of preconditioning with lipopolysaccharide on the onset of anxiety and depressive-like behaviors

Authors: *M. KOGA[1], H. TODA[1], R. NAKAGAWA[1], M. NAGAMINE[5], M. KINOSHITA[2], F. ASAI[1], M. SATO[3], Y. MITSUI[4], A. YOSHINO[1];
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Col., Tokorozawa, Japan; [5]Div. of Behavioral Sci., Natl. Def. Med. Col. Res. Inst., Tokorozawa, Japan

Abstract: In peripheral organs such as the spleen and liver, exposure to a low dose of endotoxin causes a suppressing cytokine production in subsequent endotoxin exposure. This phenomenon is called endotoxin tolerance, a negative regulatory mechanism against acute inflammation that occurs in the body as an innate immune response. Recently, a number of studies have supported that abnormal inflammation is involved in the pathogenesis of various diseases. Therefore, the inhibitory effects of endotoxin tolerance inflammation are suggested to be beneficial for treatment and prevention. In the brain, abnormal inflammation has also been implicated in the pathogenesis of psychiatric symptoms. Still, to date, there are no reports of the application of endotoxin tolerance in the brain to inhibit onset of psychiatric symptoms. In the present study, we investigated whether inducing endotoxin tolerance can suppress an onset of psychiatric impairments, such as lipopolysaccharide (LPS)-induced social deficits, depression-like behavior induced by water immersion restraint stress, and anxiety-like behavior caused by social defeat stress. We also assessed the biochemical changes in the brain induced by LPS preconditioning, focusing on the condition of microglia, cells responsible for brain immunity. LPS preconditioning suppressed the occurrence of behavioral abnormalities in mice subjected to LPS administration, water immersion restraint stress, and social defeat stress. Biochemical analysis indicated that the LPS administration and social defeat stress increased microglial activation in the stress-subjected mice but not in the mice with LPS preconditioning. These findings suggest that induction of endotoxin tolerance effectively suppresses the onset of psychiatric symptoms derived from inflammation in the brain. In addition, since inhibition of microglial responses is involved in the mechanism of endotoxin tolerance in the brain, microglia may be a valuable point of action for preventing the onset and relapse of psychiatric disorders.

Disclosures: **M. Koga:** None. **H. Toda:** None. **R. Nakagawa:** None. **M. Nagamine:** None. **M. Kinoshita:** None. **F. Asai:** None. **M. Sato:** None. **Y. Mitsui:** None. **A. Yoshino:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: European Union Grant 305340
Swedish Research Council Grant 20150338
NIH Grant P20GM113123
NIH Grant U54GM128729
NIH/NIGMS Grant P20GM103442

Title: Sex differences in the skin-brain axis in an hla class II mouse model of group a streptococcal skin infections

Authors: A. SATPATI, S. MUKUNDAN, B. GROVE, C. K. COMBS, ***S. NOOKALA**;
Univ. of North Dakota Sch. of Med., Univ. of North Dakota Sch. of Med., Grand Forks, ND

Abstract: Infections caused by *Streptococcus pyogenes*, commonly known as beta-hemolytic group A Streptococci (GAS), are heterogeneous, ranging from pharyngitis to life-threatening invasive Necrotizing Fasciitis (NF), a subset of necrotizing soft tissue infections, and toxic shock. GAS superantigens directly crosslink HLA-class II (HLA-II) on antigen presenting cells and the variable beta chain of the TCR on T cells resulting in simultaneous activation of both cell types and potent stimulation of inflammatory mediators instigating a "cytokine storm". Based upon the growing recognition of complex immune cell environments in the brain, we predicted that GAS NF communicates changes directly to the brain through an influx of inflammatory mediators. More importantly, we hypothesized that the brain consequences of GAS NF are HLA-II dependent. To examine changes in the skin-brain axis during skin GAS NF, HLA-II DR3, DR4, or DQ8 expressing transgenic mice were randomly divided into 3 groups: uninfected controls, GAS infected, and GAS infected with resolution by CLN treatment (10mg/kg/5 days) and monitored for 15 days post-infection. DQ8 mice showed a significantly higher GAS burden in the skin, adipose tissue, and cortical and hippocampal brain regions. Sex differences in GAS burden in DQ8 mice were apparent with increased cortical GAS burden in the males that was significantly reduced with CLN. Immunostaining showed the internalization of GAS in neurons. Surprisingly, GAS infection-mediated cortical cytokine changes were modest, except for IL-22, whose levels were significantly higher in CLN treated females. Western blot analysis of cortical and hippocampal homogenates showed no significant differences in GFAP levels among the male and female groups. However, significantly higher cortical Iba-1 levels were observed in both GAS infected, and CLN treated male mice. Our clinically relevant HLA-II GAS NF mouse model demonstrates that GAS infection communicates to the brain in an HLA-II and sex-dependent fashion despite antibiotic intervention and is characterized by microgliosis and intraneuronal sequestration, perhaps serving as a latent reservoir for future reactivation in susceptible individuals.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.03

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P20 GM103427
NIH Grant GM139762

Title: Transcriptional regulation of IRF7 mediated by a novel long non-coding RNA in microglia

Authors: *S. CIECHANOWSKI[1], O. BURLEIGH[1], N. MATHY[4], X.-M. CHEN[2], K. DRESCHER[2], A. SEGISMUNDO[4], A. SHIBATA[3];
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Abstract: Systemic immune responses due to viral infection significantly contribute to neurodegeneration seen in various diseases. When activated in response to viral infection, microglia participate in pathogen clearance and promote both neurorecovery and neurotoxicity. Increased understanding of the regulatory factors for microglial activation may be critical to influencing antiviral immunity in the CNS. Long noncoding RNAs (lncRNAs) are transcripts that lack coding potential and perform regulatory activities through interactions with RNA-binding proteins, such as transcription factors. Our previous work shows that lncRNAs can regulate microglial antibacterial responses, so we hypothesize that lncRNAs are also important in microglial antiviral immunity. In vivo and in vitro FBV/nJ mouse systems were used to study whether Theiler's murine encephalomyelitis virus (TMEV) altered lncRNA expression in microglia. Post-TMEV infection, lncRNA Nostrill expression significantly increases 3.87±0.76 fold in TMEV-infected and chronically demyelinated brain, 3.0±0.04 fold in infected primary microglia, and 2.75±0.14 fold in infected microglial cell lines, compared to uninfected controls (n=3, ±=SEM, $p<0.05$). Upregulation of Nostrill in response to TMEV is dependent upon NFκB signaling, since NFκB inhibitors block TMEV upregulation of Nostrill. TMEV-mediated NFκB signaling significantly upregulates gene transcription of interferon response factor 7 (IRF7) to ~22fold in primary microglia, and to ~14fold in microglial cell lines as compared to unstimulated controls (n=3, $p<0.05$). Silencing of Nostrill using siRNA constructs blocks upregulation of IRF7 following infection of microglial cell lines with TMEV. Overexpression of Nostrill significantly increases IRF7 gene transcription without (~2fold) or with (~8fold) TMEV infection, as compared to controls (n=3, $p<0.05$). Following TMEV infection in microglial cell lines, qRT-PCR analysis showed an increase in infection burden with silencing of Nostrill (2.72±0.32fold), and a reduced viral burden with the over expression of Nostrill (0.35±0.09fold), as compared to control (n=3, ±=SEM, $p<0.05$). These data suggest that lncRNA Nostrill upregulation following viral infection is necessary and sufficient for regulation of IRF7 gene transcription and microglial antiviral immune responses. Regulation of these immune responses is important for modulating neurodegenerative disease processes associated with viral infection within the CNS.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIAAA R01AA025591

Title: Double-binge alcohol exposure does not provoke microglial response in adolescent rats

Authors: *J. WOODEN, J. MELBOURNE, C. ANASOOYA SHAJI, K. NIXON;
Univ. of Texas, Austin, Austin, TX

Abstract: Alcohol use disorders (AUDs) remain a prevalent public health concern, with drinking often initiated during adolescence. The adolescent brain is especially sensitive to excessive alcohol consumption, showing changes to structure, function, and developmental trajectory. Some hypothesize that microglia contribute to these changes through the upregulation of proinflammatory cytokines and mediators, however, they can also initiate protective functions that limit damage and aid repair. Microglia also display innate immune memory (IIM), whereby prior microglial activation results in altered response to subsequent stimuli. Our lab has previously shown that 2-day binge alcohol exposure elevates activation markers and increases responsiveness to immune challenge in isolated microglia, indicating that priming (a form of IIM) has taken place. However, whether ethanol primes adolescent microglia *in vivo* is unknown. Adolescent male Sprague-Dawley rats were administered 25% (w/v) ethanol or isocaloric control diet via intragastric gavage every 8 hours for two days. After 1 week of abstinence a second 2-day binge was conducted. Rats received either control diet for both binges (CC, *n*=9), control diet for the first binge and ethanol diet for the second binge (CE, *n*=9), or ethanol diet for both binges (EE, *n*=8). Rats were sacrificed at 2 days after the last dose by saline perfusion, and one hemisphere of the brain was post-fixed in 4% paraformaldehyde for histological studies (MHCII, CD68). For the other hemisphere, the hippocampus was dissected out, snap frozen, and used for ELISA assays (TNF-α, IL-6, IL-10, BDNF). The mean ethanol dose administered was 12.7 ± 1.6 g/kg/day yielding a peak blood ethanol concentration of 383.6 ±19.32 mg/dL (measured after the last dose). There was no change to proinflammatory cytokines TNF-α or IL-6 following single or double-binge alcohol exposure. Additionally, no CD68 phagocyte marker expression was observed in the parenchyma of the hippocampus of any rat. Activated macrophage marker MHCII was detected in the hippocampus of a subset of rats, but the CE or EE groups were not significantly higher than the CC group. We also did not see changes indicating alternative, anti-inflammatory type activation, as IL-10 and BDNF following single or double-binge was not different between CC, CE, or EE groups. Therefore, despite prior evidence that 2-day binge alcohol exposure primes microglia to over-respond, no enhanced response was observed in the second binge according to cytokine expression or proinflammatory marker immunoreactivity *in vivo*.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.05

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS T32NS041218
NMSS (JF-1806-31381)
NIH (1R01NS107523-01)

Title: Clearance of senescent-like microglia improves remyelination in a mouse model of focal demyelination

Authors: *P. S. GROSS[1], Z. MANAVI[2], J. LEE[2], W. E. BARCLAY[3], M. FU[3], J. K. HUANG[1,2];
[1]Interdisciplinary Program In Neurosci., [2]Dept. of Biol., Georgetown Univ., Washington, DC; [3]Natl. Inst. of Neurolog. Disorders and Stroke (NINDS), Bethesda, MD

Abstract: Multiple Sclerosis (MS) is a demyelinating and degenerative disease that is the number one neurological disability among young adults. Myelin degeneration results in denuded axons that consequently conduct signals inefficiently and eventually degenerate. In early stages of the disease, oligodendrocytes, the myelinating cells of the central nervous system, and their progenitors (OPCs), can regenerate myelin, resulting in effective remyelination. However, at later stages of the disease and with increasing age, remyelination becomes inefficient, leading to degeneration of axons and worsening of symptoms. One of the key characteristics of aging is the accumulation of senescent cells, which are apoptosis-resistant cells locked in permanent cell cycle arrest that secrete an inflammatory milieu termed the senescence associated secretory phenotype (SASP). Here, we hypothesized that the age-associated accumulation of senescent cells limits the ability of OPCs to remyelinate. Using a murine reporter line with a fluorochrome indicator under the expression of the p16INK4a locus (p16-tdt), a common marker of senescence, we found increased markers of senescent cells present after toxin-induced demyelination and throughout the process of remyelination. As early as 5 days post lesion (dpl), we found large increases in p16-tdt positive cells and other senescence markers, with expression progressively decreasing throughout remyelination before plateauing, with low lingering levels present at later timepoints. These markers predominantly colocalized with microglia. Additionally, aged mice showed markedly increased markers of senescence in the lesion compared to their young counterparts at both early and late timepoints post lesion, suggesting the accumulation and subsequent failed clearance of senescent cells might contribute to their reduced remyelination. Interestingly, clearance of senescent cells in young mice using the transgenic INK-ATTAC mouse line, which selectively ablates p16INK4a expressing cells, resulted in increased remyelination in the lesion compared to vehicle-treated controls. These results suggest that therapeutic targeting of cellular senescence, i.e. by senolytics or senomorphics, might promote remyelination in MS.

Disclosures: P.S. Gross: None. **Z. Manavi:** None. **J. Lee:** None. **W.E. Barclay:** None. **M. Fu:** None. **J.K. Huang:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.06

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR
Scottish Rite Charitable Foundation
Fondation CHU Ste-Justine

Title: Perinatal cerebellar injuries induce altered long-term anxious behavior in male mice

Authors: E. GUARNIERI[1], M. MENGUS[2], ***S. TREMBLAY**[3];
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Abstract: Background and Methods: Cerebellar development of extremely preterm infants is highly vulnerable during the third trimester to perinatal insult. To explore the pathophysiology and long-term neurobehavioral consequences of cerebellar hemorrhages (CBH) and early systemic inflammation (EIS), we induce in 2 days-old transgenic mice (B6.129P2(Cg)-C*x3Cr1*$^{\text{CreERT2-EYFP/iDTR}}$) perinatal cerebellar insults in combination with microglia depletion (iDTR$^{+/WT}$) or not (iDTR$^{WT/WT}$) to measure microglial cell contribution. Four groups were randomly obtained: control (Ctrl), CBH, EIS and double insults (CBH+EIS). Neurobehavioral tests were blindly performed in 60-85 days-old mice. **Results:** Based on our univariate statistical analyses results stratified by sex, male mice spent more time (EIS alone: 13.5±6.2%, ***P<0.01, n=14*; CBH+EIS: 15.3±8.7%, ***P<0.01, n=13*) and enter more often in the open-arm (EIS alone: 10.0±3.4 entries, **P<0.05*; CBH+EIS: 11.1±6.2 entries, ***P<0.01*) of the elevated-plus maze compared to controls (4.8±2.7% and 6.4±2.9 entries, *respectively, n=11*). They also cover further distance (EIS alone: 3783±615cm, *P=0.5490*; CBH+EIS: 4221±768cm, **P=0.0467*) and stay less immobile during open-field testing (EIS alone: 24.9±12.6%, **P=0.0362*; CBH+EIS: 29.3±15.6%, *P=0.0784*) compared to controls (3506±755cm and 33.3±10.9%, *respectively*). If we depleted microglial cells at the time of the insult, male mice are becoming more anxious reaching back anxious responses (time spent in open arm: EIS alone 7.8±3.5%, ***P=0.0049, n=13*; CBH+EIS: 12.9±10.3%, *P=0.4496, n=14*) similar to controls not exposed to insults (4.8±2.7%) while being tested in the elevated plus maze. Females did not show significant alterations of their long-term anxious responses after being exposed to perinatal cerebellar injuries. **Conclusions:** Our results suggest that sex-specific behavioral phenotypes are induced in mice suffering from perinatal cerebellar injuries. Indeed, male mice present altered anxious responses while exploring a new environment. Microglial cells seem to contribute to long-term behavioral phenotypic alterations and, if depleted prior to perinatal insult, may improve neurodevelopmental outcomes. Further analyses are required to characterize sex-differences of microglia phenotypic responses over time after injury which may contribute to neurodevelopmental alterations.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.07

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSF 1906690

Title: Deep Learning-based Classification of Microglia Proliferation

Authors: *P. R. MOUTON[1], H. MORERA[2], S. ALAHMARI[3], P. DAVE[2], A. S. ANDERSON[4], L. O. HALL[2], Y. KOLINKO[5], A. BECKER[6], W. C. MOBLEY[6], D. GOLDGOF[2];
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Abstract: Microglia are cells of myeloid origin that play key roles in the maintenance, repair, and immunosurveillance of neural tissues in vertebrates and invertebrates. There is also widespread evidence of microglia-mediated neuropathology in traumatic brain injury, neurotoxicological exposures, neurodegenerative diseases, and other neurological disorders. Currently, cell-level stereology of microglia requires manual counting (clicking) on hundreds of highly magnified cells in stained tissue sections through each defined region of interest in each case (subject, animal). Though theoretically accurate, the limitations of this approach include low efficiency (1-2 hours per region/case) and high inter-rater error due to human factors (subjectivity, training, experience, fatigue). To overcome these limitations, we developed a deep learning (DL)-stereology approach using snapshot ensembles of convolutional neural networks (CNN). Conventional stereology (optical fractionator) was used at high magnification (high mag, 100x oil) to quantify the total numbers of Iba1-immunostained microglial cells in neocortex (NCTX) of 54 brains from mice aged 6 to 14 months. After sorting these cases from lowest (~500,000) to highest (~900,000) based on total number of microglial cells in NCTX, we selected the cases with the highest (n=7), middle (n=7), and lowest (n=7) cell counts. In these 21 cases a systematic-random set of images was automatically collected through NCTX at 20x magnification (low mag) and assigned the correct global label (low, middle, high). These labeled low-mag images served as ground truth for training an ensemble of CNNs to automatically predict the correct global class for low mag images from test cases not previously used for training the model. Cross validation was done for each class using 6 mice for training and 1 mouse for testing. The ensemble predictions were averaged, and a global prediction (low, middle, high) generated for each test brain based on the class most often assigned to low-mag images from that brain. This fully automatic approach correctly identified the global class in 18 of 21 of the test cases (86% accuracy), without the need for any cell-level segmentation or manual stereology. These predictions of global class required less than one minute per case and showed 100% repeatability (Test-Retest). Our ongoing studies focus on adding more cases to achieve accuracy of 95% or greater; increasing resolution by training with classes that vary by 100,000 or fewer microglial cells; expanding the approach to immunofluorescent images; and tuning the model as needed for assessing microglia in more brain regions.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Csf1r inhibition modulates microglia and macrophage response to disease in the mouse EAE spinal cord

Authors: *M. LAMORTE, N. A. HAGAN, T. R. HAMMOND, D. OFENGEIM, J. PROTO, J. KANE, A. BLAZIER, J. SALEH;
Sanofi, Cambridge, MA

Abstract: At high doses CSF1R inhibitors rapidly deplete microglia in the mouse brain and have been used to study microglia function in the health and disease. However, at lower concentrations CSF1R inhibition suppresses microglia inflammatory responses in disease (Hagan et al., 2020; Jafari et al., 2021) without depleting microglia. To better understand the effect of CSF1R modulation on microglia and macrophage responses and cell state in the central nervous system we performed transcriptomic analysis on two distinct mouse models of neuroinflammation. In an acute LPS neuroinflammation model, bulk RNA sequencing revealed that CSF1R inhibition shifted the inflammatory profile of the brain. To better understand the

impact of CSF1R inhibition on specific cell types, we performed single cell RNA sequencing (scRNAseq) on mice with experimental autoimmune encephalitis (EAE), a demyelinating autoimmune disease that triggers infiltration of T-cells and monocytes and causes microglia and astrocyte activation. Animals were treated with a novel CSF1R inhibitor (CSF1Ri) following induction of disease. CSF1Ri treatment significantly reduced disease score compared to vehicle treated animals. scRNAseq analysis of the spinal cords uncovered a major shift in the spinal cord cellular milieu including increased numbers of activated microglia and infiltration of dendritic cells, multiple monocyte populations, and T cells. The CSF1Ri had little or no effect on the dendritic cells, astrocytes, and oligodendrocytes/OPCs and modest effects on the T cells transcriptional signature. However, the CSF1Ri significantly shifted the microglia and monocyte cell state, possibly contributing to the reduction in the inflammatory disease response and improved disease score. Microglia and monocytes cell states were also examined by flow cytometry which demonstrated a shift in microglia and monocytes cell subpopulations with CSF1Ri treatment. To explore the relevance of this therapeutic approach in human, an iPSC-derived tri-culture system was used to model the complex CNS micro-environment *in vitro*. Following CSF1R inhibitor treatment, these tri-cultures were then exposed to LPS or α-synuclein pre-formed fibrils (PFFs). As in our mouse studies, CSF1R inhibition shifted the transcriptomic profile of these cultures suggesting that therapeutically targeting this key signaling node could modulate microglia and macrophage cell state in inflammatory and neurodegenerative disease.

Disclosures: **M. LaMorte:** A. Employment/Salary (full or part-time):; Sanofi. **N.A. Hagan:** A. Employment/Salary (full or part-time):; Sanofi. **T.R. Hammond:** A. Employment/Salary (full or part-time):; Sanofi. **D. Ofengeim:** A. Employment/Salary (full or part-time):; Sanofi. **J. Proto:** A. Employment/Salary (full or part-time):; Sanofi. **J. Kane:** None. **A. Blazier:** A. Employment/Salary (full or part-time):; Sanofi. **J. Saleh:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 2020-MSCRFL-5427

Title: Role of PARP-1 in microglia-mediate neuroinflammation

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Abstract: Neuroinflammation is a common pathway in neurodegenerative disease. We recently revealed that PARP-1 [Poly (ADP-ribose) polymerase-1) activation in neurons has a crucial role in α-synuclein (α-syn) neurodegeneration of Parkinson's disease (PD). However, less effort has been spent on understanding the contribution of microglial PARP-1 activation to neurodegeneration. We found that activation of microglia can convert the neurotoxic reactive astrocyte in models of Alzheimer's disease and PD. Genetic depletion and pharmacological inhibition of PARP-1 in primary microglia prevented the pathologic α-syn-induced microglial activation, which result in the suppression of neurotoxic reactive astrocyte formation. As an action mechanism, we further found that PARP-1 is required for α-syn PFF-induced inflammasome activation in microglia. Consistently, intrastriatal injection of α-syn preformed fibril (PFF) as a model of sporadic PD increased microglial and astrocyte activation in WT mice, while PARP-1 KO mice injected with α-syn PFF failed to show the microglial and astrocyte activation. Taken together, PARP-1 is required for pathologic α-syn-induced microglia activation and neurotoxic reactive astrocytes formation via regulating inflammasome activation in microglia, which contributes to non-cell autonomous neurodegeneration in a model of PD.

Disclosures: Y. Choi: None. **V.L. Dawson:** None. **T.M. Dawson:** None. **T. Kam:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Sleep disruption alters microglial function leading to lethal neuroinflammation during septic like inflammation

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Abstract: Sleep disruption alters microglial function leading to lethal neuroinflammation during sepsis-like inflammation Abubakar Wani[1], Piyush Sharma[1], Bradlee Heckmann[2], Douglas R Green[1*1] Department of Immunology, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA.[2] USF Health Byrd Alzheimer's Center and Neuroscience Institute, Department of Molecular Medicine, Morsani College of Medicine, Tampa, FL, USA.* Corresponding author

Abstract

It is well established that infection can promote slow wave sleep, but the functional importance of this phenomenon remains unclear. In this study, we utilized sleep fragmentation, i.e., intermittent disruption of sleep throughout the day, to explore the relationships between sleep and inflammatory disease. We found that sub-lethal injection of a bacterial lipopolysaccharide (LPS), prior to sleep fragmentation (SF) invariably resulted in dramatically increased mortality

compared to LPS only or SF only controls, highlighting the importance of sleep following an inflammatory challenge. Further investigation revealed hyperactivation of microglia in the hypothalamus and other brain areas in mice subjected to LPS plus sleep fragmentation. As Toll-like receptor-4 (TLR4) is crucial for responses to LPS, we examined animals with microglia-specific deletion of TLR4 and found that it completely protected mice from the combination treatment. To further explore the role of sleep, we generated Dec2 (BHLHE41) mutant mice that display decreased sleep and found that these animals are protected from low dose LPS plus SF. We are currently extending our results to bacterial infection.

Disclosures: **A. Wani:** None. **P. Sharma:** None. **B. Heckmann:** None. **D. Green:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 286.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Plan Propio de Investigación y Transferencia de la Universidad de Málaga (Predoctoral Grant)

Title: Enhanced stress response in rats that suffered acute neuroinflammation induced by neuraminidase three months before

Authors: *A. LEÓN-RODRÍGUEZ[1,2], J. M. GRONDONA[1,2], M. FERNÁNDEZ-ARJONA[3,2], C. PEDRAZA[4,2], M. D. LÓPEZ-ÁVALOS[1,2];
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Abstract: Microglial cells are protagonists in neuroinflammatory processes and their activation is a notorious feature of such events. In acute inflammation, microglial cells return to their basal surveillant state in few days. However, sometimes they evolve towards a primed state, characterized by hypersensitivity to new stimuli and an exacerbated response which may jeopardize brain functions. Because the hypothalamus is a pivotal hub for neuroendocrine and autonomic functions, we have been exploring evidences of microglial priming in this region and its consequences. We used a model of acute ventricular neuroinflammation consisting in the

intracerebroventricular (ICV) injection of neuraminidase (NA). This enzyme is found in the cover of neurotropic bacteria and viruses, e.g. influenza, mumps or measles viruses, thus mimicking a brain infection. Three months after inducing neuroinflammation with NA to rats, an acute stressor was applied to investigate the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the stress response elicited, as well as the inflammatory activation of hypothalamic microglial cells. The acute stressor was forced swimming for 6 minutes. Afterwards, blood samples were retrieved to determine corticosterone levels by ELISA, and the brains extracted to analyze microglial cells in histological sections by immunohistochemistry with IBA1 and inflammatory markers by qPCR. Stressed rats previously injected with NA had increased levels of corticosterone compared with control rats that were equally stressed but had been ICV injected with saline. Also, qPCR studies in hypothalamic tissue revealed that NA-treated rats presented an increased expression of the genes for the inflammasome protein NLR family pyring domain containing 3 (NLRP3) and the microglial marker IBA1. Concomitantly, the morphological analysis of microglial cells located in the paraventricular nucleus (PVN) showed a morphological bias towards a slightly activated state in microglia of NA-injected rats compared to those of saline injected controls. These results suggest the long-term priming of hypothalamic microglial cells, and particularly those located in the PVN, after the acute ventricular inflammation induced by NA. Interestingly, increased corticosterone levels in NA-treated rats after exposure to an acute stressor point to an enhanced activation of the HPA axis. Thus, prior neuroinflammatory episodes, and in particular those associated to viral/bacterial infections, might result in subtle but persistent changes that condition the response to future challenges such as stressful events. Microglial cells could underlie those alterations.

Disclosures: A. León-Rodríguez: None. **J.M. Grondona:** None. **M. Fernández-Arjona:** None. **C. Pedraza:** None. **M.D. López-Ávalos:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.12

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: The effects of fructose on inflammation and metabolism in primary microglia cell cultures

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Abstract: Microglia are found in the central nervous system (CNS) and are known to be involved in a variety of processes such as brain development and plasticity. As the immune cells of the CNS, microglia are tasked with regulating inflammation in response to acute and chronic stressors, and are key cells involved in the role of chronic inflammation in psychiatric illnesses

such as depression and anxiety. While glucose is the primary energy source of the brain, recent research suggests that microglia can use other energy sources, such as fructose, a sugar that is found in corn syrup and has been implicated in psychiatric illness and metabolic disorders and may have inflammatory effects. The objective was to determine the metabolic and inflammatory effects of fructose on primary microglia cell cultures. It was hypothesized that fructose leads to microglia releasing pro-inflammatory cytokines such as interleukin 6 (IL-6), interleukin 1β (IL1β), and tumour necrosis factor (TNF) as compared to glucose. For the experiment, primary microglia expressing green fluorescent protein (CX3CR1+/eGFP) were isolated from mouse brains and cultured. Microglia were cultured with various concentrations of fructose in glucose-free media and stimulated with lipopolysaccharide (LPS) and interferon γ (IFNγ) and compared to microglia cultured with glucose (17.5mM). It was expected that as concentrations of fructose increased, there would also be an increase in the release of cytokines. The results of a two-way ANOVA demonstrated that microglia treated with fructose have a statistically significant lower release of IL1β, TNF, and IL6 than glucose and LPS ($p < 0.05$). These results demonstrate that fructose is less pro-inflammatory than glucose and LPS. Future studies will focus on microglia fructose uptake through the GLUT5 transporter and how LPS or IFNγ could influence this. Overall, this work opens an exciting avenue to continue exploring how microglia respond and utilize a variety of metabolic resources.

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286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Life Sciences CURA Brigham Young University
Internal Support from Brigham Young University

Title: Effects of microglial-stimulated neuroinflammation on dopamine terminal function in the nucleus accumbens

Authors: *S. C. LINDERMAN, L. H. FORD, H. A. WADSWORTH, E. R. WHITE, E. B. TAYLOR, S. T. JONES, S. C. STEFFENSEN, J. T. YORGASON;
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Abstract: Methamphetamine induces dopamine release in striatal subregions through direct effects on dopamine terminals. Microglia are the immune cells of the brain and are observed throughout the striatum. Using immunohistochemistry and confocal microscopy, we demonstrate the presence of dopamine receptors on striatal microglia. In consideration of dopamine receptor localization on microglia, the present study hypothesizes that microglia react to

methamphetamine-induced dopamine release. The presence of dopamine receptors on microglia also suggests that microglia may signal to dopamine terminals. Therefore, the present study also examines the effects of microglia activation on dopamine terminal function. The interactions between microglia and dopamine terminal function were also examined in the presence of methamphetamine throughout. Multiphoton microscopy was used to measure microglia motility and ramification and thus immune activation. Dopamine terminal function (e.g. release and uptake) were examined using fast-scan cyclic voltammetry (FSCV) techniques in mouse brain slices. The bacterial coat protein lipopolysaccharide (LPS) reduced microglia ramification across a 4-hour time period. In contrast, methamphetamine increased microglia ramification. Consistent with previous work from our lab and others, dopamine transporter antagonist methamphetamine decreased dopamine uptake, which was marked by a large reduction in dopamine transporter function. Interestingly, LPS appeared to increase dopamine uptake, which may be a secondary effect from the increased evoked dopamine release. LPS also increased the amplitude of dopamine release, which was in contrast to aCSF controls. These results suggest that microglial neuroinflammation induced by LPS administration significantly alters dopamine terminal function. Current experiments are examining methamphetamine effects on dopamine release after LPS exposure.

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286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.14

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Canadian Institute for Health Research
CGS-D Doctoral Scholarship (ADR)
Ontario Graduate Scholarship (SJM)

Title: Plasma derived extracellular vesicle biomarkers of post-stroke microglia activation

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Abstract: Microglia, the resident macrophages of the CNS, play a dual role in the response to ischemic stroke. While the acute microglia response is largely neuroprotective, chronic pro-inflammatory microglia activation post-stroke is associated with secondary neurodegeneration and poorer neurological outcomes. However, our ability to reliably detect ongoing microglia

activation post-stroke is currently limited. Plasma derived extracellular vesicles (EVs) represent promising indicators of cell-specific activity that may allow for the non-invasive measurement of microglia activation. This study identified activation-state specific EVs released from microglia following pro-inflammatory stimulus *in vitro* and validated them using an experimental stroke model. Following LPS exposure, cultured microglia release EVs expressing the microglia protein TMEM119 alongside increased expression of the toll-like co-receptor CD14. Immunoprecipitation and nanoparticle tracking analysis (ONI Nanoimager) of $TMEM119^+$ $CD14^+$ EVs from wildtype Fischer344 rat plasma confirmed co-localization on EVs with an average diameter of 209.7 nm (SD=64.12 nm). Transmission electron microscopy with immunogold labeling of TMEM119 and CD14 confirmed their localization to the EV membrane. To model focal ischemia, 3-month-old wildtype Fischer344 rats (n=8/group) received either a saline or endothelin-1 injection into the dorsal right striatum. Plasma was collected at baseline and 28 days after surgery at which point brains were harvested for stroke volume and histological analyses. Immunohistochemistry confirmed persistent $CD14^+$ and $MHC\text{-}II^+$ pro-inflammatory microglia in the stroke site at 28 days. To directly measure circulating microglial EVs, fluorescent antibodies against TMEM119, CD14 and MHC Class II were incubated with plasma and EVs were quantified using Apogee A60 Micro plus nanoflow cytometer. At 28-days circulating $TMEM119^+/CD14^+$ EVs are significantly increased post-stroke in comparison to baseline levels ($p=0.0078$) and saline injected animals ($p=0.0401$). Circulating $TMEM119^+/CD14^+$ EVs at 28 days are weakly correlated with stroke volume ($r=0.66$, $p=0.0775$). $TMEM119^+/MHC\text{-}II^+$ EVs were also increased in post-stroke rats in comparison to baseline ($p=0.0156$) and saline injected animals ($p=0.0151$). For the first time, we report detection of EVs from activated microglia in the peripheral circulation following stroke which represent a future tool for the non-invasive and rapid measurement of microglia activity *in vivo.* Future work is required to investigate associations between circulating microglia EVs and neurological outcomes post-stroke.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 286.15

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01NS083704
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Title: Il-10 deficiency fails to alter tau pathology in 3-6-month-old htau mice

Authors: *S. DADRAS, L. WESTON, K. BHASKAR;
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Abstract: Research objective and rational: The presence of hyperphosphorylated microtubule associated protein tau is strongly correlated with cognitive decline and neuroinflammation in tauopathies. However, the role of anti-inflammatory molecules in regulating tau-induced neurodegenerative sequel is unclear. Here, we specifically investigated if interleukin-10 (IL-10) has a role in regulating the early events of pro-inflammatory signaling and tau phosphorylation in hTau mice with IL-10 deficiency. **Methods:** We used a hTau mouse model expressing all six isoforms of non-mutant human tau with complete knockout of endogenous mouse tau to recapitulate the age-dependent development of tau pathology seen in Alzheimer's disease (AD). We crossed hTau mice with IL-$10^{-/-}$ mice to create hTau-IL-$10^{-/-}$ mice and aged them to 3 and 6 months. We quantified Tau phosphorylation in hippocampal homogenates and sagittal brain sections by Western blot and immunohistochemistry respectively. Microglial morphology in brain sections was quantified by skeleton analysis. We determined cytokine gene expression and protein levels in cortical homogenates by RT-qPCR and MesoScale Discovery Multiplex assays. **Results:** Our findings revealed that genetic deletion of IL-10 in hTau mice appears to be ineffective in altering neuroinflammation and tau phosphorylation. Both tau hyperphosphorylation and proinflammatory cytokines were present at low levels in hTau mice and not altered by the IL-10 deficiency. Only the chemokine CXCL1 was significantly reduced ($p<0.05$) in hTau-IL-$10^{-/-}$ mice compared to age-matched non-transgenic control or hTau mice. In contrast, CXCL1 was rather increased in IL-$10^{-/-}$ mice challenged with lipopolysaccharide (LPS) – reported in our previous study (PMID: 34275478). Finally, microglial morphology appeared unchanged in hTau and hTau-IL-$10^{-/-}$ mice relative to non-Tg controls and hTau mice. **Conclusion:** Together, these results suggest contrasting roles for IL-10 in LPS-induced systemic inflammatory model of tauopathy versus hTau-based genomic mouse model of tauopathy. Non-reciprocal roles of CXCL1 in LPS vs hTau models may likely explain these differential effects.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.16

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VA BLR&D Grant BX003486

Title: Thrombospondin-1 signaling induces neuronal dysfunction and neuroinflammation during hepatic encephalopathy

Authors: *A. JHAWER[1], G. FRAMPTON[1], S. BHATTARAI[1], E. TROYANOVSKAYA[2], M. MCMILLIN[1];
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Abstract: Hepatic encephalopathy (HE) is a neurological complication that arises from loss of liver function that is associated with cognitive deficits, cerebral edema, and neuroinflammation. Thrombospondin-1 (TSP1) is a homotrimeric protein involved in many disease processes, and we have shown that TSP1$^{-/-}$ mice are protected from HE-associated neurological decline. CD47 is a TSP1 receptor that has been shown to influence inflammation. Therefore, we hypothesize that TSP1 activates CD47, which exacerbates HE pathology by inducing neuroinflammation. HE was induced in CD47$^{-/-}$ or C57Bl/6 (wild-type control) mice by intraperitoneal injection of 100 mg/kg of azoxymethane (AOM). Tissue was collected once mice progressed to coma, defined by loss of righting reflex. Neurological decline was assessed by measuring reflex responses and ataxia. Liver injury was determined by hematoxylin and eosin staining and measurement of serum transaminases. Cerebral edema was calculated by wet weight/dry weight of brain tissue. TSP1, CD47, CYP2E1, IBA1 and cleaved caspase 3 expression in liver, cortex, hippocampus or cerebellum was assessed by real-time PCR, immunohistochemistry, or immunofluorescence. IL-1β, CCL2, TNFα and IL-6 were assessed in liver, cortex, hippocampus and cerebellum by real-time PCR and ELISA analyses. TSP1 and CD47 expression was increased in the liver, cortex, hippocampus and cerebellum of AOM-treated mice when compared to vehicle-treated mice. CD47$^{-/-}$ AOM-treated mice had reduced neurological decline, cerebral edema and liver injury compared to wild-type AOM-treated mice. Hepatic CYP2E1 expression was unchanged between groups, suggesting no changes in AOM metabolism. Microglia activation, as assessed by IBA1 staining, was more prevalent in AOM-treated mice, but CD47$^{-/-}$ had less incidence of microglia activation compared to wild-type mice. Pro-inflammatory cytokine expression was unchanged in the liver but was significantly decreased in all brain regions of CD47$^{-/-}$ mice compared to wild-type mice administered AOM. In conclusion, therapeutic strategies to target TSP1 signaling via CD47 may prove effective at alleviating HE-induced neurological decline and neuroinflammation.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

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Program #/Poster #: 286.17

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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R01 EY018606 (RTL)
R01 EY027701 (RTL)
T32 EY007125 (KMA)

Title: Gpnmb expression alters microglia responses to optic nerve crush injury

Authors: *A. M. FEIDLER, K. M. ANDERSH, R. T. LIBBY, A. K. MAJEWSKA; Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Glaucoma is an irreversible progressive disease often diagnosed too late to be effectively treated. In glaucoma, blockage of the aqueous humor leads to an increase in intraocular pressure, damaging retinal ganglion cell (RGC) axons which leave the retina to form the optic nerve. Insult to these axons leads to anterograde and retrograde RGC degeneration and death, an irreversible process in mammals. Many glaucoma studies focus only on RGCs, but recent evidence shows that many non-neuronal cells, such as microglia, play critical roles in disease progression. Microglia are the immune cells of the central nervous system, and contribute to many processes relevant to injury and regenerative capacity seen in glaucoma. One of the most common genetic mouse models of glaucoma, the DBA/2J mouse has a mutation in two genes, one of which is *Gpnmb*, which encodes a type 1 transmembrane glycoprotein that is highly expressed in microglia. Under homeostatic conditions, GPNMB has neuroprotective effects against oxidative stress, and during inflammation, GPNMB decreases pro-inflammatory cytokines and increases anti-inflammatory cytokines. Given that GPNMB can reduce inflammation and is expressed in microglia, its presence or absence could influence the role of microglia in disease. Here, we investigate how loss of *Gpnmb* affects the microglial response to optic nerve crush (ONC), a manipulation often used to model glaucoma in animals. We used *Gpnmb* knockout ($Gpnmb^{-/-}$) and wildtype littermate ($Gpnmb^{+/+}$) mice of both sexes that underwent a manual ONC unilaterally, with the contralateral eye serving as a sham control. The brain and both eyes were harvested 7 days after injury and immunoreacted for microglia marker Iba1, homeostatic microglial marker P2Y12, and phagocytic marker CD68. We quantified the expression of each of these markers, as well as microglial morphology in the crushed and sham eyes of the same animal. We found that in sham eyes, microglia in the ganglion cell (GCL) and inner plexiform layers (IPL) were hyper-ramified in $Gpnmb^{-/-}$ animals, but showed similar expression of Iba1, CD68 and P2Y12 to those in $Gpnmb^{+/+}$ animals. Smaller increases in ramification were also observed in the outer plexiform layer and in the visual cortex of $Gpnmb^{-/-}$ animals. In crushed eyes, microglia in the GCL and IPL of both genotypes showed changes indicative of immune activation, including de-ramification, increased expression of Iba1 and CD68, and decreased expression of P2Y12, however the magnitude of these changes was more pronounced in $Gpnmb^{-/-}$ animals. These data suggest that the loss of *Gpnmb* may change microglial phenotypes, possibly priming these cells to respond more robustly to injury.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 286.18

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR
Scottish Rite Charitable Foundation
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Title: Perinatal cerebellar injuries perturb tissue repair responses of microglial cells in the developing brain

Authors: *M. MENGUS[1], E. GUARNIERI[3], R. IMANE[3], S. TREMBLAY[2];
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Abstract: Background: Extreme preterm infants are exposed to multiple inflammatory stressors including perinatal cerebellar hemorrhage (CBH) and postnatal infection, two major risk factors for neurodevelopmental impairments. Given the dual involvement of microglia in immune and non-immune functions across the central nervous system during development, they may play a central role in the pathogenesis of cerebellar injury. During the recovery phase post-injury, microglial cells presenting a pro-repair phenotype will facilitate healing and homeostasis of the damaged tissue. Melatonin treatment have been demonstrated to improve pro-repair responses of microglial cells and will be studied to maintain or restore tissue healing responses altered in our translational model. **Methods:** Mice were exposed to CBH at postnatal day 2 (P2) combined or not with early inflammation (LPS). Melatonin treatment was administered for three consecutive days starting 2 hours after insult exposure. Microglia phenotypic changes across time (P2, P3, P7 and P15) were analyzed by flow cytometry with a panel of markers. **Results**: Our data showed that two weeks after being exposed to perinatal insults (P15), M2b phenotype from microglial cells are significantly decreased in mouse pups exposed to a systemic inflammatory stress alone (LPS: 7,04%, ***P*=0.0025, *n*=11) and exposed to a combined insult (CBH+LPS: 6,51%, ***P*=0.0025, *n*=10) compared to controls (28,56%, *n*=10). Mice exposed to cerebellar haemorrhage alone showed a trend to a lower amount of M2b cells at P15, but this result did not reach significance (CBH: 17,24%, *P=0,2800, n=5*). Our preliminary results regarding melatonin treatment started rapidly after insult exposure restored percentage of activated microglial cells exhibiting M2b profile at P15 to 14,49% in mouse pups exposed to systemic inflammation (*n*=4, *****P*<0,0001). To date, alterations of M2-cell responses after CBH alone or combined with LPS are not restored by melatonin treatment (CBH alone: 8,393%, *n*=2; CBH+LPS: 7,658%, *n=3)*. **Conclusions**: Perinatal insults exposure induces a reduced level of activated M2b-microglia cells, which translate to altered microglia proliferation and tissue remodeling responses and may create a vulnerability window during the recovery phase of injury. Melatonin treatment may restore normal tissue repair responses after perinatal systemic inflammation.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

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Program #/Poster #: 286.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AA025591
R01AA016959
F32AA029928

Title: Decreased microglial sensitivity to binge ethanol upon repeat exposure in adolescent male rats

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Abstract: Microglia, the macrophages of the brain, support a wide range of functions in CNS development and homeostasis. Microglial activation leads to context-dependent shifts in morphology and function and cells display innate immune memory (IIM), such that prior activation may lead to changes in response to subsequent stimuli. In both humans and animal models, binge alcohol exposure leads to microglial activation alongside tissue damage. Here, we focus on adolescence, a critical period of increased sensitivity to alcohols effects. Previous data from our group shows that microglia isolated from adolescent rats 2 days following 2-day binge ethanol exposure have elevated activation markers and increased responsivity to immune challenge, indicative of a type of IIM called priming. However, it is unknown whether ethanol primes adolescent microglia *in vivo*. Therefore, adolescent males were exposed to either a single (CE) or double (EE) 2-day binge. Rats in the CE group (n=8) were gavaged with isocaloric control diet every 8 h for 2 days (modified Majchrowicz model), recovered for 7 days, then gavaged with 25% (w/v) ethanol diet for a further 2 days. The EE group (n=8) received ethanol diet in both binges, and a third group control diet (CC; n=8) throughout. A separate control group remained on chow (UBC; n=3). Tail bloods collected immediately following the final binge produced mean blood ethanol concentrations of 383.6 ± 19.32 mg/dL that weren't significantly difference between groups. Following the last gavage, rats were transcardially perfused with phosphate buffered saline and brains post-fixed in paraformaldehyde, sectioned at 40μm on a vibrating microtome, and fluorescence immunohistochemistry performed for microglia-specific marker, IBA1. Images from the hippocampus (molecular layer of the dentate gyrus and CA1) were acquired with a laser scanning confocal microscope (Olympus FV3000), 3D reconstructed and volumetric analyses and microglia estimates were conducted using Imaris (Bitplane). No differences in microglia number were observed across groups. Total microglia coverage volume was increased in CE compared to CC, though no differences in average microglia volume were present when quantifying individual cells. Soma volume was increased significantly in CE compared to all other groups. Thus, microglia demonstrated shifts in morphology indicative of activation (increased soma size) 2 days following a single, but not repeat, 2-day binge. These results support previous data that isolated microglia are activated 2 days following adolescent binge exposure but show that microglia are less sensitive to morphological shifts upon repeat exposure.

Disclosures: **J. Melbourne:** None. **J. Wooden:** None. **K. Nixon:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.20

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIEHS T32 Training Grant T32ES007254

Title: Intranasal Treatment with Nicotine-derived Nitrosamine Ketone (NNK) Alters Microglia Activity and Causes Disruption of Blood-Brain Barrier (BBB) on Mice

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Abstract: NNK is a toxin and nicotine metabolite found in Cigarette Smoke (CS). CS continues to be a leading cause for decline of quality of life as well as deaths globally. Epidemiological assessments have suggested that CS is associated with neuroinflammation and several neurological disorders, including Alzheimer's disease, stroke, and multiple sclerosis. While direct effects of CS have not been fully understood, studies in a humanized flow-based *in vitro* blood-brain barrier model used CS extract to show resultant pro-inflammatory effects and loss of BBB function and endothelial cell viability. NNK delivered IP in mice induced glial activation sustained from 4 to 12 days as evaluated immunohistologically. Specific effects of NNK on the BBB, vascular dynamics, or *in vivo* microglial dynamics as they relate to the vasculature and across multiple areas are unknown. Here, we show that NNK when given through an intranasal route, leads to disruption of the BBB and vessel-localized microglia activity for acute (4 days) exposure and is further enhanced over chronic (12 weeks) exposure. To investigate microglial and vascular responses to NNK *in vivo*, we combined the use of transgenic mouse line (CX3CR1-GFP) with intravital neuroimaging by two-photon microscopy. Single timepoint volumetric scans and time series of up to an hour were taken to assess spatial, dynamic observations and vasospasms. A machine learning algorithm was employed to segment microglial soma from processes. *Ex vivo* immunofluorescence (IF) staining was performed on thin sagittal brain sections to corroborate *in vivo* findings. Results indicate that microglial activation was found to be heterogeneous; that is, activated microglia and ramified microglia existed within the same field of view with spatial preference observed. NNK-treated animals displayed increased vessel-associated microglia (VAM) compared to control, with analysis showing VAM sustained an ameboid appearance over time and clustered near the vessels. An increase in vascular events, including vasoconstriction, vasodilation, and microbursts, were observed in NNK treated animals suggesting a pronounced effect of NNK ion vasculature over controls. Uptake of Evans Blue increased in NNK treated animals when compared to control in both soma and processes. IF revealed that up to 93% of GFP-positive neural cells showed positive staining for a microglia exclusive marker. NNK effects were sustained and exacerbated with chronic treatment. Taken together, these results suggest an immunomodulatory effect of

NNK marked by heterogeneous localization of microglia with activated microglia confined near vessels and accompanied by vascular events.

Disclosures: L.F. Ochoa: None. **P.P. Villrreal:** None. **O.D. Solomon:** None. **M. Motamedi:** None. **G. Vargas:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.21

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CIHR
National MS Society
Canadian research chair in Glial Neuroimmunology for Dr. Jason Plemel

Title: Microglia diversity during remyelination and aging

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Abstract: Microglia diversity during remyelination and agingSameera Zia[1], Charbel Baaklini[1], Kelly Lee[1], Madelene F.S Ho[1], Sarthak Sinha[2], Mena Burr[1], Abhisha Patel[1], Andre Faria[1], Brady Hammond[1], Aislinn Maguire[1], Olivia La Caprara[3], Timothy Friedman[1], Bradley J. Kerr[1,3], Anastassia Voronova[1,4], Jeff Biernaskie[2,5], Jason R. Plemel[1,6,7]
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Multiple sclerosis (MS) is a neurodegenerative disease characterized by demyelinating lesions resulting from loss of myelin. Lost myelin may be regenerated through remyelination in MS, where greater remyelination positively correlates with reduced disability but is prone to failure and becomes less efficient with age. Remyelination is promoted by microglia, who secrete pro-remyelination factors and clear inhibitory molecules. Microglia are also a heterogeneous cell type, forming subpopulations that vary across development, anatomical regions, and disease. We hypothesize that microglia subpopulations are diverse during remyelination and altered with age. We used the lysolecithin mouse model wherein acute demyelination is followed by spontaneous, but robust, remyelination. We isolated microglia from LPC lesioned tissue and used single cell RNA sequencing coupled with advanced bioinformatics techniques. We described diverse

remyelination-associated microglia (RAM) subpopulations that change their transcriptional state throughout remyelination, moving from an *Igf1 or Irf7* RAM dominated state to one that is characterized by *Meg3* and *Plp1*. We used RNAscope to validate this shift of *Igf1* and *Irf7* subpopulations within mouse remyelinating lesions. As mice age, we found the kinetics of RAM subpopulations changes. We found a delayed accumulation of the *Igf1* RAM subpopulations in middle aged mice, suggesting a delay in the RAM phenotype induction with age. Taken together, we characterized diverse microglial states during remyelination that are delayed with aging. Understanding how microglia transition between these different states that are impaired with again may provide new targets to promote remyelination.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS R01NS091575
CNRM-70-9238
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USU Startup Funds

Title: Microglia reactivity is altered by SMN deficiency

Authors: *E. M. BERGMAN, B. MELVIN, G. KHAYRULLINA, S. IZADJOO, S. PAULSON, M. STINSON, J. ROTTY, B. G. BURNETT;
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Abstract: Spinal muscular atrophy (SMA) is a neurodegenerative disease caused by deletions or mutations of the *SMN1* gene resulting in insufficient production of survival motor neuron (SMN) protein and loss of ventral horn neurons. We and others have shown that SMA microglia adopt a reactive profile, including production of inflammatory cytokines, increased phagocytosis, and activation of the complement system. However, it remains unclear if these are cell-autonomous features of SMN-deficiency in microglia. We confirm that the previously described changes in SMA microglia are SMN dependent, we knocked SMN down in control hiPSC-derived microglia using siRNAs and analyzed cell morphology and gene expression of inflammatory genes. To further explore the temporal requirement for SMN in microglia reactivity we utilized a hiPSC line that constitutively expresses dCas9, allowing tight regulation of SMN expression using gRNAs that target the *SMN1* promoter. We then assessed changes in inflammatory

cytokine expression, immunoproteasome formation, phagocytosis, chemotaxis, and cytoskeletal alterations in the presence or absence of SMN gRNAs. Our initial findings indicate that several innate immune-related pathways were upregulated when SMN was suppressed, including the inflammasome and immunoproteasome. Moreover, reducing SMN reduced the number of neurite processes ($M = 0.7843$, $SD = 0.9447$) compared to controls ($M = 2.333$, $SD = 1.306$) ($t(100) = 6.862$, $p < 0.0001$), consistent with a transition from a ramified to a reactive state. Our ongoing studies suggest that SMN-deficiency primes microglia to become reactive, potentially exacerbating the motor neuron loss and contributing to SMA disease progression.
DISCLAIMER: The views presented here are those of the author and are not to be construed as official or reflecting the views of the Uniformed Services University of the Health Sciences, the Department of Defense or the U.S. Government.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF Grant 2021R1I1A1A01049783

Title: A distinct microglial cell population expressing both CD86 and CD206 constitutes a dominant type and executes phagocytosis in two mouse models of retinal degeneration

Authors: *Y. ZHANG[1], Y.-S. PARK[1], S.-S. PAIK[1], J.-H. SONG[2], K.-W. SUNG[3], I.-B. KIM[1]; [1]Dept. of Anat., [2]Dept. of Orthopedic Surgery, St. Vincent Hosp., [3]Dept. of pharmacology, The Catholic Univ. of Korea, Seoul, Korea, Republic of

Abstract: Retinal degeneration (RD) is a leading cause of blindness with irreversible loss of photoreceptors. Microglial cells are the key regulators of inflammation during RD and are conventionally classified as M1 (pro-inflammatory) or M2 (anti-inflammatory). However, recent studies have shown that this conventional classification of microglial cells has not been appropriately applied in RD. In this study, we examined the spatiotemporal distribution and characteristics of microglial cells to identify their subpopulations and understand their function. RD was induced in BALB/c and C57BL/6 mice by blue LED exposure and NaIO3 injection, respectively. Mouse eyecups were prepared for terminal deoxynucleotidyl transferase dUTP nick end labeling and immunohistochemistry with anti-IBA1, anti-P2RY12, anti-CD86, and anti-CD206 antibodies at 12 h, 24 h, 72 h, and 120 h after RD induction. Fluorescence-activated cell sorting (FACS) was performed using anti-CX3CR1 and anti-CD206 antibodies to isolate the microglial cell subtypes. Expression levels of Il-10, Il-6, Trem-2, ApoE, Lyz2 were evaluated by quantitative real time-PCR. IBA1-labeled microglial cells located in the inner and outer

plexiform layers of the normal retina migrated to the outer nuclear layer (ONL) and subretinal space (SRS) and increased in number from 12 to 72 h after RD. However, P2RY12-labeled innate microglial cells decreased in number 24 h post RD in the ONL and SRS and almost disappeared at 72 h. Microglial cells expressing M1 marker CD86 gradually increased from 12 to 72 h after RD in the ONL and SRS, whereas microglial cells expressing M2 marker CD206 abruptly increased at 72 h. Among microglial cells at 72 h after RD, CD86/CD206-double-labeled microglial cells accounted for approximately 90% of the microglial cells in the ONL and SRS. This subpopulation, sorted by FACS, exhibited a significant increase in of Apoe, Trem2, and Lyz2 levels but not in Il-6 and Il-10 levels. Our results suggest that CD86/CD206-double-labeled microglial cells are a distinct and dominant microglial type during RD and mainly function as a phagocytic executor rather than a pro- or anti-inflammatory modulator.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

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Program #/Poster #: 286.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PSC-CUNY 52-394

Title: Phagocytic microglia and adult neurogenesis in the dentate gyrus are correlated with spatial and recognition memory in a subregion- and hemisphere-specific manner of aging mice

Authors: S. JETT[1], K. CHANG[2], A. FERDINAND[1], A. BRAND[3], N. WOLF[3], R. INIRIO[1], J. GOLDBLATT[1], A. ISKHAKOVA[1], A. BOOK[1], *C. PYTTE[1,2];
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Abstract: Microglia, the resident immune cells of the brain, can be either protective or detrimental to neurons depending on their activation state. The dentate gyrus of the hippocampus is functionally sensitive to microglial activity, which plays a role in adult neurogenesis and impacts hippocampal-dependent learning and memory. Moreover, the dentate gyrus is composed of distinct subregions including the granule cell layer (GCL) and hilus, and has been reported to show functional lateralization in the rodent brain. Therefore, consideration of subregion and hemisphere is necessary to understand the effects of aging on inter-related changes in microglial activity, adult neurogenesis, and hippocampal-dependent memory associated with age-related cognitive decline. We used the intracellular receptor CD68 as a marker of phagocytic microglia and doublecortin (DCX) as a marker of young neurons. Here we report a positive correlation between CD68+ density in both the left and right hemisphere GCL and measures of performance

in the Barnes Maze, suggesting that increased CD68+ cells in the GCL corresponded to improved long term spatial memory. On the contrary, CD68+ density in the right hemisphere GCL only was associated with impaired memory, tested by object novelty preference. When we divided our subjects by age, we found that that in middle-aged mice, CD68+ density in the left hilus was associated with better spatial memory performance in the Barnes Maze. However, in old mice this relationship shifted such that CD68+ density in the left hilus was associated with poorer performance in the Barnes Maze. Interestingly, in old mice, DCX+ in left GCL, but not the right GCL, was associated with better performance during the Morris Water Maze probe trial. In sum, we found that correlations between CD68+ density and measures of hippocampal-dependent memory performance differed by task, mouse age, dentate gyrus subregion, and hemisphere. This underscores the complexity of activated microglia in potentially contributing to cognitive performance.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 286.25

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant P20 GM103429

Title: Myeloperoxidase is a mediator of inflammation in cultured mouse microglial cells in response to amyloid-beta 42

Authors: *T. APPLETON, S. EWING, D. DONLEY;
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Abstract: Free radical signaling and oxidation-reduction pathways are important inflammatory signaling mechanisms in microglial cells. Dysregulation of these pathways results in oxidative stress that is associated with neuronal degeneration diseases such as Alzheimer's disease. Pathways by which oxidative stress promotes neuroinflammation during disease are not fully understood. Recently, the pro-oxidant enzyme, myeloperoxidase (MPO), has been associated with Alzheimer's disease progression but little is known about its role in neuroinflammation. The goal of this study was to characterize the role of MPO on inflammatory responses to disease-relevant stimuli. During peripheral inflammation, MPO is an inflammatory mediator and important for clearing foreign matter such as bacteria. Microglia are brain-resident immune cells that have many similarities with peripheral macrophages. Upon encountering damage or disease signals, microglia activate. In Alzheimer's disease, amyloid-beta-42 (Aβ42) and elevated iron are both associated with increased microglial activation and neuroinflammation. We found that MPO

protein and activity is increased in cultured microglia after Aβ42 and iron stimulation. To address the role of MPO, we treated immortalized microglial cells with the MPO inhibitor, 4-aminobenzoic hydrazide (MPOi) in the presence and absence of activating stimuli. We found that the MPO decreases glycolic activity in both Aβ42-stimulated and unstimulated cells. Since metabolic activity is a determinant of activation state, we measured the effect of MPO inhibition on pro-inflammatory activation. We found that MPO promotes release of pro-inflammatory cytokines. These results suggest that MPO may be contributing to inflammatory processes via modulating cellular oxidative stress. Interestingly, we found that MPO inhibition had little effect on the production of reactive oxygen species after iron and amyloid-beta stimulation. These results indicate that MPO may be an indirect mediator of inflammation in microglia. Further research is needed to determine the interplay between MPO and disease-relevant stimuli and how MPO shapes the redox state of the cell. This work will impact our understanding of neuroinflammatory pathways as well as the role of MPO as a biomarker for disease and mediator of inflammation.

Disclosures: T. Appleton: None. **S. Ewing:** None. **D. Donley:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 286.26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01EY029913
GM061655

Title: Microglia depletion and repopulation alleviates vascular damage and neuronal loss in the diabetic retina

Authors: *K. A. CHURCH, D. RODRIGUEZ, I. LOPEZ-GUTIERREZ, D. VANEGAS, S. M. CARDONA, A. E. CARDONA;
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Abstract: Diabetic retinopathy (DR), is the leading cause of blindness amongst working age adults. Microglia, the resident phagocytes of the retina, are believed to influence the development of DR, but their exact contributions to vascular integrity and neuronal loss are unknown. Previous studies have shown that diabetic retinas with aberrantly activated microglia in the absence of *CX3CR1 or CX3CL1*, show (1) robust microglial activation and elevated release of pro-inflammatory mediators-IL-1β and TNF-α and decreased production of anti-inflammatory cytokines IL-10 and IL-13, (2) cellular clustering around blood vessels and (3) robust fibrinogen deposition and vascular damage. Therefore we hypothesize that **acute depletion** and ***repopulation*** of $CX3CR1^{CreER}$:$R26^{iDTR}$ microglia during the course of disease when microglia receive the early-stage environmental cues of retinal inflammation due to

hyperglycemia will induce neuroprotective cues to alleviate neuronal and axonal loss and prevent fibrinogen deposition into the diabetic retina. Utilizing a genetic model to express diphtheria toxin receptor (DTR) under the tamoxifen (TAM) inducible *CX3CR1*-promoter, microglia become susceptible to the effects of diphtheria toxin (DTx). **Microglia were depleted in diabetic and control mice for two weeks from six to eight weeks of diabetes**. ***Following a two week microglia repopulation***, vascular and neuronal damage were assessed at ten weeks of diabetes. Our findings revealed that transient depletion of microglia induced neuroprotective cues to prevent $NeuN^+RBPMS^+$ neuronal loss and induced thicker $TUJ1^+$ axons. Transient depletion of microglia also decreased fibrinogen extravasation into the diabetic retina. Repopulating microglia displayed a resting phenotype mirroring those of non-diabetic TAM controls in contrast to diabetic mice displaying microglia with amoeboid morphology and decreased the percentage of $Ly6C^+$ infiltrating monocytes into brain and spinal cord tissues. Microglia depletion and repopulation during the course of disease when microglia receive early-stage activation cues, is neuroprotective by inducing the proliferation of a homeostatic microglia cell population that prevents neuronal loss and supports vascular repair.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.27

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01EY029913

Title: Fractalkine isoforms differentially regulate microglia activation and vascular damage in the diabetic retina

Authors: *D. RODRIGUEZ[1], K. A. CHURCH[1], A. N. PIETRAMALE[1], S. M. CARDONA[1], D. VANEGAS[1], I. A. MUZZIO[2], K. R. NASH[3], A. E. CARDONA[1];
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Abstract: Diabetic retinopathy (DR) is a microvascular complication and leading cause of blindness worldwide due to loss of neurons, microgliosis, and vasculature damage. Inflammation caused by microglia exacerbates the retinal integrity, resulting in glial cell dysfunction. The microglia-neuronal crosstalk mediated by CX3CR1 and fractalkine (FKN) signaling provides a neuroprotective environment in several neurological diseases. CX3CR1 polymorphic variants present in about 30% of the population decreases FKN binding affinity to CX3CR1, and their role in microglia function in the retina during diabetes is unknown. FKN is a membrane-bound

(mFKN) adhesion molecule. When cleaved by proteases, the soluble (sFKN) form is released with chemoattractant properties. Studies show that diabetic CX3CR1-KO and FKN-KO mice display worse retinal pathology, and intra-vitreal administration of recombinant FKN to diabetic FKN-KO mice rescued vascular pathology. Mechanisms by which mFKN and sFKN regulate retinal function are still unknown. We hypothesize that during diabetes, the over-expression of sFKN using recombinant adeno-associated viruses (rAAVs) will prevent vascular and neuronal damage, and improve visual function. To test this hypothesis, rAAVs expressing mFKN or sFKN were delivered to FKN-KO retinas. FKN protein levels, microglial activation, fibrinogen leakage, neuronal densities, and visual acuity were compared in diabetic and non-diabetic controls. Immunofluorescence and high-resolution confocal imaging show that sFKN minimizes microglial activation, reduces fibrinogen deposition, and rescues neuronal loss, compared to mice administered with rAAV-mFKN. Retinas with rAAV-sFKN of diabetic and non-diabetic mice was sufficient in improving visual function by implementing a two-choice visual discrimination task through learning-based behavior. In conclusion, our data suggest that delivery of rAAV-sFKN, compared to rAAV-mFKN, ameliorates activation of microglia, vascular damage and prevents neuronal loss. rAAV-sFKN correlates with the success rate of the mice finding the reward based on their ability to distinguish between spatial gradients. FKN appears to act as a neuroprotective agent in the diabetic retina, potentially serving as an alternative pathway to implement translational and therapeutic approaches to minimize retinal pathology and improve visual function.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

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Program #/Poster #: 286.28

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2017M3A9G6068257
HT13C1015

Title: Longitudinal quantification of neuroinflammation in chronic and infectious disease models using a novel fluorescent reporter mouse model

Authors: M. BOITET[1,2], H. EUN[1], A. ACHEK[1], ***R. GRAILHE**[1];
[1]Inst. Pasteur Korea, Seongnam-si, Korea, Republic of; [2]Dept. of Biol. Chem., Univ. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Today, there are few reports of successful in vivo fluorescence imaging due to strong light absorption and scattering mainly by the tissue at wavelengths below 600 nm and auto-

fluorescence impeding the signal-to-noise ratio. Fortunately, as a new generation of red and far-red fluorescent proteins has emerged, one can select fluorescent proteins with excitation wavelengths above 600 nm, adapted to in vivo imaging based on their spectral properties, brightness, stock shift, and maturation. In our study, we characterize a new transgenic mouse expressing the far-red fluorescent protein E2-Crimson under the control of a human GFAP promoter. Direct fluorescence histology of brain slices revealed the presence of E2-crimson positive cells across all brain regions. Counter-staining confirmed the absence of co-localization of E2-crimson within microglial or neuronal cells, but a specific expression of E2-Crimson in astrocyte cell population as its signal overlaps with GFAP immunostaining. Furthermore, in vivo fluorescent imaging of GFAP-E2-Crimson mouse shows that the endogenous expression of E2-Crimson was bright enough to be quantified during the entire animal lifespan and up-regulated upon neuro-inflammation. Finally, we will present in vivo study cases involving astrocyte reactivity triggered upon (i) amyloid deposit from Alzheimer's disease model, (ii) encephalitis virus and (iii) pharmacologically induced seizures and neurotoxic action of kainic acid.

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Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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HT13C1015
UST YOUNG SCIENTIST RESEARCH PROGRAM 2018

Title: Development and validation of a cost-effective in vivo fluorescent imaging system applied to brain inflammation

Authors: *M. BOITET[1,2], A. ACHEK[1], R. GRAILHE[1];
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Abstract: Optical fluorescent imaging is a functional technology based on light emitted by a fluorescent reporter probe either injected or expressed in living animals. This standard tool enables a dynamic investigation of diseases' pathophysiology and therapeutic activity in animals throughout their lifespan. Unfortunately, in vivo fluorescence imaging instrumentations are costly and might not always be optimized to the reporter spectral properties. In this study, we propose an alternative, do-it-yourself equipment, called BrightMice, based on a digital color camera modified to detect far-red fluorescent signals. BrightMice in vivo imaging system takes advantage of two widespread and affordable technologies: 3D printing and consumer cameras. BrightMice was designed to provide (1) top-acquisition far-red imaging, (2) total chamber

darkness to limit background noise, (3) instrumentation compactness, and (4) low cost. As a proof of concept, we used a novel GFAP-E2-Crimson transgenic mouse, expressing a far-red protein in brain tissue shown to report neuroinflammation in vivo. Using this reporter mouse strain, we demonstrated that BrightMice provides a comparable signal-to-noise ratio as found with expensive commercial instruments. BrightMice is suitable for rapid phenotypic screening of freely moving transgenic pups, and distinguishing hemizygous and homozygous genotypes in GFAP-E2-crimson transgenic animals. Furthermore, BrightMice compactness was suitable for Animal Biosafety Level-2/3 facility, as demonstrated upon successful neuro-inflammation tracking in adult GFAP-E2crimson mice infected with Japanese Encephalitis Virus.

Disclosures: **M. Boitet:** None. **A. Achek:** None. **R. Grailhe:** None.

Poster

286. Microglia, Neuroinflammation, and Immune Function: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 286.30

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CAPES
CNPQ

Title: Mesenchymal Stromal Cells Protect the Blood-Brain Barrier, Reduce Astrogliosis, and Prevent Cognitive and Behavioral impairments in an Experimental Model of Sepsis

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Abstract: Sepsis is a systemic inflammatory host-response against a pathogen; it is the largest cause of admission to intensive care units (ICUs) in the United States. Sepsis-associated encephalopathies (SAEs) are neurological complications that occur during or after sepsis events. SAE causes long-term neurological consequences affecting memory, cognition and mood. Currently, there is no prevention or treatment for neurological damage resulting from SAEs. Thus, investigating new therapies is necessary. Both clinical and experimental studies have been show the effects of cell therapy in neurodegenerative diseases. Indeed, the immunomodulatory capacity of mesenchymal stromal cells (MSCs) is well established. The aim of the present study was to evaluate the effects of MSC therapy on blood-brain barrier (BBB) maintenance, astrocyte activation, neuroinflammation and cognitive/ behavioral damage in an experimental model of sepsis. In order to investigate that, we used male adult Swiss mice and cecal ligation and puncture (CLP) surgery for sepsis induction and sham-operated animals were used as control. Six hours after surgery, mice were treated with MSC intravenously (1×105 cells in 0.05 mL of

saline/mouse) or saline. At day 1 the clinical score and plasma levels of Interleukin (IL) -1β, IL-6, and MCP-1 (assessed by ELISA) were increased in CLP animals. Cell therapy did not affect clinical score or survival rate, but led to a reduction in systemic levels of IL-1β, IL-6, and MCP-1 and also led a reduction in cortical and hippocampal cytokine levels. MSC therapy led a BBB protection (evaluated by Evans Blue Dye Assay) and a reduction in astrogliosis (evaluated by immunofluorescence) at 72h. In cultured astrocytes stimulated with lipopolysaccharide (LPS) conditioned media from MSCs reduced astrogliosis at 24h, suggesting a paracrine mechanism of action. These results might be related with an improvement in spatial memory (Morris Water Maze test), aversive memory (fear-conditioned test) and anxiety-like behavior (Elevated plus maze test). Thus, we can conclude that a single dose- therapy with MSC led to an improvement in cognitive damage, behavioral impairments, protection of the BBB, reduction in local levels of cytokines and astrogliosis and these effects might be associated with a decrease in systemic levels of inflammatory mediators.

Disclosures: M. Barbosa-Silva: None. **A.Y.O. Silva:** None. **É.A. Amorim:** None. **M.N. Lima:** None. **H.A. Oliveira:** None. **M.G. Granja:** None. **R.M.P. Campos:** None. **P.R.M. Rocco:** None. **H.C. Castro-Faria-Neto:** None. **T. Maron-Gutierrez:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.01

Topic: C.08. Ischemia

Support: NIH DSPAN Grant: 5K00NS105220-04
NIH: Medical University of South Carolina COBRE in Stroke Recovery Research Pilot Project Program
College of Charleston Major Academic Year Support (MAYS) for Undergraduate Student
1R01NS099595-01A1, NIH NINDS (Robinson)

Title: Intranasal Administration of BDNF Improves Cognitive Recovery and Promote Synaptic and Dendritic growth in a Neonatal Mouse Model of Hypoxic Ischemia

Authors: *S.-K. SIMS[1], L. MCGONEGAL[2], M. SADDOW[2], C. ROBINSON[1];
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Abstract: INTRODUCTION: Neonatal stroke, which occurs as frequently as 1 in every 2,500 live births is caused by oxygen deprivation to the brain. Premature infants have an increased risk of experiencing a hypoxic ischemic event and subsequent lifelong neurological disabilities. While treatments for adults with stroke include anticoagulants, motor and cognitive rehabilitation, neonatal stroke care is limited to supportive care, such as hyperthermia and

hyperbaric oxygen therapy. Previous studies reported that intravenous administration of brain derived neurotrophic factor (BDNF) in the acute post-stroke period reduces cell death and infarct volume in preclinical stroke model in rats. Hence, while neurotrophic factors offer neuroprotection, hence there is a need to evaluate potential strategies to increase neurotrophic factors in the brain. Intranasal delivery is attractive in that it can promote widespread distribution of BDNF within both the brain and spinal cord. Our overall hypothesis is that intranasal BDNF will improve functional recovery including overall brain health and development following neonatal stroke. We explored the benefit of intranasal BDNF treatment on functional recovery in a neonatal P7 hypoxic mouse model.METHODS: For our model of hypoxic ischemic, a ligation of the right carotid artery was induced which is followed by a two-hour exposure to an 8% oxygen/ 92% nitrogen in an enclosed chamber. Male and female pups were subjected to a 2 h hypothermia in a temperature-controlled chamber as a standard of care. A solution of saline (control) or recombinant human BDNF (Harlan Laboratories .1uM in saline) was administered with a Gilson pipette at the same time each day for 7 days into each nasal cavity in awake mice. We evaluated cognitive recovery using novel tactile recognition (NTR) and novel object recognition (NOR) and western analysis to analyze neuro-markers and brain health such as synaptophysin and microtubule associated protein -2 (MAP2). RESULTS: The objective of these studies is to address these gaps in knowledge and evaluate the role and therapeutic potential of BDNF in neonatal stroke recovery. Our results suggest a differential impact of intranasal BDNF on pro and mature BDNF in cortical and hippocampal brain regions, which correlate with cognitive and motor outcomes. Our results suggest that higher levels of mature BDNF are predictive of better improvements at day 42 on cognitive and motor assessments. Our results also suggest that greater cognitive improvement correlated with higher levels of synaptophysin and MAP2 which suggests greater neuroplasticity after injury.

Disclosures: S. Sims: None. **L. McGonegal:** None. **M. Saddow:** None. **C. robinson:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.02

Topic: C.08. Ischemia

Support: R21NS123814
R21AG061643

Title: Human mutant APP/PS1 transgenes do not influence touchscreen-assessed cognitive function outcome in adult mice with early-life hypoxia-ischemia

Authors: *K. M. CARLIN[1], L. L. JANTZIE[2], V. TURNBILL[5], S. ROBINSON[6], L. DOUCETTE[3], M. ST. PIERRE[3], R. CHAVEZ-VALDEZ[7], L. J. MARTIN[4], F. J. NORTHINGTON[8];
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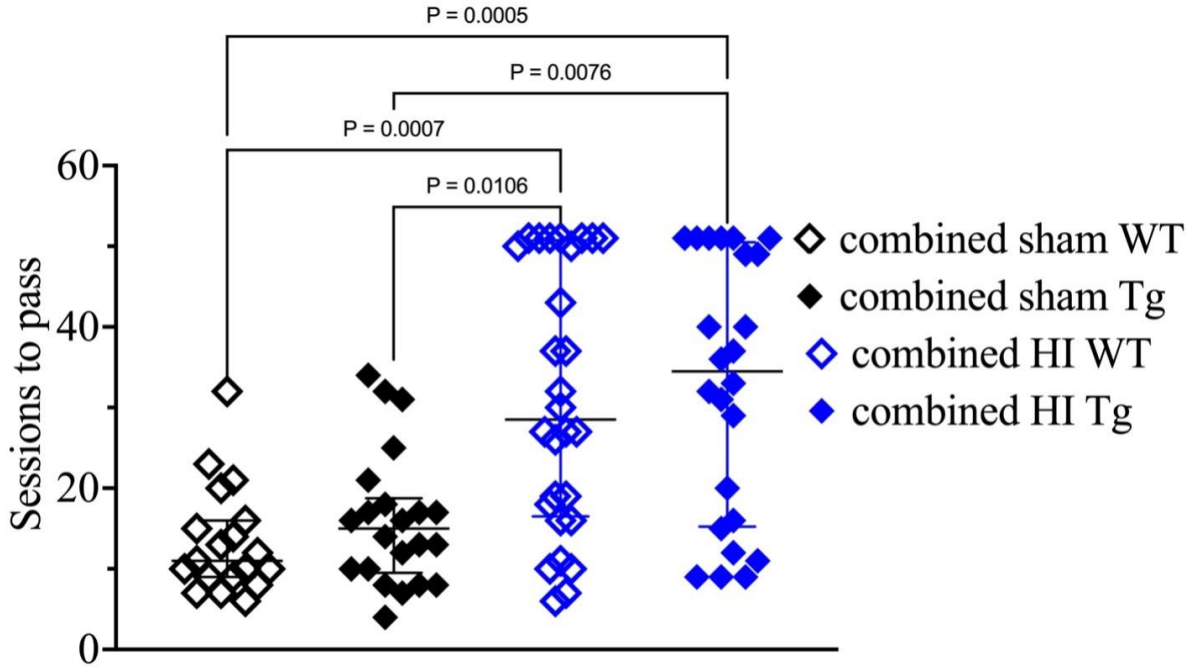
Hopkins Univ. Sch. of Med., Baltimore, MD; [5]Dept. of Pediatrics, [6]Neurosurg., [7]Pediatrics, Johns Hopkins Univ., Baltimore, MD; [8]Pediatrics and Neonatalogy, Sch. of Med., Baltimore, MD

Abstract: Hypoxic-Ischemic Encephalopathy (HIE) during development permanently alters cerebral function. Despite the use of therapeutic hypothermia, HIE survivors have executive dysfunction and cognitive deficits like adults with neurodegenerative diseases. More discriminatory testing will improve sensitivity of cognitive testing. Mice expressing transgenes for many adult-onset neurodegenerative diseases have higher mortality after early brain injury. We examined cognitive function after neonatal HI in mice with and without human mutant APP/PS1 transgenes to test the hypotheses that neonatal HI in mice results in adult cognitive impairments, which is worse in mice expressing human familial Alzheimer's disease mutations in amyloid precursor protein (APP) and presenilin-1 (PS1). APP/PS1 transgenic (Tg) mice, and non-transgenic (nTg) littermates received neonatal HI (carotid ligation/ 45m of FiO_2 0.08) or anesthesia alone (sham) at P10. We tested all survivors for visual discrimination (VD) using a touchscreen testing platform starting at P70 after a week of feeding restriction to no more than 15% of loss of body weight. Data from 22 Sham nTg, 24 Sham Tg, 28 HI nTg and 27 HI Tg mice were analyzed using Brown-Forsythe and Welch ANOVA. HI mice required increased number of total trials ($P<0.0001$ (HI nTg vs Sham nTG) and $P<0.001$ (HI Tg vs Sham Tg), increased number of correction trials ($P<0.001$ and $P<0.005$), increased incorrect trials ($p<0.0005$ and $p<0.005$) and increased number of sessions to pass ($P<0.001$ and $P<0.01$) respectively compared to sham mice. This study is the first to show impaired VD after HI, but there was no effect of APP/PS1 genotype. Effect is primarily driven by males. Assessment of cognitive function after neonatal HI in mice with genetic risk for adult neurodegeneration is novel. Despite strong drivers for APP/PS1 transgenes, there is a decisive lack of transgene effect on VD. Further studies about learning acquisition, cognitive flexibility and executive dysfunction will provide insights into long-term effects of HIE in neonates of different genetic backgrounds.



Disclosures: **K.M. Carlin:** None. **L.L. Jantzie:** None. **V. Turnbill:** None. **S. Robinson:** None. **L. Doucette:** None. **M. St. Pierre:** None. **R. Chavez-Valdez:** None. **L.J. Martin:** None. **F.J. Northington:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.03

Topic: C.08. Ischemia

Support: 19gk0110033h0002
JP20K16875

Title: Lox-1 mediates the inflammatory activation of microglia under hypoxic ischemic conditions

Authors: *Y. AOKI[1,2], M. ITOH[2];
[1]Pediatrics, Univ. of Miyazaki, Miyazaki-shi Kiyotake-cho Kihara, Japan; [2]Natl. Ctr. of Neurol. and Psychiatry, Kodaira/Tokyo, Japan

Abstract: Microglia play an important role in the immune system in the brain. Activated microglia are not only injurious but also neuroprotective. We previously confirmed marked lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression in microglia in pathological lesions in the neonatal hypoxic-ischemic encephalopathy brain model. LOX-1 is known to be an activator of cytokines and chemokines through intracellular pathways. Here, we investigated a novel role of LOX-1 in microglia under hypoxic and ischemic conditions. We isolated microglia from brains of 0-3 day-old SD rats and confirmed each cultured well, which was occupied over 98% with Iba-1 positive cells. Then, we performed oxygen-glucose deprivation (OGD) insult to the cultured microglia for 6 hours and evaluated the expression levels of LOX-1, cytokines and chemokines. As a result, we found that defects in oxygen and nutrition induced LOX-1 expression and led to the production of inflammatory mediators, such as the cytokines IL-1β, IL-6 and TNF-α, the chemokines CCL2, CCL5 and CCL3. Then, the LOX-1 signal transduction pathway was blocked by LOX-1 siRNA and the production of inflammatory mediators was suppressed. Next, we investigated signal transduction pathway of LOX-1 in microglia. As the intracellular signaling pathway of LOX-1 is known, we investigated p38-MAPK and ERK1/2 activity and phosphorylation. OGD insult induced phosphorylation of p38-MAPK and ERK1/2. LOX-1 inhibition recovered the p38-MAPK phosphorylation level but not the ERK1/2 phosphorylation level. We also investigated the NF-κB distribution pattern in OGD-treated microglia. NF-κB p65 expression was examined in the cytoplasm in primary microglia. After OGD induction, NF-κB p65 expression was mainly in the nucleus. However, LOX-1 knockdown in OGD-treated microglia recovered LOX-1 expression in the cytoplasm. In conclusion, the expression of LOX-1 was induced in microglia and was related to cytotoxic inflammatory process under hypoxic ischemic conditions. The upregulation of LOX-1 was

related to the activation of p38-MAPK and NF-κB, but not that of ERK 1/2. LOX-1 and its related molecules may be therapeutic targets in neonatal hypoxic-ischemic encephalopathy

Disclosures: Y. Aoki: None. **M. Itoh:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.04

Topic: C.08. Ischemia

Support: ISSSTE Grant E015-233.2017.

Title: Effect of Cerebrolysin treatment of neonatal hypoxic-ischemic encephalopathy rat model on the HDAC gene family expression

Authors: *E. BARRIENTOS ZAVALZA[1], C. PIÑA LEYVA[2], M. LARA-LOZANO[3], G. FLORES[4], D. MARTINEZ-FONG[2], B. LEON-CHAVEZ[5], A. JIMENEZ-ANGUIANO[6], J. A. GONZALEZ-BARRIOS[7];
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Abstract: Hypoxic-ischemic encephalopathy (HIE) is one of the main causes of neonatal death, and in survivors it generates long-term neurological sequelae that include learning and memory deficits, motor damage and cerebral palsy. Among the mechanisms that contribute to neurological dysfunction are excitotoxicity, apoptosis, necrosis and inflammatory process; all associated with epigenetic modifications that maintain control of gene expression during HIE. Histone deacetylases (HDAC) are enzymes that catalyze the deacetylation of histone lysine residues and their activity is related to gene repression. Furthermore, changes in HDAC gene expression have been described after hypoxia-ischemia injury. Previous studies in our laboratory have shown that Cerebrolysin administration in rats with HIE improves motor skills, learning and memory. The aim of this study was to evaluate the effect of Cerebrolysin administration on the HDAC gene family expression in neonatal rat model of HIE. We analyzed cerebral cortex and hippocampal samples obtained from HIE rats, 24 hours and 21 days after cerebral injury. Total RNA extraction and reverse transcription were performed; subsequently, the expression of the HDAC family genes was evaluated by q-PCR. The expression of HDAC genes family after 24 hours of hypoxia-ischemia injury, did not show statistical differences between experimental groups. Whereas, at 21 days after the brain injury, we found an increase in expression of HDAC2

and HDAC3 genes in cortex; and increase in expression of HDAC2, HDAC3 and HDAC7 genes in the ipsilateral hippocampus of HIE animals treated with Cerebrolysin. Where the HDAC4, HDAC5, HDAC6, HDAC8, HDAC10 and HDAC11 genes expression were not modified. Our findings demonstrate that Cerebrolysin administration in HIE neonatal rats exerts its epigenetic effect on learning, memory and motor system by up-regulation of HDAC2, HDAC3 and HDAC7 genes.

Disclosures: **E. Barrientos Zavalza:** None. **C. Piña Leyva:** None. **M. Lara-Lozano:** None. **G. Flores:** None. **D. Martinez-Fong:** None. **B. Leon-Chavez:** None. **A. Jimenez-Anguiano:** None. **J.A. Gonzalez-Barrios:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.05

Topic: C.08. Ischemia

Support: NIH Grant 1R21NS109838-01A1
NIH Grant 1R01NS119251-01A1

Title: Long term effects of intestinal microbiota composition on cortical excitability and behavior after neonatal intermittent hypoxia

Authors: I. GOUSSAKOV, S. SYNOWIEC, ***A. DROBYSHEVSKY**;
NorthShore Univ. HealthSystems, Evanston, IL

Abstract: Background: Premature infants are often lacking normal microbial community in their intestines that naturally should have come from mothers. Premature infants are also often at risk of perinatal hypoxic brain injury, considered as one of the major factors leading to motor, sensory and cognitive deficits. We hypothesize that neonatal gut microbiota composition can modulate cortical maturation of excitatory/inhibitory circuit and long term cognitive and behavioral outcome in response to neonatal brain to hypoxic brain injury. Methods. C57BL/6 mouse pups were exposed to intermittent hypoxia (IH) regiment consisting 12 to 20 hypoxic episode daily of 5% oxygen exposure for 2 min at 37C from P3 to P7. Microbial manipulation groups consisted of triple-antibiotic treatment from E18 (maternal) + postnatal gavage till P9 of 1) antibiotics, 2) E.coli, 4) B. infantis, 4) saline, 5) naïve pups. Behavioral tests were conducted at P60-P80, followed by MRI and patch clamp recording in cortical slices. Results. Amplitudes and frequencies of mEPSC's and IPSC's in motor cortex were downregulated after IH relative to normoxia, except antibiotics group where both mEPSC's and IPSC's were decreased in hypoxia and normoxia. Open field test revealed a significant increase in traveled distance after neonatal hypoxia, but there was difference between microbiome groups. No difference either between hypoxia or microbiome groups was found on elevated plus maze, novel objects recognition and spontaneous alteration Y-maze tests. Both cued and context fear conditioned memory were

decreased after IH with mixed effects due to microbial composition. Conclusion. The data suggest that both excitatory and inhibitory transmission in adults is reduced after neonatal IH. Long-term changes in cortical network maturation are likely affect cognitive and behavioral outcome. The effect is modulated by microbial gut composition in neonates during IH and is likely complex.

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.06

Topic: C.08. Ischemia

Support: RO1 NS105905
K08 NS086969

Title: Hippocampal disfunction following a novel model of global cerebral ischemia

Authors: *E. TIEMEIER, J. VIGIL, C. MINJAREZ, A. DINGMAN, R. DIETZ, N. QUILLINAN;
Univ. of Colorado, Denver, CO

Abstract: Hippocampal injury and cognitive impairment produced by cardiac arrest in neonatal children effects significant developmental delays later in life. Current study of ischemic injury in neonates centers around established MCAO and photothrombotic models of focal injury with neonatal global ischemic models of asphyxial induction, superior aortic artery occlusion, and electrical induction via transesophageal AC stimulation producing imperfect, unsustained global injury or undesired secondary injury. To address these challenges, a novel rodent model of global cerebral ischemia was developed in neonatal rats. Cardiac arrest was induced in post-natal day 10 rats via intravenous 0.5M potassium chloride. Following 10-14-min of asystole, cardiopulmonary resuscitation CPR was performed with chest compressions and intravenous epinephrine with return of spontaneous resuscitation within 3 minutes. Left ventricular whole blood samples were collected and analyzed 30 minutes after CA/CPR. Neuronal injury was assessed 3 days after CA/CPR by Fluorojade and NeuN staining in coronal brain sections. To assess synaptic plasticity of surviving neurons, extracellular recording from hippocampal CA1 region were performed 14 days after CA/CPR (taken from Schaffer-collateral pathway in acute hippocampal slices following a theta-burst stimulation). Increase in field excitatory post-synaptic potential (fEPSP) slope 60 min after theta burst stimulation of the Schaffer-collateral pathway (40 pulses 100Hz) was analyzed as a measurement of long-term potentiation (LTP). Results reported as mean±SD. Within these experiments, 10/14 rats were successfully resuscitated and survived to their endpoint. Blood gas analysis 30 min after CA/CPR revealed pH 6.7±0.14 compared to 7.3±0.05 in sham rats (n=3, p=0.02). In addition, lactate was increased 30 min

following GCI (10.3±1.4 mmol/L vs 2.4±0.4 mmol/L in sham, n=3, p=0.03), as was troponin (GCI: 8.1±2.3 ng/ml vs Sham 1.2±0.4 ng/ml, n=3, p=0.04). Fluorojade revealed neuronal cell death in the hippocampus and striatum at 3 days after CA/CPR. To assess cognitive function, hippocampal LTP was assessed 14 days following GCI. In sham mice, LTP was 179±45% (n=5) of baseline (set to 100%) 14 days following surgery. In contrast, LTP was impaired 14 days after GCI (106±20%, n=5, p=0.01 compared to sham mice). These results demonstrate acute cardiac injury, neuronal cell death and hippocampal dysfunction following neonatal CA/CPR. These results are consistent with clinical outcomes in perinatal brain injury. Future studies will extend these results to evaluate cognitive-affective outcomes in the juvenile period of development.

Disclosures: E. Tiemeier: None. **J. Vigil:** None. **C. Minjarez:** None. **A. Dingman:** None. **R. Dietz:** None. **N. Quillinan:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.07

Topic: C.08. Ischemia

Support: NIH NINDS R35-NS116852

Title: Role of neuronal cation-chloride cotransporters in tissue shrinkage after acute brain injury

Authors: *F. BAHARI[1], K. J. STALEY[2];
[1]Massachusetts Gen. Hosp., Charlestown, MA; [2]Neurol., Massachusetts Gen. Hosp., Boston, MA

Abstract: Brain injury in very low birth-weight (VLBW) babies is extremely common. Intraventricular hemorrhage (IVH) is one of the more severe complications in these neonates. In United States alone about 12000 VLBW infants develop IVH every year. A large number of infants with IVH (50% to 75%) later develop cerebral palsy, intellectual disability, and/or hydrocephalus. The pathophysiology of brain injury leading to IVH in this patient population is unclear, therefore providing effective intervention is challenging. We hypothesize that because of the unique salt and water transport systems expressed in immature neurons, unlike mature neurons, they shrink in response to injury. The neuronal volume loss leads to local tissue shrinkage which then triggers blood vessel displacement and rupture, and IVH.To test our hypothesis we measured the neuronal volume in immature and mature tissue before and after acute injury, and correlated the volume changes with the expression of membrane salt and water transporters. Further, we investigated whether genetic or pharmacological manipulation of transporter expression or activity alters neuronal volume response to injury.Neuronal volume responses as well as blood vessel displacement were monitored using *in vivo* and *in vitro* multi-photon imaging of transgenic mice. Age-dependency of neuronal membrane salt and water transporter expression was clarified using immunohistochemical techniques. We found that the injury-induced volume changes were correlated with neuronal age-dependent expression of salt

and water transporters: Injured immature neurons shrink, leading to large blood vessel displacement. This displacement represents a candidate mechanism for blood vessel rupture and hemorrhage. Pre-injury pharmacological or genetic alterations in transporter activity or expression stabilized the volume response to injury and thus would reduce the chance of hemorrhage. Our findings open a new avenue of investigation for development of new clinical techniques to detect acute brain injury and prevent neuronal shrinkage and the ensuing IVH in VLBW babies.

Disclosures: F. Bahari: None. **K.J. Staley:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.08

Title: WITHDRAWN

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.09

Topic: C.08. Ischemia

Support: NIH NINDS K08 NS101122

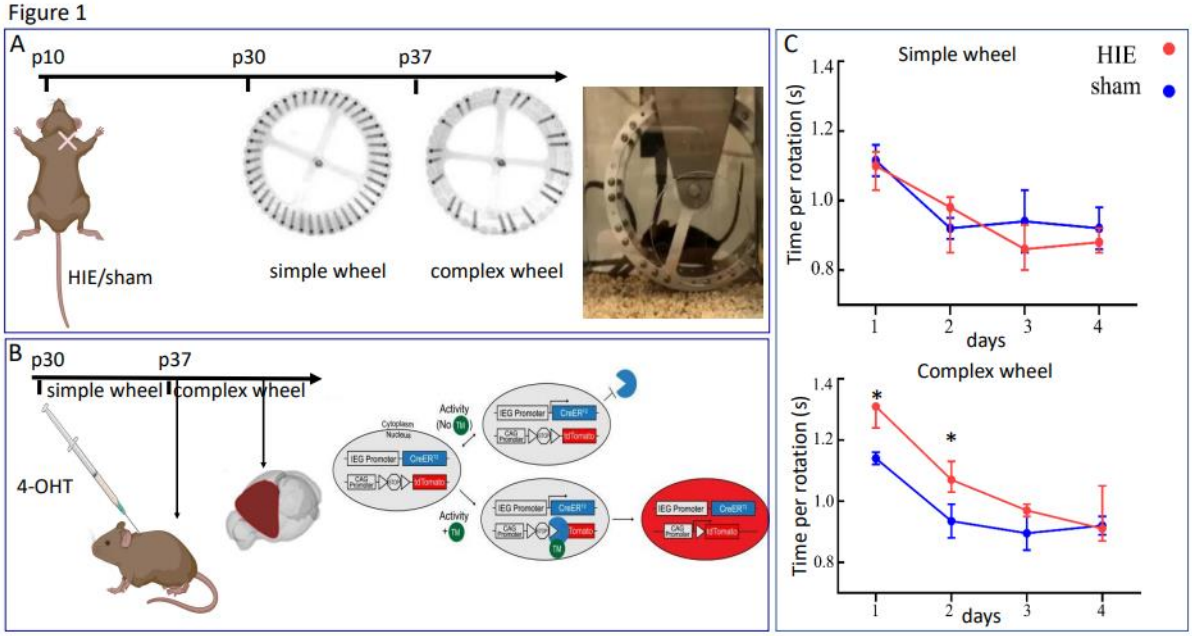
Title: Motor learning and the motor engram following neonatal hypoxic ischemic encephalopathy

Authors: *W. MATYSIK[1,2], J. KAPUR[3], J. BURNSED[4];
[1]Univ. of Virgina, Charlottesville, VA; [2]Biol. Sci., UCSD, San Diego, CA; [3]Dept Neurol., Univ. Virginai Hlth. Sci. Ctr., Charlottesvle, VA; [4]Pediatrics, Univ. of Virginia, Crozet, VA

Abstract: Motor deficits are common following neonatal brain injury. Even school-age children without gross motor impairment, are at risk for problems with fine motor coordination and processing following neonatal hypoxic-ischemic encephalopathy (HIE). These deficits may result from disruption of the complex cascade of events necessary for motor learning. The formation of a motor engram, an ensemble of neurons that contain a specific motor memory, is critical in motor learning. The aim of our study was to test motor learning and map motor engram formation in a mouse model of neonatal HIE. HI is created using unilateral carotid ligation+60 min of 8%O2 in Cre-tamoxifen mice (TRAP2) on p10. Shams receive

incision+anesthesia without hypoxia/ligation. Starting on p30 performance is measured on a simple wheel (gross motor testing) and complex wheel tasks (motor learning over several days) (McKenzie 2014). 4-OHT injection allows expression of fluorescent protein (tdTomato) in active neurons (expressing cfos). To capture neuronal activity in early and late training, mice were injected on day 1 and then perfused on day 4 to costain with cfos. There were no differences in performance between HI and sham on the simple running wheel (n=5/group, p=0.7 day 1, p=0.6 day 4). On the complex running wheel, sham mice exhibited faster performance than HI on days 1 and 2, but by day 4 were similar (n=5/group, p=0.018 day 1, p=0.67 day 4). Neuronal activity in uninjured mice decreased in both primary and secondary motor cortex (MC) between day 1 (tdTomato) and 4 (tdtomato+C-fos). 33% of neurons on day 1 were reactivated with subsequent learning on day 4. Young adult mice exposed to neonatal HI exhibit deficits in motor learning, but not gross motor skills. Preliminary data show neuronal activity decreased in MC with subsequent motor learning. A subset of neurons active on day 1 of learning are reactivated with ongoing learning. These neurons may represent motor engram neurons in the MC. Ongoing work examines changes in the motor engram after neonatal HI.

Figure 1



Disclosures: **W. Matysik:** None. **J. Kapur:** None. **J. Burnsed:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.10

Topic: C.08. Ischemia

Support: AMED- CREST 18gm1210005h0001
JSPS KAKENHI 21H05049

Title: Intravital imaging of neutrophil recruitment in the peri-infarct area of a photothrombotic stroke model

Authors: *N. SHIBUYA, T. ITOKAZU, T. UEDA, T. YAMASHITA;
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Abstract: While neutrophils are known to be recruited to peri-infarct area after the ischemic brain injury and affect neural tissues in deleterious ways, the precise mechanism of the recruitment of neutrophils remains to be elucidated. This study aimed to explore potential therapeutic agents for modifying neutrophil behaviors through intravital imaging of an experimental stroke mouse model. The combination of in vivo two photon (2P) imaging and a tightly controlled photothrombotic stroke mouse model enabled us to investigate the temporal dynamics of the evolution of ischemic brain lesions in a highly reproducible manner. Taking advantage of this system, we showed that neutrophil depletion by a neutrophil specific antibody mitigated the infarct expansion, confirming the noxious effect of neutrophils against the ischemic brain. To identify therapeutic approaches targeting neutrophils in ischemic brain injury, various agents were screened, and colchicine and an anti-P-selectin antibody were found to be potent in blocking neutrophil recruitment. In the early phase (6h post-infarction), both agents effectively inhibited neutrophil attachment to the vessel wall. On the other hand, in the later phase (16h post-infarction), only colchicine was capable of inhibiting neutrophil infiltration into the peri-infarct area. Subsequent analysis revealed that the effect of the anti-P-selectin antibody against neutrophil attachment to the vessel wall was transient and thus insufficient for mitigating neutrophil infiltration. Finally, colchicine treatment was shown to ameliorate infarct expansion. Our results suggest that colchicine may be a candidate for novel stroke therapy, and that our intravital strategy is a potent tool for the direct investigation of pathophysiology in the ischemic border zone.

Disclosures: **N. Shibuya:** None. **T. Itokazu:** None. **T. Ueda:** None. **T. Yamashita:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.11

Topic: C.08. Ischemia

Support: NIH R01 NS105899

Title: Evaluating Sex Differences in Cellular Function in a Rodent Photothrombotic Stroke Model

Authors: *A. WHITE[1], H. H. CHAN[2], L. CHINTADA[7], M. PORTER[2], J. ALMEIDA[3], B. FISHER[4], J. HERMANN[2], A. G. MACHADO[5], K. B. BAKER[6];
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Abstract: Introduction: The photothrombotic model of stroke (PTS) is a model of cerebral ischemia that relies on free radical production and blood brain barrier disruption after Rose Bengal dye is distributed through the vasculature, and the target vessel is illuminated with bright light. Prior studies have reported differences in lesion volume after PTS in male and female animals, which mirrors sex differences in ischemic stroke. There is a need for a better understanding of the mechanistic underpinnings of sex differences in stroke, as well as how they are reflected in commonly-used rodent stroke models.**Methods:** Eleven male and ten female Long-Evans rats underwent PTS as follows: anesthetized animals received intraperitoneal injection of 75 mg/kg Rose Bengal dye. A 3 mm craniectomy was created over sensorimotor cortex, which was then illuminated with 150 kLux white light. Post-mortem, animals underwent histologic evaluation of lesion characteristics, including lesion volume calculation, with perilesional area stained for cellular degeneration (Fluoro Jade C) as well as caspase-8 and apoptosis-inducing factor (AIF) expression at 2 and 4 weeks post-stroke (5 male and 5 female animals per timepoint).**Results:** Ten female and ten male animals survived the procedure. At two weeks post-stroke, male animals had a significantly larger lesion volume than the females (males: 12.42 mm^3 vs females: 7.89 mm^3; p=0.032)). At four weeks, males had a median lesion volume of 9.62 mm^3 compared to a median of 5.11 mm^3 (p = 0.56) in females. Male animals had a trend towards greater neurodegeneration than females as measured by Fluoro Jade C .There was a trend towards lower expression of AIF in female compared to male animals. Female animals demonstrated a trend towards greater expression of caspase-8 compared to male animals.**Conclusion:** PTS in females leads to a lesion of smaller size compared to males at 2 weeks post-stroke, with a trend towards smaller size at 4 weeks post-stroke. Differential levels of caspase-8 and AIF expression across sexes are indicative of distinct mechanisms of ischemia-induced cell death between sexes. Future investigation into the neuroprotective properties of estrogen may illuminate the mechanism underlying these differences.

Disclosures: A. White: None. **H.H. Chan:** None. **L. Chintada:** None. **M. Porter:** None. **J. Almeida:** None. **B. Fisher:** None. **J. Hermann:** None. **A.G. Machado:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS. F. Consulting Fees (e.g., advisory boards); Enspire DBS, Abbott. **K.B. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS. F. Consulting Fees (e.g., advisory boards); Enspire DBS.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.12

Topic: C.08. Ischemia

Support: 2021R1A2C2006110
2021M3E5D9021364
2019R1A5A2026045

Title: Brain endothelial angiotensin II receptor mediates BBB disruption leadingto subcortical white matter degeneration in renovascular hypertensive rats

Authors: *X. JIN[1,2], Y. OH[1], Y. CUI[3], S. KIM[4], H. LEE[4], J. CHOI[5,1], B. KIM[1,5];
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Abstract: Chronic impairment of cerebral blood flow due to vascular risk factors such as hypertension may underlie white matter degeneration in the elderly, which is a pathological hallmark in subcortical ischemic vascular dementia. Few studies have successfully established an optimal animal model that exhibits unequivocal and replicable ischemic subcortical white matter lesions. We have recently observed distinct white matter pathologies in rats with renovascular hypertension (RVHT) induced by a two-kidney, two-clip (2K2C) procedure. In this model, we detected a marked reduction in myelin basic protein and axonal degeneration within the corpus callosum and cingulum. These white matter pathologies were accompanied by white matter hyperintensities on T2 MRI and a decrease in the fractional anisotropy value in diffusion tensor imaging. A substantial extent of mature oligodendrocyte (OL) loss was evident accompanied by an increased number of immature OLs, reactive astrogliosis, and microglial activation. Furthermore, deposition of fibrinogen and IgG extravasation in the subcortical white matter indicated increased permeability of the blood-brain barrier (BBB) during the pathological progression of the white matter damage. Consistent with these findings, an endothelial tight junction protein ZO-1 showed a progressive decline in this model. Angiotensin II (AT-II), the main hormone responsible for the RVHT, was significantly elevated in the plasma and more so in the CSF. Direct application of AT-II to cultured brain endothelial cells disrupted the endothelial tight junction *in vitro*. Intracerebroventricular infusion of losartan, an AT-II receptor blocker, significantly attenuated the white matter degeneration in the 2K2C RVHT model without affecting systemic hypertension. These results suggest that elevation of brain AT-II level in RVHT may be responsible for the disruption of BBB tight junctions and thereby trigger subcortical white matter degeneration. Potent inhibition of endothelial AT-II receptors within the brain may slow the progression of subcortical white matter degeneration, ultimately preventing vascular dementia in hypertensive elderly.

Disclosures: X. Jin: None. **Y. Oh:** None. **Y. Cui:** None. **S. Kim:** None. **H. Lee:** None. **J. Choi:** None. **B. Kim:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

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Program #/Poster #: 287.13

Topic: C.08. Ischemia

Support: NIH Grant R01NS064136C
NINDS R01NS093057
AHA postdoc fellowship 916011

Title: Deleterious Effect of Acute Hyperglycemia on Immune Responses and Stroke Outcome after Ischemic Stroke

Authors: *H. CHEN[1], T. C. CHIANG[2], A. KIM[3], T. BLISS[4], M. Y. CHENG[4], G. K. STEINBERG[4];
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Abstract: Background: Over 40% of ischemic stroke patients have hyperglycemia, which raises the risk of hemorrhagic transformation (HT) and worsens stroke outcomes. Insulin treatment does not improve the outcome. Understanding how hyperglycemia exacerbates stroke injury will be vital in developing new therapeutic strategies. Immune responses are critical in stroke injury, but the impact of acute hyperglycemia on post-stroke immune responses remains elusive. In this study we investigated the effects of acute hyperglycemia on immune responses and stroke outcome.**Method**: C57BL6 male mice were subjected to ischemic stroke by 30 min intraluminal middle cerebral artery occlusion (MCAO) followed by reperfusion. Sham controls underwent the same surgical treatment but without MCAO. To induce hyperglycemia, glucose was given intraperitoneally 10 min before MCAO. Cohort 1 was sacrificed at 4.5hr to evaluate BBB leakage with Evans blue assay; cohort 2 was sacrificed at 24hr to quantify brain infarct, HT and brain edema; cohort 3 was used for evaluating neurological deficits on post-stroke days 1, 3 and 7.
Cohorts 4 and 5 were euthanized at 4.5hr and 24hr, and their brain immune cells were analyzed with flow cytometry.**Result**: Acute hyperglycemia significantly increased BBB leakage and induced HT by 4.5hr, and worsened HT and brain edema by 24hr; there was no effect on brain infarct. These pathological events translated into higher overall mortality, greater body weight loss and more severe neurological deficit at post-stroke day 1, 3 and 7. The severity of HT was associated with worsened neurological deficit and shorter survival time. Flow cytometry analysis revealed that at 24 hr after stroke, hyperglycemia significantly increased the number of $Ly6c^+$ microglia, and also the intensity of the Ly6c and CD3 signal in microglia in the ischemic hemisphere. The number of neutrophils, B cells, and $CD8^+$ T cells in the ischemic brain was also increased. Pearson correlation matrix revealed that the quantity of infiltrating neutrophils significantly correlated with microglia Ly6c ($r=0.91$, $p<0.001$) and CD3 ($r=0.86$; $p<0.001$) intensity, suggesting that microglia activation and neutrophil infiltration are orchestrated in the ischemic brains. These immune changes were not significantly altered at 4.5 hr.**Conclusion**: Acute hyperglycemia adversely impacts stroke outcome, with hemorrhagic transformation and

brain edema as key pathological events, associated with higher mortality, and more severe neurological deficit. Acute hyperglycemia-induced microglia activation and infiltration of immune cells may play a key role in mediating the detrimental effects of hyperglycemia.

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.14

Topic: C.08. Ischemia

Support: CONACYT NO. 930329

Title: Generation of optical biosensors for the detection of IL-6 and IL-10 in animal blood serum with acute global cerebral ischemia

Authors: *O. MONTES[1,2], M. GARCIA[1], O. GONZALEZ[1], R. DELGADO[3];
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Abstract: Cerebrovascular accidents (CVA) are all neuropathies characterized by a decrease in blood flow and its nutrients to the brain; mainly oxygen and glucose. These diseases are considered the leading cause of death and disability in adults and the elderly. In CVAs there are molecules that decrease or increase brain damage. Interleukins (IL) are proteins that regulate inflammatory processes. IL-6 is characterized by facilitating the inflammatory response, whereas IL-10 is known for its anti-inflammatory effects. Plasma concentrations of both ILs are essential to generate more effective clinical protocols. In this study we developed optical biosensors for the detection of these ILs (IL-6 and IL-10) in the blood serum of ischemic male rats. For this project we used 90 male rats of the Sprague Dawley strain. The animals were divided into groups with; pharmacological treatment of estradiol benzoate (BE), control groups and sham groups. All animals were included in a global cerebral ischemia protocol and sacrificed: 2,4,6,12 and 24 hours post ischemia. The general objective of this work was to create specific optical biosensors for the detection of IL-6 and IL-10 in the blood serum of ischemic individuals; we also measured the concentrations of these ILs at different postischemia times and contrasted them with animals that received a famacological Tx of BE. Our work group has achieved the development of BO by means of a new methodology and the plasma measurements of ILs have shown a distinction between the experimental groups; this allows us to conclude that plasma concentrations of ILs vary as a function of postischemia time as well as of BE administration. Our work is a first approach to the development of commercial kits that, by using BO, allow the immediate and

specific detection of these ILs. Furthermore, these BO are low cost and do not require specialized personnel for their use.
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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

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Topic: C.08. Ischemia

Support: NIH Grant
RISE program

Title: Acid-sensing ion channels in long-term stroke recovery

Authors: *A. S. ARMSTRONG, T. YANG, T. LENG, Z.-G. XIONG;
Neurosci., Morehouse Sch. of Med., Atlanta, GA

Abstract: Stroke is a leading cause of death and long-term disabilities worldwide. Tissue plasminogen activator (tPA) is currently the only FDA approved therapeutic for ischemic stroke but has very limited success. Searching for new targets and more effective therapeutic strategies is still the focus of stroke research. Acid sensing ion channels (ASICs) are proton-gated cation channels that have well-established roles in ischemic brain injury. Applications of ASIC1a antagonists or ASIC1a deletion reduced infarct size after middle cerebral artery occlusion (MCAO). However, most studies done to establish ASICs' role in stroke so far are short-termed and only demonstrate their acute impact. It is unknown if the reduction in infarct size by ASIC1a inhibition or deletion translates to improved long-term survival and behavioral recovery. The objective of this study is to determine the effect of ASIC1a deletion on long-term stroke survival and behavioral recovery in mice. 30 min MCAO was performed in wild-type and ASIC1$^{-/-}$ mice. 24 hours after MCAO, mice were assessed for neurological deficits, and then underwent behavioral analysis weekly following MCAO for 4 weeks. Neurological deficit and behavioral analysis scores were analyzed for relative improvement over the 4 weeks and the differences between wild-type and ASIC1$^{-/-}$ mice were compared. We show that ASIC1$^{-/-}$ mice have a lower focal and general neurological deficit following MCAO than wild-type mice. In addition, ASIC1$^{-/-}$ mice are better able to maintain motor coordination and strength measured via pole test than wild-type mice. These findings suggest that ASIC1a deletion has a significant impact on long-term recovery of motor activities following ischemic stroke. Additional analysis of long-term survival and behavior recovery following MCAO is ongoing.

Disclosures: A.S. Armstrong: None. **T. Yang:** None. **T. Leng:** None. **Z. Xiong:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

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Program #/Poster #: 287.16

Topic: C.08. Ischemia

Title: The TRPV4 Channel and Ischemic Spreading Depolarization

Authors: *D. A. HANCOCK[1], R. D. ANDREW[1,2];
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Abstract: Ischemia induces spreading depolarization (SD) in the higher brain leading to associated swelling and injury of the participating neurons. There is debate about whether ischemic swelling is osmotically generated[1]. Certain TRP ion channels are Ca^{2+}-dependent with a role in mechano-osmotic transduction. The TRPV4 channel is a non-selective cation channel in some neurons. We investigated its potential role in SD generation and consequent cell swelling evoked by oxygen-glucose deprivation (OGD) in mouse brain slices. Imaging change in light transmittance (ΔLT) detects SD initiation and propagation through brain slices. ΔLT imaging revealed that brain slices from CD57 mice (3-5 months, n=10) incubated in artificial CSF (aCSF) with 10 μM of the TRPV4 antagonist HC-067047 for 20 minutes displayed neocortical and hippocampal SD onset times delayed by 32% ($p \leq 0.001$) as compared to controls. Neocortical SD propagation speed was also decreased by 45% ($p \leq 0.01$) compared to untreated controls. The CD57 mice TRPV4 antagonist group also showed a decreased LT by 34% ($p \leq 0.001$) from controls, suggesting reduced damage. The CD1 mice (1-2 months, n=6) brain slices when incubated in aCSF + HC-067047 (10μM or 20μM) for 20 minutes surprisingly showed non-significant differences from controls regarding SD onset time and propagation speed. This suggests that TRPV4 channel activation may contribute to SD and related cell swelling in the ischemic CD57, but not CD1 mouse brain. Perhaps there is a strain difference in the expression of TRPV4 channels in CD57 vs CD1 mice. These results show that while TRPV4 is not solely responsible for ischemic SD generation in either mouse, it has a role in promoting SD in one (CD57), but not another (CD1), strain of mouse. This builds on our previous unpublished work showing that the inhibitor of TRPM7 (FTY720 at 2 uM) does not affect ischemic SD in CD57 mice. Future research will use two photon laser scanning microscopy to differentiate the swelling of neuronal cell bodies from astrocytes. Blocking the TRPV4 channel has proven to be a promising therapeutic avenue in preventing damage post-ischemic stroke. 1, Neurocrit Care (2021) 35:S112-S134 https://doi.org/10.1007/s12028-021-01326-whttp://doi.org/10.1007/s12028-021-01326-w

Disclosures: D.A. Hancock: None. **R.D. Andrew:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

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Topic: C.08. Ischemia

Support: Key scientific innovation grant 2019JZZY020909
CIFMS 2021-I2M-1-071

Title: The impact of retrosplenial cortex ischemia on motor cognitive functions and neuronal dynamics

Authors: *L. DU[1,2], S. TU[1], Y. WANG[1], X. HE[1];
[1]Inst. of Medicinal Plant Develop., Peking Union Med. Col., Beijing, China; [2]Shandong Yikang Pharmaceut. Co.,Ltd, Shandong, China

Abstract: It has been demonstrated that ischemic brain injury causes cognitive and movement disorders. Much recent discussion about ischemic brain injury associated with locomotor and cognitive disorders has focused on the corticocortical circuit around the retrosplenial cortex (RSC), that commonly affected by brain ischemia. Here in mice that underwent middle cerebral artery occlusion (MCAO), the sensory, motor, and balance abilities of ischemic experimental animals were evaluated by modified neurological severity scores (mNSS). The cortical connectivity between the RSC and other cortical regions that are affected by MCAO modeling has been tested with retrograde tracers. The simultaneous neuronal activities were recorded and analyzed from bilateral RSC of freely moving mice with MCAO modeling. This study addressed the impact of ischemic injury on the RSC significantly increased the mNSS and indicated neurological deficits. The cortico-cortical connectivity between the RSC and other cortical regions was impaired. We also found pathological neuronal activities that have emerged differently in brain hemispheres affected by MCAO modeling. This study summarizes that the cognitive impairments induced by MCAO are by disturbing the projections on the RSC-related corticocortical connections and neuronal activities.

Disclosures: L. Du: None. **S. Tu:** None. **Y. Wang:** None. **X. He:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

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Topic: C.08. Ischemia

Support: James and Esther King Biomedical Research Grant 9JK08

Title: Chronic nicotine exposure increases blood-brain barrier permeability by decreasing tight junction protein levels and increases inflammation in aged male rats

Authors: *S. CHO, A. K. REHNI, K. R. DAVE;
Neurol., Univ. of Miami Miller Sch. of Med., Miami, FL

Abstract: The deleterious health effects of cigarette smoking are worse in the elderly than in the younger population. Smokers have a higher risk of premature mortality compared to never smokers at an older age[1]. Furthermore, both epidemiological and animal studies found that intracerebral hemorrhage (ICH) in elderly subjects results in larger hematomas and worse outcomes compared to younger subjects[2,3]. We previously showed that chronic nicotine exposure increased hematoma volumes and neurological deficits after collagenase-induced ICH, increased blood-brain barrier (BBB) impairment, and increased tumor necrosis factor-α (TNF-α) in young male rat brain microvessels. However, as ICH risk increases with age, we aimed to verify these findings in aged animals[4]. Our hypothesis is that chronic nicotine increases the risk of ICH in aged male rats by increasing BBB permeability and inflammation to a greater extent compared to young male rats. To test this hypothesis, aged Sprague-Dawley males (~18 months) were subcutaneously implanted with an osmotic pump containing either saline or nicotine (4.5 mg/kg/day) for about two weeks. Evans Blue dye (EBD) was intravenously injected into the rats, and after one hour, animals were perfused with saline. One half of the brain was used to spectrophotometrically analyze for EBD. The other half was used to isolate brain microvessels. The rate of hydrogen peroxide production was measured using Amplex Red assay. Tight junction proteins were measured using Western blot and normalized to GAPDH. Statistical analysis was performed using Student's t-test, and results are presented as mean ± SEM. EBD content in nicotine-treated aged males (280.1±60.6 ng/g of brain tissue) was 139% ($p<0.05$) higher than saline-treated aged males (117.7±78.2 ng/g of brain tissue). The rate of H_2O_2 production was 93% ($p<0.05$) higher in nicotine treated animals than saline-treated animals. Claudin 3 and Claudin 5 levels were 37% and 36% lower ($p<0.05$ for both), respectively, in the nicotine group than the saline group. Levels of PECAM-1 and ZO-1 were 57% ($p<0.01$) and 61% ($p<0.05$) lower, respectively, in the nicotine-treated rats than saline-treated rats. TNF-α levels were 33% higher ($p<0.01$) in nicotine group than saline group. Our results show that nicotine exposure increases BBB permeability and inflammation in aged male rats. This increased BBB permeability and inflammation may be responsible for the increased risk of and worsened outcomes following ICH in older tobacco users. References: 1) Arch Intern Med. 2012;172(11):837-44. 2) J Stroke Cerebrovasc Dis. 2018;27(1):97-102. 3) Stroke. 2004;35(11)2571-5. 4) Stroke. 2007;38(10):2718-25.

Disclosures: S. Cho: A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; James and Esther King Biomedical Research Grant - Study Staff. **A.K. Rehni:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; James and Esther King Biomedical Research Grant - Study Staff. **K.R. Dave:** A. Employment/Salary (full or part-time):; University of Miami Miller School of

Medicine. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; James and Esther King Biomedical Research Grant - PI.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.19

Topic: C.08. Ischemia

Title: A relevant model of thromboembolic stroke in diabetic mice for a preclinical evaluation of the risk of hemorrhagic transformations.

Authors: *F. LEBRUN[1,2], A. LETOURNEUR[2], N. VIOLLE[2], J.-F. BISSON[2], C. ORSET[1], D. VIVIEN[1,3];
[1]Physiopathology and Imaging of Neurolog. Disorders (PhIND), INSERM U1237, Caen, France; [2]ETAP-Lab, Strok@lliance, Vandœuvre-lès-Nancy, France; [3]Dept. of clinical research, Caen-Normandie Univ. Hosp., Caen, France

Abstract: Current pharmacological strategies to promote recanalization at the acute phase of thrombotic stroke relies on the use of recombinant tissue-type plasminogen activator (rt-PA), the only authorized thrombolytic treatment. The clinical development of thrombolytics - including rt-PA – must deal with the issue of Hemorrhagic Transformations (HT) as a side effect correlated with poor outcomes in patients. Thus, assessing HT risk from the preclinical stage is a major safety concern for any new drug targeting the early phases of ischemic stroke. As HT are correlated with comorbidities in humans, we hypothesized that a new model of thromboembolic stroke in diabetic (Db) mice could detect the deleterious effects of rt-PA and aspirin used as reference drugs. In blinded randomized studies, we induced diabetes using repeated injections of streptozotocin toxic to the insulin-producing beta cells of the pancreas to obtain male diabetic Swiss mice. Then we performed thromboembolic stroke, with or without rt-PA, aspirin, N-Acetyl-Cystein (NAC) and Glunomab® (an antibody blocking the tPA-NMDA receptor interaction) either alone or in combination.Thanks to brain imaging enabled by MRI performed at 24h post stroke onset, we revealed that Db animals display significantly larger infarct volumes than non-Db mice. Both laser speckle and MRI data also showed that rt-PA is able to quickly improve brain tissue reperfusion and middle cerebral artery recanalization in all animals including Db mice. Thus as expected, rt-PA significantly reduces lesion size in non-Db animals by 55% (n=13/group, $p<0.005$, Holm-Šídák's multiple comparisons test), with no effect in Db mice (n=12-13, $p=0.28$, Vehicle *vs* rt-PA in Db mice). This non-beneficial effect of rt-PA is associated with a high level of HT assessed by T2* MRI, a phenomenon not observed in non-Db animals. Grip-test also revealed a lack of functional deficit recovery after rt-PA treatment in Db animals.In this model, NAC also promotes lysis of arterial thrombi, reduces ischemic lesion sizes and improves risk of HT and neurological outcomes in Db mice. However, the combination of

NAC with rt-PA do not lead to such beneficial effects. In contrast, blocking endothelial rt-PA-NMDAR signaling with Glunomab® counteracts the side effects of rt-PA in Db mice, especially HT. Thus, this combination of treatments might be an alternative to increase eligibility of patients to fibrinolysis.In conclusion, this preclinical model, by reproducing known effects of diabetes on ischemic stroke in humans, can predict the risks of HT after ischemic stroke for reference drugs and to explore innovative therapeutic solutions according to STAIR guidelines.

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

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Program #/Poster #: 287.20

Topic: C.08. Ischemia

Support: NIH Grant 5R01NS117428-02
ANR

Title: Neuroprotective and neurogenesis Effects of A Purine Derivative Drug for the treatment of Hypoxia Ischemia Brain Injury.

Authors: A. MAÏZA[1], X. CHENG[2], C. DISDIER[2], N. COSTA[1], A.-C. GUYOT[1], R. GASTON-BRETON[1], B. S. STONESTREET[2], *A. MABONDZO[1];
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Abstract: Hypoxia-ischemia (HI) is one of the leading causes of neurodevelopmental morbidities in preterm and full-term infants. Several molecular mechanisms involved in HI injury result from abnormalities in blood-brain barrier (BBB) function and neuronal death. The only therapeutic strategy to treat HI encephalopathy in full term infants is hypothermia, which is only partially protective. HI related brain injury is characterized by inflammatory responses along with early alterations in the BBB. Inflammation and BBB abnormalities predispose to neuronal injury and loss. A novel purine derivative drug (PDD303), acting through GSK-3β and prohibitin pathways could be a promising therapeutic strategy against HI related brain injury. We investigated the beneficial effects of PDD303 by targeting the BBB and inflammation after HI brain injury in neonatal rats. The Rice-Vannucci model was used for pharmacokinetic studies and evaluation of neuroprotective effects of PDD303. 30 mg/kg of PDD303 were administrated i.p. after HI. PDD303 was quantified in brain and blood by LC-MS/MS. The infarct volume was quantified 72h after HI using Nissl staining (n = 26 animals/group). Inflammatory responses were examined by: (i) quantification of cytokines in brain and blood of treated and control animals and, (ii) astrocyte activation (GFAP) and (iii) modulation of neurogenesis (Sox2 and Pax6) by qPCR in both animal groups (n = 12 animals/group). A rat *in vitro* BBB model was

used to examine [^{14}C]-sucrose-BBB permeability after LPS (10 ng/ml) and PDD303 exposure (n=3). One-Way Anova and Tukey or Bonferroni's post-hoc were used for statistical analysis. PDD303 exhibited brain penetration with the partition coefficient Kp =0.9 in sham and HI groups with a half-life $T_{1/2}$ =3 h. PDD303 decreased the infarct volumes (40%, p<0.0001) in HI treated animals. Cytokines analysis showed an anti-inflammatory effect of PDD303 (decreases in Mip1-α, P=0.0069) in brain of HI animals. This anti-inflammatory effect was associated with a decrease in astrocyte activation (GFAP, 64%, p=0.0080) and downregulation of progenitor marker expression (Sox2, 44%, p<0.0001; Pax6, 60%, p=0.0105). *In vitro*, PDD303 prevented increases in [^{14}C]-sucrose-BBB permeability (56%, p <0.0003). In conclusion, PDD303 crosses the BBB and is distributed into the left and right brain hemispheres in HI treated animals, prevents alterations in BBB function and exhibits anti-inflammatory effects. PDD303 is neuroprotective and normalizes neurogenesis induced by HI. By targeting the BBB and neuroinflammation PDD303 might improve standard of care for neonates after HI.

Disclosures: A. Maïza: None. **X. Cheng:** None. **C. Disdier:** None. **N. Costa:** None. **A. Guyot:** None. **R. Gaston-Breton:** None. **B.S. Stonestreet:** None. **A. Mabondzo:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.21

Topic: C.08. Ischemia

Support: NIH R01 166199

Title: Spak inhibitor zt-1a protects white matter integrity and improves cognitive function in mouse model of vcid

Authors: *M. BHUIYAN[1,3,4], F. CHEN[1], I. JAHAN[1,3], Z. WENG[1], L. FOLEY[2], T. HITCHENS[2], X. DENG[5], D. SUN[1,3,6], G. CAO[1,6];
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Abstract: Background: The key features of vascular contributions to cognitive impairment and dementia (VCID) are white matter damage including myelin loss, astrogliosis, and BBB breakdown. However, the underlying molecular and cellular mechanisms of VCID are not well understood and no effective treatment is available. Stimulation of Na-K-Cl cotransport 1 (NKCC1) and its downstream kinase SPAK (the STE20/SPS1-related proline/alanine-rich kinase) contribute to intracellular Na^+ overload, astrocytic hypertrophy, and swelling. In this study, we investigated the role of SPAK-NKCC1 in astrogliosis, white matter demyelination, and cognitive function in murine models of VCID with bilateral carotid artery stenosis (BCAS).

Methods: BCAS was induced in male mice by suture ligation of both carotid arteries (CA) guided by needles or by using two metal micro-coils twined around both CA. Sham or BCAS mice receiving vehicle or a selective SPAK inhibitor ZT-1a were monitored for changes in the regional cerebral blood flow (CBF) and neurological and cognitive functions. Ex vivo MRI-DTI was subsequently conducted to detect brain injury and demyelination. Expression of WNK-SPAK-NKCC1 cascade proteins, astrogliosis, and demyelination were further examined by immunofluorescence staining. **Results:** BCAS mice displayed chronic CBF reduction and cognitive function deficits, along with significantly reduced mean fractional anisotropy and increased radial diffusivity and mean diffusivity values in the corpus callosum, and external capsule in MRI DTI analysis. Compared with the Sham control mice, the BCAS mice showed increased expression and activation of WNK-SPAK-NKCC1 signaling, $NKCC1^{+}GFAP^{+}$ astrocytes, and demyelination in white matter tracts. Interestingly, early inhibition (2-4 wks) or delayed inhibition (4-8 wks) of SPAK kinase with ZT-1a prevented BCAS-induced NKCC1 expression, astrogliosis, damage of white matter tracts, and significantly improved CBF and cognitive functions. **Conclusion:** Our study demonstrates that the WNK-SPAK-NKCC1 cascade is upregulated in BCAS brains and contributes to white matter astrogliosis, demyelination, and cognitive impairment. Pharmacological inhibition of the WNK-SPAK complex has therapeutic potential for VCID therapy. *This research was supported by NIH R01 166199 Grant (M.I.H.B.).*

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.22

Topic: C.08. Ischemia

Title: An NF-kB targeted biologic localizes to the brain and reduces infarct size in the middle cerebral artery occlusion model of ischemic stroke in spontaneously hypertensive rats

Authors: *J. A. HOWELL[1], M. LOPEZ[2], S. BURKE[2], E. PERKINS[3], G. BIDWELL, III[2]; [1]Program in Neurosci., Univ. Of Mississippi Med. Ctr., Jackson, MS; [2]Neurol., [3]Neurosurg., Univ. of Mississippi Med. Ctr., Jackson, MS

Abstract: Strokes are the 5th leading cause of death in the United States, and ischemic strokes comprise ~87% of stroke incidence. In addition to the lack of oxygen and energy reaching the tissue, the occlusion also leads to increases in intracellular calcium and glutamate release, weakening of the blood-brain barrier, and the activation of inflammatory processes. The nuclear factor kappa B (NF-κB) cascade is a major regulatory pathway of inflammation in the body and is known to be activated in response to ischemic stroke in humans and in the rodent middle cerebral artery occlusion (MCAO) model. Using a drug carrier called elastin-like polypeptide (ELP), we have designed a specific inhibitor of the NF-κB cascade, SynB1-ELP-p50i, that we

hypothesize will reduce neuroinflammation and improve outcomes following ischemic stroke. To begin characterizing SynB1-ELP-p50i, we assessed its localization in SH-SY5Y neurons and BV2 microglia by treating cells with 50 μM of rhodamine labeled SynB1-ELP-p50i. In both neurons and microglia, SynB1-ELP-p50i is able to enter the cell and localizes primarily to the cytoplasm of the cell. We next conducted *in vivo* biodistribution and efficacy studies using the MCAO model of ischemic stroke in spontaneously hypertensive rats. Following 2 hours of MCAO, rats received a 50 mg/kg intravenous injection of rhodamine labeled SynB1-ELP-p50i. Key organs were harvested and imaged, and the amount of rhodamine labeled SynB1-ELP-p50i within the brain was calculated using fluorescent histology. SynB1-ELP-p50i significantly localized to the ischemic hemisphere of the brain at a concentration of 38.44 μg/mL compared to the contralateral hemisphere, which had a concentration of only 4.97 μg/mL (two-way ANOVA; $F(1,12) = 63$, Sidak's post-hoc, $p < 0.01$). For the efficacy study, brains were harvested 24 hours after MCAO to allow for reperfusion. Brains were sectioned and stained with tetrazolium chloride for visualization of infarcted tissue. Blinded observers calculated infarct volume, and SynB1-ELP-p50i (50 mg/kg) treatment significantly reduced infarct size to 329.43 mm^3 compared to saline treated controls, which had an infarct volume of 372.75 mm^3 (two-tailed, unpaired t-test; $t(24) = 2.073$, $p = 0.0491$). Studies are ongoing to assess the effects of SynB1-ELP-p50i treatment on neurological outcome and motor behavior following MCAO in spontaneously hypertensive rats.

Disclosures: J.A. Howell: None. **M. Lopez:** None. **S. Burke:** None. **E. Perkins:** None. **G. Bidwell:** None.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.23

Topic: C.08. Ischemia

Support: NIH R01 NS102495
AHA postdoctoral fellowship 903383

Title: Ogerin potentiates GPR68 signaling and offers protection against ischemic brain injury in mice.

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Abstract: Persistent pH reduction, or acidosis, occurs in a wide range of neurological diseases, including brain ischemia. To better interpret how acidosis regulates neuronal injury, we have examined the expression of the proton-sensitive G-protein coupled receptors (GPCRs) in the brain. Our previous data showed that GPR68 (also known as ovarian cancer G-protein coupled

receptor; OGR1) exhibits robust expression in brain neurons and mediates a neuroprotective pathway in acidotic and ischemic conditions. Here, we asked whether potentiating GPR68 function offers protection against ischemic brain injury. Since there is no known activator of GPR68, we used Ogerin, which was previously identified as a positive allosteric modulator of GPR68 and increased its pH sensitivity. We first analyzed the effect of Ogerin in organotypic cortical slices. Ogerin induces phosphorylation of protein kinase C (PKC) substrates in wild-type but not GPR68-/- slices. Next, we assessed its effect on neuronal injury. We induced oxygen-glucose deprivation (OGD) in slices and analyzed delayed neuronal injury at 24 hr with propidium iodide (PI)/Syto-13 staining. OGD induced the intake of PI. Ogerin (10 μM) led to a significant reduction in PI:Syto-13 fluorescence ratio. Further, we asked whether Ogerin reduces brain injury induced by transient middle cerebral artery occlusion (tMCAO). Intraperitoneal injection of Ogerin has no significant effect on 60 min tMCAO-induced brain infarct size. In contrast, both intracerebroventricular and intravenous injection of Ogerin led to significant reduction in tMCAO-induced brain infarct. These data suggest that Ogerin can be a promising reagent to protect the brain from ischemic injury. Funding: NIH R01NS102495 (XZ) AHA postdoctoral fellowship #903383 (TW)

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.24

Topic: C.08. Ischemia

Support: Joe and Marie Field Foundation

Title: Intracerebral transplantation of autologous bone marrow stem cells produces functional recovery in rats with long term stable strokes.

Authors: *M. I. MYERS[1,3,4], K. J. HINES[2], G. SPAGNUOLO[1,3,4], A. GRAY[1,3,4], Y. GÓMEZ-GÁLVEZ[1,3,4], J. CAI[1,3,4], R. ROSENWASSER[2], L. IACOVITTI[1,2,3,4];
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Abstract: While treatment options exist for the acute phase of stroke, there are limited options for patients with stable infarcts and long-term disability. Allogenic mesenchymal stem cells (MSCs) have shown promise for the treatment of stroke when delivered systemically soon after ischemic injury. There is, however, limited data on the use of a) autologous MSCs, b) delivered via subcortical stereotactic transplantation c) in rats with a stable infarct. This study seeks to evaluate the efficacy of intracerebral transplantation of autologous MSCs in a rat middle cerebral artery occlusion (MCAO) model of chronic stroke. Male Sprague-Dawley rats underwent right

middle cerebral artery occlusion for 120 minutes to induce stroke. 16 days following stroke, rats underwent tibial bone marrow aspiration. Autologous MSCs were then cultured and expanded in a closed system sterile growth bioreactor. 1 month following stroke, MSCs were harvested, brain MRI was obtained, and a stereotactic injection robot was used to implant various doses of stem cells via three trajectories in the peri-infarct region. The total number of cells in the three treatment groups consisted of ($1x10^6$ cells, $2.5x10^6$ cells, $5x10^6$ cells, n=6 in each group). The control group received an MCA stroke and was saline injected (n=9). In a second cohort of animals, fluorescent tagged quantum dots (QD) were used to label autologous MSCs, which were tracked in the brain at 1 week, 1 month, and 2 months post-transplantation**.** Behavior was assessed using the modified neurological severity score (mNSS) (0-16), revealing highly significant neurological improvement at 1 and 2 months following MSC transplantation in all treatment animals, compared to controls. No apparent dose response in efficacy measures was observed, since $1X10^6$ cells was likely beyond the threshold needed for treatment efficacy. As expected, no difference in terms of ischemic lesion volume or MRI aspect were observed between MSC-treated and control animals, as cells were transplanted 1 month after the acute injury of MCAO. Immunocytochemistry revealed increased astrocyte and microglia reactivity along the peri-infarct region. Surprisingly, quantum dot analysis displayed the continued long-term presence of MSCs in the MCAO brain, possibly increasing their long-term effectiveness. Thus, these studies suggest that intracerebral transplantation of autologous MSCs may be a promising treatment for chronic MCA stroke.

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

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Program #/Poster #: 287.25

Topic: C.09.Stroke

Support: NIH Grant K08NS105914

Title: Elevated intracranial pressure in the setting of intraventricular hemorrhage leads to synaptic elimination in the dentate gyrus in a rodent model

Authors: C. H. PUGLISI[1], J. '. KEITER[3], C. PETERSON[1], C. HAWK[1], V. KALISTRATOVA[1], A. IZADI[1], G. G. GURKOFF[4], F. R. SHARP[2], **B. WALDAU**[1];
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Abstract: BACKGROUND: Long-term memory loss is frequently seen after intraventricular hemorrhage in the setting of a ruptured brain aneurysm or intraventricular expansion of a parenchymal hemorrhage. We have established a rodent model of intraventricular hemorrhage

with controlled infusions of autologous blood followed by artificial elevation of the intracranial pressure to 50 mm Hg for 2 hours that leads to long-term memory deficits in the Morris water maze. We have found microglial activation that was most pronounced with intraventricular hemorrhage and elevated intracranial pressure. For this study, we investigated the working hypothesis that the long-term memory deficit is caused by excessive synaptic elimination by microglia in the hippocampus after increased intracranial pressure in the setting of intraventricular hemorrhage.
METHODS: Experimental animals were divided into 4 groups: Intraventricular hemorrhage followed by elevated intracranial pressure to 50 mm Hg for 2 hours (IVH+ICP), intraventricular hemorrhage alone (IVH), volume control and sham control surgery. Synaptic integrity based on co-localization of markers was assessed with a PSD95/VGLut2 dual stain in the hippocampus and compared between groups.
RESULTS: The IVH+ICP group showed qualitatively a lower number of intact pre- and postsynaptic terminals compared to the sham group in the dentate gyrus. Quantitative data will be presented during the meeting.
CONCLUSION: IVH + ICP leads to a reduction in intact synapses in the hippocampus possibly through microglial activation and synaptic elimination.

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Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.26

Topic: C.08. Ischemia

Support: NIH grant NS122808

Title: Insulin-treated diabetic rats exhibit enhanced stroke risk for at least 7 days post-recurrent hypoglycemia exposure

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Abstract: Diabetes is a chronic metabolic disease responsible for a major fraction of global healthcare expenditure, mortality, and morbidity. Cardiovascular complications are a leading cause of mortality in subjects suffering from diabetes. The major side effect of intensive anti-diabetic therapy is hypoglycemia. Recurrent hypoglycemia (RH) episodes are common among type 1 and type 2 diabetes patients. Previous findings from our laboratory have shown that RH

exposure for at least a minimum duration of 2 h per day for 5 consecutive days increases stroke risk when evaluated a day after the last hypoglycemia episode in insulin-treated diabetic (ITD) rats. However, how long the prothrombotic effect of mild/moderate RH lasts in ITD rats is not known. Thus, the aim of the present study was to define the maximum detrimental window of hypoglycemia exposure-induced increased risk of thrombosis (a surrogate for stroke risk) in ITD rats. Hyperglycemia in streptozotocin diabetic rats was corrected using insulin pellet treatment. ITD rats were randomly assigned to either ITD + RH + Glucose (hyperinsulinemic euglycemia: control) or ITD + RH groups (hyperinsulinemic RH) for 3h / day exposure for 5 consecutive days. Either 3 or 7 days after the last episode of hypoglycemia, the right carotid artery and left jugular vein of the rats were linked via a shunt containing a pre-weighed 4-0 silk suture, and the shunt was opened for 15 minutes. The suture was then weighed to quantify thrombosis. Thrombosis was determined 3 and 7 days after RH in different sets of animals. We observed that the clot weights in the ITD + RH + Glucose group and ITD + RH group quantified 7 days after RH were 14 ± 2 mg (n = 7) and 25 ± 5 mg (n = 6), respectively. The clot weight in the ITD + RH group was 80% greater ($p<0.05$) than in the RH + Glucose group. However, the clot weights in the RH-exposed ITD rats quantified 3 days after RH (33 ± 11 mg, n=4, p=0.15) were not significantly different from the control group (11 ± 1 mg, n=3), possibly due to currently employed small sample size. We are in the process of increasing sample size. We are also planning to test intervals longer than 7 days. Our results show that RH exposure increases stroke risk in ITD rats for at least 7 days after exposure. In future studies, we aim to identify the minimum frequency of episodes of hypoglycemia that increases stroke risk in treated diabetic rats. Acknowledgement: NIH grant NS122808.

Disclosures: **A.K. Rehni:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **S. Cho:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine. **K.R. Dave:** A. Employment/Salary (full or part-time):; University of Miami Miller School of Medicine.

Poster

287. Mechanisms and Treatments for Ischemic Stroke II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 287.27

Topic: F.03. Stress and the Brain

Support: R01 NS113921
R01 NS107417

Title: Rna and dna methylation signatures are globally distributed in the neonatal pig brain

Authors: *V. L. OLBERDING, A. AMREIN ALMIRA, C. JAVDAN, J. K. LEE, L. J. MARTIN;
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Abstract: Epigenetic mechanisms could contribute to the delayed neural degeneration, neural network dysfunction, and long-term cognitive disabilities in children that have experienced neonatal hypoxia-ischemia. However, the distributions and cellular localizations of epigenetic writers, erasers, readers, and signatory markers in different brain regions of clinically relevant large animal models of neonatal encephalopathy have not been studied. In neonatal pig brain, we studied using western blotting and immunohistochemistry the distributions of: nucleolar protein-Sad1/UNC84 domain protein 2 (NSUN2), a methyltransferase that catalyzes methylation of various RNAs; N6-methyladenosine (m6A), the most abundant mRNA modification; and 5-methylcytosine (5mC), a methylated form of the DNA base cytosine. The NSUN2 antibody detected a highly specific protein at ~ 90 kDa in western blots of piglet brain homogenates. By immunohistochemistry, NSUN2 was highly enriched in the nucleus of neurons and glia throughout the brain. Granule neurons in the cerebellum, but not in hippocampal dentate gyrus, were the exception by having low NSUN2 immunoreactivity. m6A immunoreactivity, fully abolished by competition of antibody with purified m6A and diminished by RNase treatment, was also localized to the nucleus of neurons and glia throughout the brain. m6A immunoreactivity was particularly enriched in glial white matter cell nuclei and in cells of the ganglionic eminence. 5mC immunoreactivity had an abundant global distribution in neurons and glia and was discretely localized to fine strands throughout the nucleus of neurons and in larger neuronal intranuclear formations. RNA and DNA epigenetic patterns in piglets with encephalopathy are being assessed for aberrancy. These data show that salient epigenetic markers and their regulatory mechanisms have broad distributions and neuronal and glial cell nuclear enrichment throughout neonatal pig brain gray and white matter regions and are potentially poised for acquired injury-induced rewriting of the epigenetic landscape.

Disclosures: V.L. Olberding: None. **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

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.01

Topic: C.10. Brain Injury and Trauma

Title: Structural abnormality in juvenile myoclonic epilepsy

Authors: *S. LIM[1], Y.-M. SHON[2];
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Abstract: Objective: This study examined not only the gray and white matter changes of Juvenile Myoclonic Epilepsy(JME) patients by using tract-based spatial statistics (TBSS),

constrained laplacian-based anatomic segmentation with proximity (CLASP) algorithm, but also the subcortical volume loss from manual volumetry and 3D shape analysis using spherical harmonic based functions (SPHARM) algorithm. In addition, our study is intended to elucidate the changes of cortical thickness that is correlated to volume reduction of thalamus and hippocampus. Background: JME patients do not appear to have structural abnormalities following conventional neuroimaging. However, several studies that investigated JME patients have revealed microstructural and functional changes when using state-of-the-art imaging techniques. Methods: We included 27 JME patients and 22 normal controls. Cortical thickness analysis : The CLASP algorithm was applied. 3D shape analysis of hippocampus and thalamus : Performed automatically using SPHARM algorithm. Results: Cortical structural changes: JME patients exhibited a significant reduction in cortical thickness in the dorsolateral frontal and anterolateral temporal cortex. Relevant subcortical structural changes: Significant reduction in volume in the bilateral thalami and hippocampi. Changes of the surface thickness by SPHARM analysis: JME subjects showed a few clusters with more decreased surface thickness at middle and dorsal part of hippocampi and thalamic nucleus. Correlation of cortical structures with the hippocampal or thalamic volume loss: Significantly reduced cortical thickness correlated with the volume reduction of both hippocampi. It encompassed widespread cortical gray matters including superior and inferior temporal and orbitofrontal, and medial frontal region. Cortical regions correlated with the clinical variables: We found a cluster on the parietal region showing statistically negative correlation of where in surface thickness with the seizure frequency. Conclusions: The use of combined imaging analyses in this study demonstrated widespread structural abnormalities in JME. Our results suggest that structural MRI changes of JME may be widespread, rather than confined to well-known frontothalamic circuit.

Disclosures: S. Lim: None. **Y. Shon:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.02

Topic: C.10. Brain Injury and Trauma

Support: MH094258
NS103780
F31NS086254
Kiwanis Neuroscience Research Foundation

Title: Assessing lesion network recovery following temporal lobe resection in individuals with mesial temporal lobe epilepsy

Authors: *C. DEIFELT STREESE[1], M. J. SUTTERER[1], J. E. BRUSS[2], D. TRANEL[2], M. A. HOWARD, III[1];
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Abstract: The mechanism by which recovery occurs following a brain injury is not fully understood. Recent work has shown a relationship between recovery and brain network characteristics returning to baseline metrics. To provide further support to this conceptualization of recovery, we collected resting state (RS) functional magnetic resonance imaging data on 15 human participants with mesial temporal lobe epilepsy before and at several timepoints within the first year after temporal lobe resection (TLR). Individual lesion networks were generated based on RS data from each participant prior to surgery. Lesion networks were defined as the network of brain regions functionally connected to the resected area prior to TLR. To assess network recovery, we calculated 5 key network properties (strength, sparsity, energetic cost, clustering coefficient, and modularity) of lesion networks over time. Mixed effects models were used to determine which lesion network properties were significantly related with post-operative chronicity. Lesion network strength and sparsity models were influenced by both a negative linear component (β=-0.432, t(26.10)=-3.80, p=.001; β=-0.477, t(22.61)= -4.05, p<.001) and positive quadratic component (β=0.042, t(27.71)=3.35, p=.002; β=0.051, t(23.44)=3.30, p<.001), indicating that in the first months following TLR, there are decreases in lesion network strength and sparsity, but that over time these network metrics begin returning to baseline. We concluded that there are post-operative decreases in the size and interconnectedness of the lesion network which recover to their pre-operative levels over time, while the overall shape and modular structure of the lesion network is maintained throughout. Models run on whole-brain data did not yield significant results, suggesting this pattern may be specific to the lesion network. These findings provide further support for approaching the study of focal brain injury and recovery from a network perspective and underscore the value of within-subjects lesion-based network analyses in understanding the impacts of brain injury.

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Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.03

Topic: C.10. Brain Injury and Trauma

Support: NIH IRACDA- JHU ASPIRE
R01 NS125897-01
R01 NS122927

Title: Virtual Stimulation of Interictal Stereo-EEG to Localize the Epileptogenic Network

Authors: *R. SMITH[1], S. ZHAI[2], K. GUNNARSDOTTIR[2], D. EHRENS[2], A. LI[3], J. A. GONZÁLEZ-MARTÍNEZ[4], S. V. SARMA[1];
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Abstract: For patients with drug-resistant epilepsy, localizing and surgically treating the epileptogenic network can bring seizure freedom. However, surgical success rates vary from 30-70% because no clinical biomarker of epileptogenicity exists. We hypothesize that epileptogenic nodes act as powerful sources of pathological activity during seizures but are actively inhibited during interictal periods. Thus, although stimuli of various forms enter these regions during interictal times, the network response to these stimuli are subdued. We tested this hypothesis *in silico* by performing virtual stimulation of stereo-EEG (sEEG) channels during interictal periods and used the magnitude of the network response to localize the epileptogenic network. We performed virtual stimulation in sEEG data gathered from 34 epilepsy patients that were assessed for clinical outcome. The data were first divided into non-overlapping 500 ms windows, and a linear time-invariant model, $\mathbf{x}(t+1) = \mathbf{A}\mathbf{x}(t)$, was constructed for each window. Then, we added an exogenous perturbation for each channel: $\mathbf{x\_stim}(t+1) = \mathbf{A}\mathbf{x\_stim}(t)+\mathbf{B}(t)$ where **B** is a unit vector with the 1 corresponding to the index of the channel being virtually stimulated. Next, we measured the simulated network response, $\mathbf{x\_stim}(t)$, for 500 time steps, and calculated the L-2 norm ("size") of this network response. This process was repeated for every channel in the window and every window in the dataset, creating a heat map of network responses as a function of virtually stimulating each electrode over time. We found that in surgical success patients, stimulating regions within the hypothesized epileptogenic network (EN) evoked a smaller network response than stimulating regions outside of the EN in 9/14 success cases, with 5/14 being statistically significant differences in response magnitudes. In failed surgery cases, stimulating the EN resulted in a statistically significantly reduced response in only one case (1/20), but followed a trend of lower EN-stimulated network responses in 9/20 cases. These results support our hypothesis that nodes of the epileptogenic network are being inhibited during interictal periods, producing smaller network responses when stimulated than non-EN regions. We believe that a better understanding of how the epileptogenic network interacts with surrounding brain regions in both ictal and interictal periods may provide valuable insight into the mechanisms that give rise to seizures. The development of an interictal biomarker of epileptogenic tissue would add information to ictal data gathered from capturing seizures in the hospital while simultaneously improving surgical outcomes.

Disclosures: R. Smith: None. **S. Zhai:** None. **K. Gunnarsdottir:** None. **D. Ehrens:** None. **A. Li:** None. **J.A. González-Martínez:** None. **S.V. Sarma:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.04

Topic: C.10. Brain Injury and Trauma

Support: T32-NS061788-13
State of Alabama General Funds

Title: Brain temperature elevations: a biomarker of microstructural tissue damage in focal epilepsy.

Authors: *A. SHARMA, R. NENERT, A. GOODMAN, J. P. SZAFLARSKI;
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Abstract: Background. Neuroinflammation (NI) is a key component of many neurological disorders, including epilepsy. NI can lead to microstructural tissue damage that may worsen with increasing brain temperature. Atypically high brain temperatures may thus be a surrogate measure of the biochemical consequences of NI. Using volumetric magnetic resonance spectroscopic imaging (MRSI-t), brain temperature can be non-invasively calculated (T_{CRE} = $-102.61(\Delta_{H20-CRE)} + 206.1°C$) and mapped in vivo. Preliminary studies of focal epilepsy have found T_{CRE} increases ipsilateral to seizure focus. Since T_{CRE} mapping is a new method with few applications in neurological populations, it must be evaluated in conjunction with more established techniques. This multimodal imaging study combined T_{CRE} mapping by MRSI-t with neurite orientation and density imaging (NODDI), a histopathologically-verified tool for modeling the brain's microstructure. The fusion of MRSI-t and NODDI allowed investigating whether T_{CRE} elevations were spatially coupled with microstructural damage and/or edema. **Methods**. Twenty adults with temporal lobe epilepsy (TLE) and 20 healthy controls were scanned at 3-Tesla. Structural images were processed by voxel-based morphometry. Diffusion images were processed using NODDI toolbox v1.01 to yield index maps of neurite dispersion (ODI), neurite density (FICVF), and extracellular free water (FISO). Voxelwise two-sample t-tests computed group differences in imaging data. Multimodal data fusion by joint independent component analysis modeled the spatial coupling of T_{CRE} with NODDI and GMV data. **Results.** T_{CRE} elevations were linked to regions of microstructural tissue damage, particularly in the ipsilateral temporal lobe. In this region, patients with TLE showed $T_{CRE} > 38°C$ (T_{CRE} maps), edema (FISO maps), and brain atrophy (GMV maps). For patients with TLE, T_{CRE} elevations converged with FISO elevations in the L temporal lobe. Brain atrophy (↓GMV) spatially coupled with decreased T_{CRE} ipsilateral to seizure focus. **Conclusions.** Based on multimodal data findings, elevated T_{CRE} likely indicates regions with increased levels of NI and NI-induced atrophy. T_{CRE} elevations were associated with edema ipsilateral to the seizure focus. Additionally, T_{CRE} decreases were spatially linked with brain atrophy, thus highlighting how brain temperature may change with disease progression, especially in regions impacted by cell death. These relationships were strongest in the ipsilateral to seizure onset temporal lobe, thus indicating agreement between MR measures that visualize NI.

Disclosures: A. Sharma: None. **R. Nenert:** None. **A. Goodman:** None. **J.P. Szaflarski:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.05

Topic: C.10. Brain Injury and Trauma

Support: National Institutes of Health/ National Institutes of Neurological Disease and Stroke NS083377 to SHF

Title: Global reorganization of sensorimotor representations of intact limbs after upper limb amputation

Authors: *M. MARNEWECK[1], C. GARDNER[2], J. SMITH[2], S. H. FREY[3];
[1]Dept. of Human Physiol., [2]Univ. of Oregon, Eugene, OR; [3]Program in Occup. Therapy, Univ. of Missouri, Columbia, MO

Abstract: Sensorimotor reorganization following amputation has traditionally been surveyed in the context of sensorimotor representations of the lost limb. Recently, fMRI studies employing multivoxel patterned analysis on humans with upper limb amputation report somewhat discrepantly to that shown in earlier animal studies that representations of the missing hand including its inter-digit representational structure remain relatively intact. Here we used Bayesian patterned component modelling of fMRI data to measure sensorimotor reorganization more globally by evaluating the extent which amputation modulates representational dissimilarity between left and right-sided actions of the hand and leg in the left primary motor cortex (M1) contralateral to the limb lost. Twelve right upper-limb amputees (10 males) with ages ranging from 25 to 73 (M = 57, SD = 14; age of amputation: M = 28, SD = 15) and 12 age, sex, and handedness matched healthy adults were recruited for this study. BOLD activity was measured as participants performed paced movements of their toes, and opening and closing of each hand. Two results are of interest. First, we found distinct spatial activity patterns for both left and right sided actions (irrespective of hand or leg) in amputees but not controls in left M1. Ipsilateral representations of left sided hand and foot actions in left M1 following amputation might be a result of an unmuting of inhibitory transmission from the contralateral hemisphere that is typically seen in controls during same-sided actions. Second, hand and foot actions were distinctly represented (irrespective of side) in both controls and amputees. However, in controls, this effect was driven by an interaction effect that showed arm and leg spatial activity pattern differences were specific to right-sided actions. In other words, intact distinctive arm and leg representations were present ipsilaterally in those with a missing limb. Critically, although spatial activity patterns of leg and arm movements were distinct in the amputee group, they were a lot less distinct than that seen in the control group. These findings suggest that there is global reorganization of contralateral sensorimotor representations of ipsilateral and contralateral body parts that persists years after amputation. That information content of multiple body parts is more distributed and overlapping following limb loss is a critical consideration and challenge for rehabilitation and brain-machine interfaces that have focal assumptions for restoring sensation and use of missing limb.

Disclosures: M. Marneweck: None. **C. Gardner:** None. **J. Smith:** None. **S.H. Frey:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.06

Topic: C.10. Brain Injury and Trauma

Title: Fourier transform infrared spectroscopy reveals biochemical changes in multiple sclerosis pathology

Authors: *O. GAKH[1], Y. GUO[1], B. POPESCU[2], C. LUCCHINETTI[3];
[1]Dept. of Neurol., Mayo Clin., Rochester, MN; [2]Dept. of Anat. and Cell Biol., Col. of Medicine, Univ. of Saskatchewan, Saskatoon, SK, Canada; [3]Dept. of Neurol., Mayo Clinic, Col. of Med., Rochester, MN

Abstract: Multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), is the leading cause of non-traumatic disability in young adults in the United States. Previous studies have indicated oxidative stress, mitochondrial disturbances, and energy metabolism in MS lesions and normal-appearing white matter. The involvement of these biochemical changes is suggested in MS pathogenesis. However, the progress was restricted by technological limitations like low sensitivity, low resolution, or no spatial profiling et al. To further analyze the biochemical profiling, we introduced Fourier transform infrared spectroscopy (FTIR) combined with histopathology in this MS study. FTIR spectra contain qualitative and quantitative data at a high spatial resolution which provides a unique biomolecular fingerprint. FTIR has emerged as a modern and promising tool in histopathology to provide label-free analysis of the tissue sections. We analyzed the FFPE brain tissue samples from 24 MS patients (52.4±19.6 years old) and 10 normal CNS controls (55.2±16.0 years old). Lesions were classified based on the stage of demyelinating activity. We analyzed 40 active demyelinating lesions, 23 inactive demyelinating lesions, 25 normal-appearing white matters, and 55 periplaque white matters. The data obtained from FTIR imaging were verified and topographically associated with the pathological assignments of the tissue. We found increased unsaturated lipid, unordered protein, and structure changes of nucleic acid in both active and inactive demyelinating lesions. The changes in the amide I band position and shape indicated that the protein structures in the plaques were largely affected in MS. The intensities of lipids, proteins, and nucleic acids are decreased in the inactive demyelinating lesions (~2 times) and are significantly decreased in active demyelinating lesions. The findings provide new evidence of oxidative stress which is topographically associated with MS lesions. The formation of short-chain lipids and accumulation of unsaturated lipids suggests lipid peroxidation which can subsequently cause further disbalance of lipid synthesis. The structural changes in proteins and nucleic acids can be associated with oxidation processes. The pathological changes in MS reflect biochemical alterations within the tissue. FTIR imaging bio-spectroscopy is a valuable and powerful tool for the identification of regions carrying morphological and biochemical differences.

Disclosures: O. Gakh: None. **Y. Guo:** None. **B. Popescu:** None. **C. Lucchinetti:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.07

Topic: C.10. Brain Injury and Trauma

Support: CONACYT A1-S-15323
CNEIP_C2018_P004

Title: Memory impairment in people living with HIV/AIDS

Authors: *M. L. GARCÍA-GOMAR, **A. J. CASTRO-ALAMEDA**, A. J. NEGRETE-CORTÉS, J. R. CHÁVEZ-MÉNDEZ;
Facultad de Ciencias de la Salud UABC, Tijuana BC, Mexico

Abstract: HIV can damage the hippocampus through microvascular dysfunction and hypoperfusion. The hippocampus is crucial for memory. In fact, one of the most affected cognitive domains in HIV neurocognitive impairment (NCI) is memory. Global NCI has been reported in white PLWHA from 40 to 50%. In Latino populations there have been similar rates described from 29 to 53%. There is a larger variation in NCI rates for the Latino population as some reports use norms that are not appropriate. Currently there are Latino population norms (LPN) and recently developed norms for the US-Mexico border region, in Spanish (NP-NUMBRS). The aim of this present study was to describe memory impairment in PLWHA and to determine the percentage of impairment, comparing LPN vs NP-NUMBRS. CIOMS international ethical guidelines for the participation of human subjects in health research were followed. 84 PLWHA living in Tijuana (Mexico) participated in the study (Age: Mean=39.6, SD=10.9; 28.3% Female; Years of education: Mean=8.5, SD=3.6). PLWHA were recruited from the board-and-care home “Las Memorias” (73.4% on antiretroviral therapy; Years since HIV diagnosis: Mean=9.9, SD=7.1). Participants completed a neuropsychological test battery which consisted of the Hopkins Learning Verbal Test-Revised (HLVT-R) and Paced Auditory Serial Addition Test (PASAT). Raw scores in these tests were transformed to percentiles using LPN and transformed to T-scores using NP-NUMBRS, before being averaged to calculate scores of global learning and memory. NCI was defined as percentile scores <16 and T-scores < 40. Crosstab and McNemar´s test were used to compare the rate of memory NCI utilizing LPN vs NP-NUMBRS. For HLVT-R Total and Delayed Recall Scores, PLWHA showed a mean percentile of 43.1 (SD=29.7) and 35.3 (SD=32.7) respectively, according to LPN, and a mean T-score of 40.3 (SD=12.10) and 40.4 (SD=12.7) according to NP-NUMBRS. Regarding PASAT, PLWHA showed a mean percentile of 21.4 (SD=21.3) according to Mexican norms (NEURONORMA-MX). We found rates of 34.5% memory NCI utilizing LPN vs 53.5% memory NCI with NP-NUMBRS. These differences in rates didn’t reach statistical significance (p=0.26). According to NP-NUMBRS, approximately half of PLWHA showed notable memory NCI, which is consistent with findings of prior studies. To accurately make NCI diagnosis it is important to use norms that consider specific characteristics of the population. The diagnosis of memory NCI is important since these deficits present a strong risk of concurrent problems in a wide range of health behaviors like medication non-adherence in PLWHA.

Disclosures: M.L. García-Gomar: None. **A.J. Castro-Alameda:** None. **A.J. Negrete-Cortés:** None. **J.R. Chávez-Méndez:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

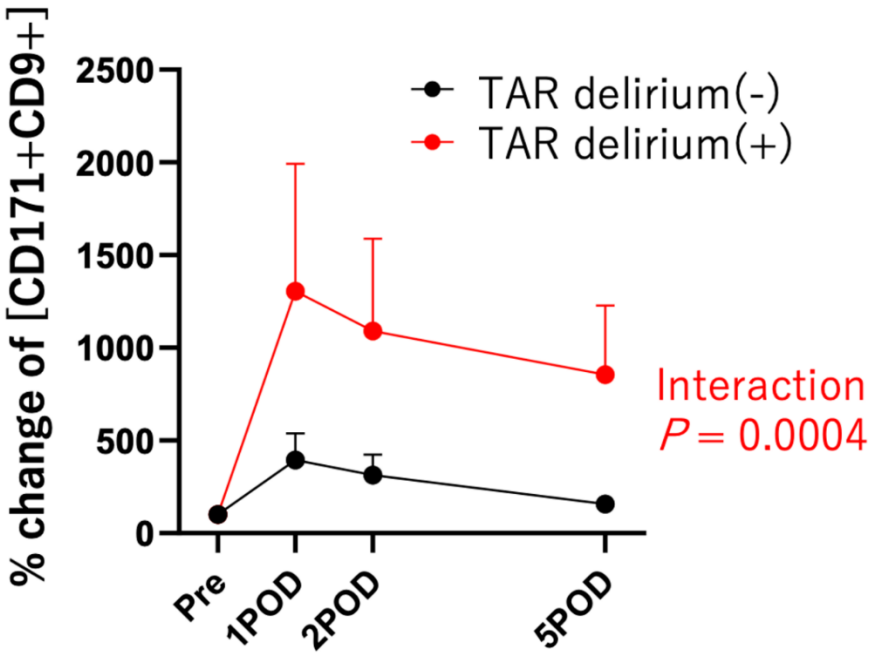
Program #/Poster #: 288.08

Topic: C.10. Brain Injury and Trauma

Title: Monitoring of brain injuries using a plasma-based sandwich immunoassay between neuron marker CD171 and exosome marker CD9.

Authors: *N. HOTTA[1], K. YOSHITANI[2], T. TADOKORO[3], M. MITSUHASHI[4];
[1]Yokohama city university hospital, Yokohama, Japan; [2]Natl. cerebral and cardiovascular center, Suita, Japan; [3]Univ. of California San Diego, La Jolla, CA; [4]Hitachi Chem. Res. Ctr., Irvine, CA

Abstract: Background: Exosomes are vesicles secreted by most of the cells that contain their cellular traits and are released into peripheral blood and other body fluids. Measuring exosomes in peripheral blood can provide intracellular information from outside the cell. However, the extraction of neuron-derived exosomes (NDEs) was not consistent depending on the isolation procedure, purity, and recovery rate. Anti CD9 antibody is one of the antibodies specific for exosomes, while anti CD171 antibody is specific for neurons. Therefore, we developed a sandwich enzyme-linked immunosorbent assay (ELISA) using anti-CD9 antibody and anti-CD171 antibody and to extract NDEs in the peripheral blood. We also examined whether this test could be used as an indicator of brain damage. **Methods:** Plasma samples were collected from the patients who underwent total arch replacement (TAR) surgery using selective cerebral perfusion at five points (pre-operation, post-operation, postoperative day 1, 2 and 5). NDEs were measured by the sandwich ELISA using anti-CD171 antibody and anti-CD9 antibody, which we developed. For the evaluation of brain damage, postoperative delirium (POD) was used (Intensive Care Delirium Screening Checklist≥4). We examined the association between NDEs and POD. **Results:** Samples were collected from patients with TAR (n=36). Thirteen patients with POD were found within postoperative day 5 after TAR. Throughout patients undergoing TAR, NDEs, [CD171+CD9+] increased postoperatively, and significant interaction (p=0.0004) of POD was observed using a mixed effect model. No correlation was found with surgical factors. **Conclusion:** [CD171+CD9+] increased rapidly after TAR, and the rate of increase was significantly greater in patients who developed POD. This assay may serve as an indicator for assessment and monitoring of brain injury.



Disclosures: **N. Hotta:** None. **K. Yoshitani:** None. **T. Tadokoro:** None. **M. Mitsuhashi:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.09

Topic: C.10. Brain Injury and Trauma

Support: NIH NINDS U54NS100064

Title: Comparison of two automated HFO detection methods in patients with traumatic brain injury.

Authors: *P.-S. LEE, M. LAI, R. GARNER, D. DUNCAN;
Lab. of Neuro Imaging, USC Stevens Neuroimaging and Informatics Inst., Los Angeles, CA

Abstract: Traumatic brain injury (TBI) can lead to seizures and subsequent secondary diagnoses of post-traumatic epilepsy (PTE). High frequency oscillations (HFO) are a promising biomarker of epileptogenesis and can be classified into ripples (80-250 Hz) and fast ripples (250-400 Hz) by their distinctive characteristics. However, the visual detection of HFOs from patient electroencephalography (EEG) is highly time consuming and subjective for multichannel EEG. This has limited the widespread use and potential impact of HFOs as a biomarker in clinical

practice. We aimed to validate whether two automatic detectors are suitable for application with scalp EEG data of patients with TBI and to investigate possible HFO correlates with seizure outcomes in a patient cohort. The ten patients were continuously monitored for a minimum of 72 hours post-injury with EEG sampling rates of 2048 Hz, 512 Hz, and 256 Hz. The scalp EEG data (12-16 electrodes) were divided into one hour windows. The datasets were visually inspected for artifacts and preprocessed using the EEGLAB toolbox (v2021.1). Preprocessing included performed notch filtering at 60 Hz to remove line noise, low-pass filtering at 400 Hz, band-pass filtering at 80-500 Hz, and independent component analysis to remove muscle, eye, heart, and channel artifacts. The clean data were analyzed using the Short Time Energy (STE) method in the RIPPLELAB toolbox in MATLAB and the HFO detector developed by Jean Gotman's lab. True positive HFO events were identified if they met the following two criteria: (1) more than 4 oscillatory cycles; and (2) circular shapes isolated from the time-frequency plots having centroids located above 80 Hz. The quantitative HFO rate is defined as the percentage of true HFO events in the total automatic detected HFO events (STE: 0.5%-12%; HFO detector: 0.8%-8%). The results of the HFO rate indicated that the STE method detected more true positive HFO events than the HFO detector did. We can further examine multimodal neurocognitive features and functional connectivity using the HFO events to characterize their seizure outcomes.

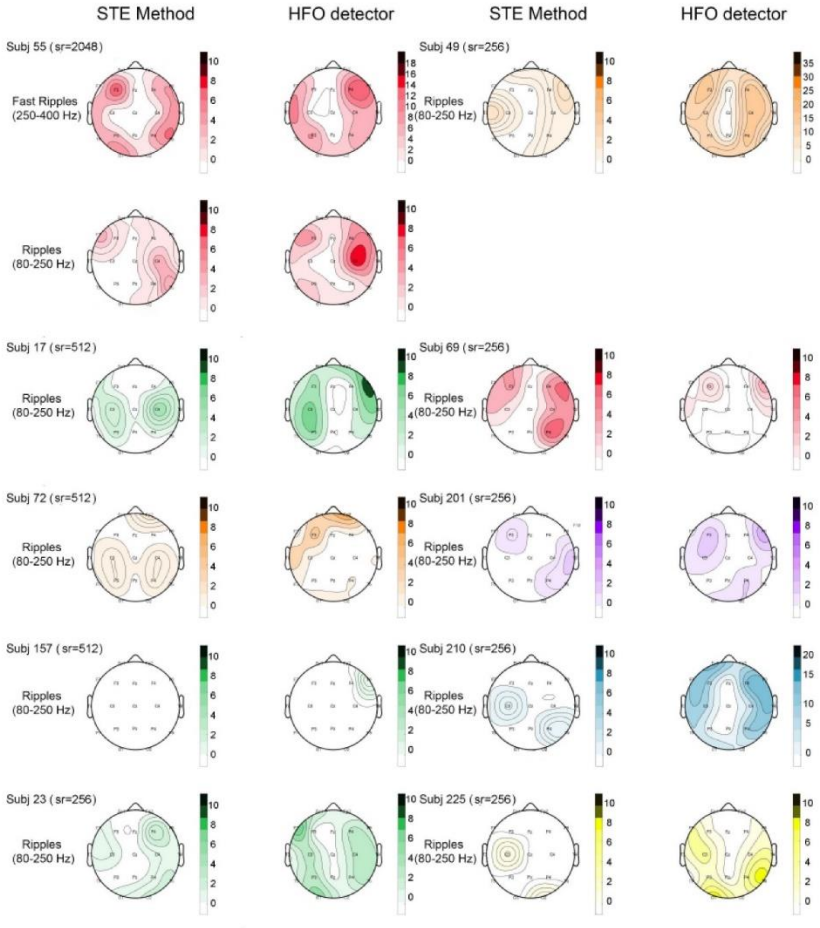


Figure 1. Topography of the number of the true HFO events of ten patients. Color denotes the number of true HFO events. STE: short-time energy; sr: sampling rate; Subj: Subject.

Disclosures: **P. Lee:** None. **M. Lai:** None. **R. Garner:** None. **D. Duncan:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

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Program #/Poster #: 288.10

Topic: C.10. Brain Injury and Trauma

Support: DOD grant W81XWH-15-1-0603
NIH grant P50 DA044121

Title: Increased connectivity within the hippocampal network after mTBI is associated with pain chronification

Authors: *P. BRANCO[1], N. BOSAK[2], A. VIGOTSKY[1], Y. GRANOVSKY[2], D. YARNITSKY[2], A. APKARIAN[1];
[1]Northwestern Univ., Chicago, IL; [2]Rappaport Fac. of Medicine, Technion – Israel Inst. of Technology, Haifa, Israel, Haifa, Israel

Abstract: The mesocorticolimbic system is thought to contribute to pain chronification, by coupling nociceptive inputs with emotional valence. In this context, the hippocampus is thought to be a key driver for these maladaptive learning processes: morphometric properties of the hippocampus have been linked with exaggerated pain recall in chronic back pain patients, and subacute back pain patients with lower hippocampal volumes are at a higher risk of developing chronic pain. Experiments with spared-nerve injury mice further show that blocking hippocampal neurogenesis stops the emergence of chronic-pain behaviors, and chemogenetic stimulation of the hippocampus alleviates mechanical allodynia. However, very little is known about what triggers this maladaptive plasticity in the first place. In this study, we examine the brains of mild-traumatic brain injury patients immediately after an emotionally charged and pain-inducing automobile accident to probe for hippocampal properties that trigger maladaptive emotional learning. We collected data from 110 patients with whiplash and mild traumatic brain injury following an automobile accident, soon after the accident (mean 41 hours, range 12-115 hours). Pain intensity for these patients was collected every month for up to a year after injury, and fifty-three percent of the patients reported clinically significant pain 12 months after the injury (NRS > 30/100).We studied resting-state functional connectivity within the hippocampal network, by parcellating the hippocampus into 6 ROIs (anterior, middle, posterior; left and right), and performing seed-based correlation analyses from each ROI to voxels within the hippocampal network.Patients who developed chronic pain showed increased functional connectivity between the medial-posterior hippocampus and bilateral posterior hippocampal network, compared to patients who fully recovered, highlighting an acute response likely related to emotional learning and memory formation. This increased functional connectivity increased linearly with the number of hours post-injury in patients developing chronic pain, whereas it was lower and stable in patients who recovered from acute pain. This time dependency was further

associated with anxiety, whereby higher post-injury anxiety led to increases in hippocampal connectivity over time. Taken together, our findings highlight a time-sensitive, maladaptive emotional learning process, consistent with the idea that that pain memory is being consolidated and, when linked with an emotionally salient incident, can place patients at risk for chronic pain.

Disclosures: P. Branco: None. **N. Bosak:** None. **A. Vigotsky:** None. **Y. Granovsky:** None. **D. Yarnitsky:** None. **A. Apkarian:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.11

Topic: C.10. Brain Injury and Trauma

Support: DoD W81XWH-20-1-0717

Title: Persistent post concussive symptoms are associated with a widespread reduction in fractional anisotropy of the major human white matter tracts

Authors: *G. BERTO[1], L. WILKINS[2], N. L. PORT[2], F. PESTILLI[1];
[1]Univ. of Texas, Austin, Austin, TX; [2]Indiana Univ., Bloomington, IN

Abstract: Concussion is a transient alteration of brain function that can occur mainly due to falls, vehicle accidents, and sport or military injuries. Although in most of the cases concussed individuals recover within a few days or weeks, in 30% of the cases their symptoms persist for a prolonged period of time after the injury. This condition is referred to as Persistent Post Concussive Symptoms (PPCS) or Post Concussive Syndrome (PCS). Predicting, at the time of injury, the individuals who are at significant risk for developing PPCS could allow for treatment interventions to reduce the risk. The aim of this study is to evaluate whether a reduction in the microstructural properties of the major human white matter tracts at the time of injury is associated with developing PPCS.
A large public dataset on student and military cadet athletes distributed from the Concussion Assessment Research and Education (CARE) consortium was used for this study. More specifically, anatomical and diffusion Magnetic Resonance Imaging (dMRI) data collected within 24-48h from injury (n=51). Participants were divided into two groups based on their Concussion Recovery Time (CRT): (Group A) 42 participants had a CRT < 28 days, and (Group B) 9 participants had a CRT >= 28 days (individuals with PPCS). A reproducible tractometry analysis was performed on 61 major white matter tracts. The mean difference in Fractional Anisotropy (FA) between group A and B was computed. The open cloud platform brainlife.io was used for all analyses.
Interestingly, 44 white matter tracts of 61 showed a reduced FA in Group B (the PPCS group; one-tailed t-test, Bonferroni corrected, $p<0.001$). We rank ordered the difference in FA between the two groups. The top-five tracts largest differences were: right posterior Arcuate fasciculus

(9.6% reduction in FA), right Middle Longitudinal Fasciculus - Angular gyrus component (8.1%), forceps Minor (7.4%), left posterior Arcuate fasciculus (7.3%), and left Uncinate fasciculus (7.2%). Furthermore, we computed the correlation between mean FA and CRT. The scatter plots of mean FA versus CRT exhibited a downward trend and negative correlation (Pearson's $r = -0.32 \pm 0.06$, $p<0.05$).
These results demonstrate that individuals with PPCS have lower FA in a majority of the white matter tracts. The result suggests that white matter is affected (likely due to inflammation) within 24-48h from injury. If the results were to be confirmed in larger samples of subjects, they could serve as a first step toward identifying a PPCS neuroimaging biomarker. Subjects with long CRT could be identified using reproducible processing pipelines such as those available on brainlife.io.

Disclosures: G. Berto: None. **L. Wilkins:** None. **N.L. Port:** None. **F. Pestilli:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.12

Topic: C.10. Brain Injury and Trauma

Support: Childress Institute for Pediatric Trauma

Title: Cognitive outcomes of head impact exposure in boys' youth ice hockey

Authors: *A. G. SWENSON[1], N. PRITCHARD[4], L. E. MILLER[2], L. A. CHAPMAN[3], L. A. FLASHMAN[3], J. E. URBAN[2], J. D. STITZEL[2];
[1]Neurosci., [2]Biomed. Engin., [3]Neuropsychology, Wake Forest Sch. of Med., Winston Salem, NC; [4]Biomed. Engin., Virginia Tech. - Wake Forest Sch. of Biomed. Engin. & Sci., Winston Salem, NC

Abstract: This study aimed to examine relationships between head impact exposure (HIE) metrics and neurocognitive outcomes in non-concussed boys' hockey athletes following a single season of play. 27 athletes ages 12-16 at preseason were recruited from a local youth hockey organization and were fitted with a custom instrumented mouthpiece for measuring head kinematics. HIE metrics recorded included mean and 95th percentile peak resultant head linear acceleration (PLA), rotational velocity (PRV), and rotational acceleration (PRA) for each athlete. Athletes completed a pre- and post-season cognitive exam which included subtests from the NIH Toolbox and ImPACT test. Preliminary relationships between HIE metrics and cognitive outcomes were assessed using linear regression models with IQ and years of hockey experience as covariates. 95th percentile accelerations were log-transformed prior to analysis to generate a normal distribution. For all regressions, Cook's D was calculated and outliers were removed using a threshold of 4/n. We recorded 1590 video-verified contact scenarios with the mouthpiece, with a median [95th percentile] PLA, PRV, and PRA of 7.35 [21.9] g, 7.15 [17.4]

rad/s, and 555 [1514] rad/s^2 respectively. Per athlete mean PLA, PRV, and PRA ranged (min – max) from 7.1 – 12.3 g, 6.9 – 10.6 rad/s, and 519 – 835 rad/s^2, respectively. All athletes experienced a decline in one or more cognitive domains; the greatest mean negative change from pre- to post-season was observed on ImPACT verbal memory composite scores (-0.26 ± 9.6). Increased mean kinematics were significantly associated with declines in ImPACT verbal memory composite scores (PLA: p=.0002, R^2=.4499; PRV: p=.0410, R^2=.1628; PRA: p=.0059, R^2=.2856), consistent across models. A decline in pre- to post-season verbal memory composite scores was negatively associated with 95th percentile PLA (p=.0080, R^2=.2584). Comparatively, there was a significant positive relationship between ImPACT reaction time scores and increasing mean PRA (p=.0247, R^2=.2090). Lower scores on the NIH Toolbox list sorting working memory task trended towards a negative association with increasing 95th percentile head accelerations, though these relationships were not significant. In summary, we observed statistically significant negative relationships between mean and 95th percentile head kinematics and verbal memory, and a significant positive relationship between reaction time and mean PRA among youth hockey athletes after one season of play. These findings suggest a relationship between head impact exposure measures and short-term effects in aspects of cognitive performance, warranting further study.

Disclosures: A.G. Swenson: None. **N. Pritchard:** None. **L.E. Miller:** None. **L.A. Chapman:** None. **L.A. Flashman:** None. **J.E. Urban:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Drs. Stitzel and Urban have a patent pending on the mouthpiece technology presented herein. **J.D. Stitzel:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Drs. Stitzel and Urban have a patent pending on the mouthpiece technology presented herein.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.13

Topic: C.10. Brain Injury and Trauma

Support: DoD Grant MR141214

Title: Brain metabolite alterations in chronic stage after traumatic brain injury and the association with pain and psychological function

Authors: *L. E. ROBAYO[1,2], V. GOVIND[3], S. SHERIFF[3], N. P. CHERUP[4,2], A. SNIPES[5,2], A. A. MAUDSLEY[3], E. WIDERSTROM-NOGA[4,2];
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Abstract: Traumatic brain injury (TBI) can lead to brain tissue metabolic alterations, which can persist for a long time. Besides cognitive, psychological, and sensory dysfunction, people with TBI may also develop chronic pain. However, metabolic alterations in pain-related brain regions are not fully understood. To address this, we used a unique in vivo magnetic resonance spectroscopic imaging (MRSI) technique to investigate brain metabolite alterations at chronic stage following TBI in adult human subjects. First, we quantified brain tissue metabolite levels in individuals with TBI, compared them with healthy individuals, and evaluated associations with psychological, pain, and sensory variables. Twenty-six participants with TBI (with and without chronic pain) and thirty-one abled-bodied healthy controls underwent a whole-brain MRSI scan at 3 Tesla. MRSI findings showed metabolic alterations in individuals with TBI, evidenced by lower N-acetyl aspartate, suggesting neuronal loss or dysfunction in the frontal, anterior cingulate cortex, and thalamus regions. These regions are implicated in the affective-motivational and cognitive aspects of pain processing and modulation. Additionally, metabolite levels in the frontal region were correlated with the severity of neuropathic pain symptoms (NPSI) and anxiety scores. The results from this study indicate the specific metabolic mechanisms of chronic pain following TBI, which can contribute to advancements in therapeutic interventions to reduce chronic pain.

Disclosures: L.E. Robayo: None. **V. Govind:** None. **S. Sheriff:** None. **N.P. Cherup:** None. **A. Snipes:** None. **A.A. Maudsley:** None. **E. Widerstrom-Noga:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.14

Topic: C.10. Brain Injury and Trauma

Support: MEDTEQ/HBHL/Fondation NeuroTrauma Marie-Robert and Saccade Analytics Mitacs Scholarship

Title: Oculomotor functions evaluation as a diagnostic tool in concussion: a pilot study using fMRI

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Abstract: Mild traumatic brain injury (mTBI), frequently referred to as concussion, is a major public health problem. Ambiguity still exists with regard to the pathophysiology and management of concussions, leaving an objective diagnosis a topical problem. Previous studies by our team demonstrated that fMRI is an objective approach that allows consistent, reproducible results in the diagnosis of concussion. However, given the limited availability of fMRI in a clinical setting, an alternative approach is needed. One of the often-reported pathologies in

mTBI, is a deficit in oculomotor function. Due to the neuroanatomical overlap between eye-movement circuitry and mTBI pathophysiology, it is expected that visual deficits would follow mTBI. In this pilot study, we aim to investigate the possibility of using an automated tool for oculomotor assessment in the concussion diagnosis.
We carried out fMRI testing with 4 tasks using an automated eye-tracking system evaluating oculomotor functions: Smooth Pursuit (SP), Saccades, Anti-Saccades, and Optokinetic Nystagmus (OKN). 6 adult concussed subjects within 1-month post-injury and 6 age- and sex-matched healthy subjects were tested. The fMRI images were preprocessed and analyzed using SPM12, and BOLD signal alterations were compared between groups.The results of a whole-brain analysis showed alterations in BOLD signal in concussed group: (1) decrease in frontal and supplementary eye fields (SEF,FEF) and visual cortex (VC), and increase in thalamus during SP, (2) decrease in SEF and inferior parietal lobule (IPL) and increase in VC during Saccades, (3) increase in FEF and VC during Anti-Saccades, (4) increase in Superior Frontal Gyrus, Middle Temporal Gyrus and IPL during OKN. The results of ROI analysis showed significant differences only in Cuneus in SP. Results from the eye-tracking system didn't show any significant differences between groups.
These preliminary results showed that concussed subjects demonstrate significant alterations in activation in brain areas involved in different aspects of oculomotor function, between the concussed group and the group of healthy controls. These results are encouraging, and they suggest that fMRI using described oculomotor tasks is a promising approach in the diagnosis of concussion.

Disclosures: E.A. Lunkova: None. **A. Ptito:** None. **R.S. Saluja:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.15

Topic: C.10. Brain Injury and Trauma

Support: This coordination is supported by the Acute Effects of Neurotrauma Consortium. Research was sponsored by the Leonard Wood Institute in cooperation with the U.S. Army Research Laboratory accomplished under Cooperative Agreement Number W911NF-14 2-0034

Title: Positive amyloid-beta positron emission tomography after repeated exposure to subconcussive blasts in healthy military personnel.

Authors: *D. BEVERSDORF[1], E. HART[2], B. FERGUSON[3], J. CASSANI[2], A. SINGH[2]; [1]Univ. of Missouri Columbia, Univ. of Missouri Columbia, Columbia, MO; [2]Univ. of Missouri, Columbia, MO; [3]Univ. of Missouri Thompson Ctr. For Autism, Univ. of Missouri Thompson Ctr. For Autism, Columbia, MO

Abstract: Background: Recurrent traumatic brain injury (TBI) is associated with psychologic, neurologic, and cognitive sequelae, which can be apparent many years after injury. Evidence of neurodegeneration has been identified in deceased military veterans and civilians even after recurrent mild trauma and subconcussive impacts. It is increasingly apparent that long-term brain structural and functional effects of TBI start long before they are clinically evident. Increased neurotoxic Aβ deposits after TBI suggest a mechanistic link between neurotrauma and the development of neuropathologic features of dementia. Military personnel are often exposed to repeated concussive forces during training exercises, such as breacher training. Understanding the impact of subconcussive blasts on brain structure and functioning can facilitate development of protective and preventative strategies tailored to the mechanism of injury. Methods: Nine instructors on the breacher training course, who instructed other solders on the proper techniques and, as a result, had repeated blast exposure, were recruited from Fort Leonard Wood. All participants were male, and ranged in age from 27-43, without other medical conditions. All had an amyloid-β positron emission tomography (Aβ-PET) scan prior to their time instructing, and a second Aβ-PET scan immediately after the end of their time instructing, 4-6 months later. The number of blast exposures were compared between instructors that had positive vs negative Aβ-PET at the end of their time instructing. All images were rated by a blinded rater who is the nuclear medicine co-investigator on this study. Results: All nine participants had negative Aβ PET studies prior to breacher training. Four of nine breacher trainers were found to have clinically significant Aβ deposition on Aβ PET after training. The four that developed a positive Aβ PET study immediately after training reported significantly more blast exposures (130.5, *SD* = 76.1) than the five that had a negative study immediately after training (average 28.2, *SD* = 12.7) ($t(7) = 2.53$, $p = 0.02$). Discussion: This is, to our knowledge, the first demonstration of changes in brain amyloid in the early timeframe after subconcussive blast exposure. In addition to confirming this finding in a larger sample size, it will be critical to examine this in other blast exposed populations, and to determine whether these changes persist over time after the end of blast exposures. Furthermore, a better understanding of the risk factors and biological mediators (such as glymphatic integrity) for these changes in amyloid, beyond increased blast frequency, is needed.

Disclosures: **D. Beversdorf:** F. Consulting Fees (e.g., advisory boards); Quadrant Biosciences, Impel Pharma, Stalicla Biosciences, Scioto Biosciences, YAMO Pharma. **E. Hart:** None. **B. Ferguson:** None. **J. Cassani:** None. **A. Singh:** None.

Poster

288. Disorders of Human Brain Function Including Epilepsy and Injury

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 288.16

Topic: C.10. Brain Injury and Trauma

Support: NSERC

Title: Assessing eye-movement performance and executive function after concussion

Authors: *E. POWNALL, L. E. BROWN;
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Abstract: After a concussion, individuals can experience subtle changes to eye-movement and cognitive performance that go unrecognized. To understand the relationship between eye-movement performance and executive functioning over the recovery trajectory, we asked healthy controls, orthopedic controls, and concussed individuals to complete tests of eye-movement performance and executive function. We documented participants' current health ratings and symptom experience, including symptom severity and time since injury. All participants completed the following standardized executive functioning tests: Digit Span, Trail-Making Test, WAIS-IV Coding, and Verbal Fluency. Eye-tracking data was also collected while participants completed both pro- and anti-saccade tasks, sinusoidal smooth pursuit trials at slow and fast speeds, and a multiple object tracking (MOT) task requiring the participants to track one to five of 10 objects. General health ratings indicated that both orthopedic and concussion injuries had a significant effect on participants' perception of general health at the group level ($p = .039$). While there were no differences between groups on performance of tests of executive function, the concussed group's performance differed from controls on the anti-saccade task as they made proportionally more two eye-movement responses. These findings, along with future analyses planned to assess relationships between executive and eye-movement performance, symptom severity and time since injury, suggest that individuals who have sustained a concussion experience eye-movement dysfunction that may interfere with cognitive performance.

Disclosures: E. Pownall: None. **L.E. Brown:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DARPA Grant D15AP00112
DARPA Grant D19AC00015
NIH Fellowship 5T32NS100663-04

Title: High Density Electrode Arrays Increase Specificity of Evoked Responses During Epidural Electrical Stimulation

Authors: *J. S. CALVERT[1], S. R. PARKER[1], E. TIWARI[1], E. SHAAYA[3], L. M. DEL VALLE[1], R. DARIE[1], J. S. FRIDLEY[2], B. MCLAUGHLIN[4], D. A. BORTON[1];
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Abstract: Traumatic spinal cord injury (SCI) is a debilitating condition that can result in permanent loss of motor, sensory, and autonomic functions below the SCI lesion. Although there

is currently no cure for chronic SCI, epidural electrical stimulation (EES) has recently emerged as a research technique to enable intentional control of motor functions that were lost after SCI. However, EES technology has yet to translate to widespread clinical use as electrode arrays used to study EES were developed for chronic pain treatment and are not optimized for the restoration of function in individuals with SCI. Here, our goal was to demonstrate the effect of high-density, 60-channel EES electrode arrays (HD-64, Micro-Leads Medical) on the specificity of EES evoked responses. Specifically, we investigated the effect of increased spatial resolution on recording evoked compound action potentials (ECAPs) from the dorsal aspect of the epidural space and evoked motor responses measured by electromyography (EMG) from the lower extremity musculature. Two female sheep were chronically implanted with two HD64 electrode arrays placed onto the lumbosacral spinal cord, spanning the L3-L6 vertebrae. The increased resolution of the HD64 electrode arrays enabled identification of stimulation locations associated with increased response specificity in both the spinal ECAP and EMG responses. Stimulation along the lateral edges of the HD64 resulted in regions of increased ECAP and motor recruitment, which were confirmed to be regions overlapping the dorsal roots of the spinal cord. Multipolar stimulation further increased the specificity of both spinal ECAP and EMG recruitment. Specifically, current steering enabled by the increased spatial resolution decreased the propagation of the ECAP responses, and reduced the simultaneous activation of multiple lower extremity muscles as measured by EMG. Overall, the increased resolution of the HD64 electrode arrays in combination with direct epidural spinal recordings enabled identification of relevant spinal anatomy to target for increased efficacy of motor activation via EES.

Disclosures: **J.S. Calvert:** None. **S.R. Parker:** None. **E. Tiwari:** None. **E. Shaaya:** None. **L.M. Del Valle:** None. **R. Darie:** None. **J.S. Fridley:** None. **B. McLaughlin:** None. **D.A. Borton:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: D15AP00112
D19AC00015
5T32NS100663-04

Title: Exploring the effects of Baclofen on electrically evoked compound action potential in the ovine spinal cord

Authors: *L. M. DEL VALLE[1], E. TIWARI[1], J. S. CALVERT[1], S. PARKER[1], R. DARIE[1], E. SHAAYA[3], A. GREGOIRE[1], R. PONCIN[1], B. MCLAUGHLIN[4], D. A. BORTON[2];
[1]Sch. of Engin., [2]Neuroengineering, Brown Univ., Providence, RI; [3]Dept. of Neurosurg., Rhode Island Hosp., Providence, RI; [4]Micro-Leads, Somerville, MA

Abstract: Traumatic spinal cord injury (SCI) is a devastating disease that afflicting hundreds of thousands of people each year. The current standard of care is to decompress the area of injury and stabilize the spinal cord to avoid secondary injury. After the patient is stabilized, pharmacological interventions and rehabilitation are prescribed to treat secondary conditions, for example loss of motor and sensory function. Recently, spinal cord epidural electrical stimulation (EES) has become a promising approach to modulate neural circuitry that is disrupted following SCI and achieve modest functional recovery. Many participants of studies using EES concurrently take pharmacological agents to treat their SCI symptoms, and it is likely that both EES and pharmacological agents will be essential to the recovery process. Thus, it is critical to understand the effects of commonly used pharmaceutical agents on the underlying spinal cord circuitry. Therefore, in this study we establish a method to explore the effects of a commonly used SCI therapeutic drug, Baclofen, in combination with and without EES on the evoked activity of the spinal cord in sheep. Baclofen, a $GABA_B$ receptor agonist, is a commonly prescribed agent used to treat conditions of SCI, including pain, muscle spasticity, and stiffness. In the spinal cord, Baclofen primarily acts on pre-synaptic terminals to hyperpolarize the cell, which in turn reduces excitability and leads to a reduction of spasticity. To study the effects of Baclofen on the spinal cord, we implanted two 60-contact HD64 (Micro-Leads Inc) electrodes arrays epidurally at L3/L4-L6 spinal segments in sheep (n=2). Implantation of vascular access port enabled for frequent drug infusion and blood collection for pharmacokinetic (PK) analysis. Blood samples were collected beginning at infusion and then at 0.5, 1, 4, 8, 24, 48, and 72 hours to obtain optimal drug concentration, and identify when the drug was no longer present within the blood. Recordings of electrically evoked compound action potentials (ECAPs) were performed pre- and post- drug infusion using the Macro+Stim data acquisition system (Ripple Neuro) during awake at-rest and treadmill walking behaviors. Here we present our preliminary results from the ongoing pharmacokinetic study. Our results provide insight into the effects of a commonly used pharmacological agent in combination with EES of the spinal cord and develop a better understanding of multimodal therapeutic integration after SCI.

Disclosures: **L.M. Del Valle:** None. **E. Tiwari:** None. **J.S. Calvert:** None. **S. Parker:** None. **R. Darie:** None. **E. Shaaya:** None. **A. Gregoire:** None. **R. Poncin:** None. **B. McLaughlin:** A. Employment/Salary (full or part-time):; Micro-Leads. **D.A. Borton:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 289.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DARPA Grant D15AP00112 Supported to Brown University
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Title: Simultaneous spinal cord epidural electrical stimulation and recording of evoked compound action potential using fully implantable high bandwidth neural interface: a pilot study in sheep

Authors: *E. TIWARI[1], S. PARKER[1], J. CALVERT[1], E. SHAAYA[1], R. DARIE[1], N. ABDELRAHMAN[2], M. MARLO[2], I. HALPERN[2], J. FRIDLEY[1], D. BORTON[1];
[1]Brown Univ., Providence, RI; [2]Modular Bionics, Inc, Berkeley, CA

Abstract: Spinal cord injury (SCI) drastically impacts the quality of life of patients. The standard of treatment for individuals with SCI focuses on rehabilitation and pharmacological treatments to maximize return of function, yet most patients do not make a full recovery. In recent years, neuromodulation techniques, specifically spinal cord epidural electrical stimulation (SCEES) have emerged as an alternative approach to restore function following SCI. Recent SCEES devices can record evoked compound action potentials (ECAPs) in the spinal cord which can be used as feedback signals to optimize spinal cord stimulation. To determine the feasibility of a closed loop feedback system, together we, with our industrial partner (Modular Bionics, Inc) tested a fully implantable Clinical Linked Neural Communication (C-LINC) system. C-LINC is a high bandwidth data communication device (UWB uplink 90Mbps data rate) with 64 recording and 16 stimulation channels. For this study, the C-LINC was fitted with 2x12 contacts and 6 double-ended electromyography (EMG) leads to perform wireless highspeed neural stimulation and recordings from the spinal cord and muscles. We sought to evaluate the functionality of the system from the sheep spinal cord. Benchtop testing was first performed in saline prior to use in animals. Electrode arrays (2x24-contacts passive or 2x60-contacts active HD64) were implanted in normal intact animals (n=2). Electrodes were then inserted into the dorsal epidural space of spinal cord at L3/L4-L6 spinal segments. The C-LINC was then implanted subcutaneously and connected to the leads via Bal-Seal connectors. Bilateral EMG recordings were made from the lower extremity muscles including biceps femoris, gracilis, peroneus longus, gastrocnemius. During awake recording sessions an external unit was placed over the implanted device to power and receive the data. Simultaneous spinal cord stimulation and electrophysiology were performed using combinations of amplitude and frequency. The on-board FPGA of the C-LINC was then programmed to control the active multiplexor circuitry of the HD64. We recorded low noise ECAPs and synchronous EMG signals via high bandwidth wireless communication between the C-LINC implant and external unit. Recorded signals were compared to ECAPs that were recorded with established benchtop data acquisition systems (Macro+Stim, Ripple Neuro). These results suggest that the C-LINC is fully capable of recording ECAPs, transmitting high-bandwidth data transcutaneously, and can be wirelessly powered for fully-implanted operation. The C-LINC and systems like it will enable new therapies to restore or replace function following SCI.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: DARPA BTO D15AP00112
DARPA BTO D19AC00015
NINDS T32 Postdoctoral Training Program in Recovery and Restoration of CNS Health and Function (5T32NS100663-04)
2020 Susan and Isaac Wakil Foundation John Monash Scholarship

Title: Leveraging increased electrode density to facilitate optimal epidural spinal stimulation location discovery

Authors: *S. PARKER[1], L. N. GOVINDARAJAN[2], J. S. CALVERT[1], R. DARIE[1], E. SHAAYA[3], T. SERRE[2], D. A. BORTON[1];
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Abstract: Recent studies have demonstrated the utility of spinal cord stimulation (SCS) in the restoration of motor function in individuals living with chronic spinal cord injury (SCI). These studies utilize epidural electrical stimulation (EES) and rely heavily on intraoperative recruitment curves to titrate stimulation parameters. Trends in EES technology point towards the adoption of higher channel-count paddle arrays. Our work has evaluated artificial neural networks to be capable of effectively selecting stimulation amplitude and frequency, when conditioned on a single electrode. As the number of electrodes on EES paddles increases, training independent models for each electrode requires an increasing amount of training data and increased computational effort. Additionally, encoding the relative positions of stimulating contacts enables networks to learn spatial dependencies between electrode locations. Herein, we evaluate a method to parameterize the stimulation location, enabling discovery of unseen stimulation sites using a single neural network model. Two high-density (60-contact, Micro-Leads Medical) epidural spinal electrode arrays were implanted in two adult sheep. Bilateral lower extremity muscle activity was recorded using 8 wireless electromyography (EMG) sensors. A low-density training dataset was collected, then used to build an amortized neural network model of spinal cord computations. Using Approximate Bayesian Computation methods, novel stimulation amplitude, frequency, and location combinations were derived from the model. EMG activations elicited from the derived parameter combinations were compared to their target activation patterns. We observed low error between summarized EMG responses when assessing network performance in stimulation locations not sampled in the training dataset. Training the network utilizing the location encoding method described here was completed in significantly less time than the current state-of-the-art, and resulted in lower error when tested on stimulation electrodes not in the training dataset. Utilizing location encoding to predict evoked EMG responses could increase the efficacy of neural network predictions of motor activation via EES and lead to greater outcomes using EES in individuals with SCI.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R21:1R21NS114982-01A1

Title: Motor-evoked Potentials in the Cortical Spinal Tract in a Porcine Spinal Cord Injury Direct Contusion Model Post Injury

Authors: M. CHOPRA[1], J. CASTLE[3], S. DAVISON[2], L. SHERWOOD[2], B. UGILIWENEZA[2], R. BERT[2], D. HOWLAND[2], *M. BOAKYE[1];
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Abstract: The presence of D-waves is thought to indicate direct corticospinal tract connectivity from the cortex to the site of recording. The objective was to compare motor evoked potentials in the lower thoracic epidural space of the Yucatan minipig before and after a T9/10 contusion SCI in the Yucatan pig. The study was performed in 8 female Yucatan minipigs with weights ranging from 22-25 kg. Pigs underwent a T9-10 mid-thoracic injury using a 50 gram weight dropped from a height of 20 cm (N=5) or 5 cm (N=3), followed by 5 min of compression by adding an additional 100 gram weight to the impactor. Two stimulation trains of stimulus intensities of 50-400 V (pulse duration 1.0 ms, ISI 0.5 ms) were delivered through stainless steel alligator clips clamped to the screws which were placed according to the international 10-20 nomenclature: FC1, FC2 was assigned to points 7.5 mm towards the nasion from the vertex and 5 mm lateral to the midline. Two double contact strip electrodes were placed in the exposed dura at dorsal epidural space 1 cm cranial and 1 cm caudal to the injury site. D waves were recorded by stimulating the motor cortex before and after the spinal cord contusion injury. Amplitudes and Latencies of evoked potentials were measured before and after SCI. Amplitudes were measured above and below the injury level. D waves were present below injury in all pigs. D wave amplitudes was significantly reduced below the injury Pre Injury and Post Injury.
Dwave amplitudes above the injury were increased after the injury. Pre-post amplitude change above injury is 16 +/- 21, p = 0.0630 This change represents an effect size of 0.76 ~ 0.8 classified as a large effect size. There was no difference between latencies between 20cm vs. 5 cm injuries or good recoverers vs. bad recoverers. There was no difference in amplitude between good recovery and bad recoverers plasticity of D-waves amplitudes above the injury was observed and may reflect sensorimotor reorganization in response to the injury. D waves presence below injury indicates injury incompleteness and importantly suggests corticospinal tract may extend to the level of measurement around T10-11. Unlike a previous study that was able to obtain motor evoked potentials in hindlimb muscles below injury, Dwaves below a 20 cm injury may simply reflect differences in impact forces despite same drop heights or that D-waves may be more sensitive than motor evoked potentials in the muscle. Further studies are needed to

compare D waves to motor evoked potentials in the Yucatan minipigs after a 20 cm injury. Limitations are the small sample size and therefore need to study more pigs to confirm findings.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 1R01NS124224
Travis Roy Foundation Boston, MA (Investigator Initiated)

Title: Intraoperative dorsal root entry zone stimulation facilitates cortical motor evoked potentials in humans

Authors: *J. R. MCINTOSH[1,4], E. F. JOINER[2], J. L. GOLDBERG[4], L. M. MURRAY[7,8], A. MENDIRATTA[3], S. C. KARCESKI[5], E. THUET[10], O. MODIK[5], N. PATEL[6], E. SHELKOV[5], J. M. LOMBARDI[1,10], Z. M. SARDAR[1,10], R. A. LEHMAN[1,10], C. MANDIGO[2,10], K. D. RIEW[4,1,10], N. Y. HAREL[9,8,7], M. S. VIRK[4], J. B. CARMEL[1,3,4];
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Abstract: Learning of skilled movement requires coincident activation of motor and sensory connections. In rats we have developed a spinal cord associative plasticity protocol which relies on synchronized stimulation of the brain and cervical spinal cord. Application of this protocol for 5-30 minutes induces lasting changes in spinal excitability and produces improvements in dexterity in rats. The strongest facilitation of muscle activation occurs when spine and brain stimulation converges in the spinal cord. We hypothesized that this finding would be reproducible in humans. Patients undergoing clinically indicated posterior or anterior cervical spine surgery for chronic spondylotic myelopathy were enrolled. During surgery, to determine the best sites of convergence for brain and cervical spinal cord stimulation, the spine was initially mapped by stimulating multiple locations and recording motor evoked potentials. In subsequent experiments, cortical stimulation was combined with bipolar epidural spinal stimulation at different latencies while muscle electromyogram responses were recorded. Subthreshold spinal cord stimulation over the dorsal root entry zones strongly augmented the muscle responses to cortical stimulation, but only when they arrived synchronously in the spinal cord. Muscles innervated by the targeted cervical segments were most strongly influenced, but muscles at more

remote segments were also facilitated. Less facilitation occurred when spinal cord stimulation was applied ventrally rather than dorsally. Strong facilitation of cortical stimulation evoked muscle responses by spinal stimulation is indicative of the convergence of these signals at the level of the spinal cord and a prerequisite for the induction of timing dependent plasticity. Future work will test whether repeated application of motor cortex and dorsal epidural stimulation timed to converge in the spinal cord will strengthen the sensorimotor connections in the spinal cord and improve recovery after spinal cord injury.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation SCIRTS 651093
CIHR PJT 180556

Title: Changes in connectivity to dI3 interneurons and spinal motoneurons following spinal cord injury in mice

Authors: *S. GOLTASH[1], T. V. BUI[2];
[1]Biol., [2]Univ. of Ottawa, Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Spinal cord injury (SCI) is a debilitating condition that disrupts the communication between the brain and the spinal cord. After an injury, commands from the brain can no longer activate neurons in the spinal cord that control muscle activity for body movements. As a result, these spinal circuits become dormant, leading to paralysis. Several studies have sought to determine how to revive these circuits to restore movements in paralyzed patients. So far, recovery levels in human patients have been modest at best. In contrast, animal models of SCI

exhibit more significant levels of recovery of lost function. Previous work from our lab has identified dI3 interneurons as a spinal neuron population central in the recovery of locomotor function in spinalized mice. We seek to determine the changes in circuitry of dI3 interneurons and motoneurons following SCI in adult mice. After a complete transection of the spinal cord at T9-T11 level in transgenic Isl1:YFP mice and subsequent treadmill training, at various time points of recovery following surgery, we examined changes in three key circuits involving dI3 interneurons and motoneurons: 1) Sensory inputs from proprioceptive and cutaneous afferents, 2) Presynaptic inhibition of sensory inputs, 3) Central excitatory glutamatergic synapses from spinal neurons onto dI3 INs and motoneurons. Furthermore, we examined the possible role of treadmill training on changes in synaptic connectivity to dI3 interneurons and motoneurons. Our data suggests that in the absence of treadmill training, there was a decrease in sensory inputs to dI3 INs at 3 weeks post injury but no change was observed in motoneurons after SCI. Treadmill training seemed to prevent the decrease of sensory inputs to dI3 INs after injury. The level of presynaptic inhibition of sensory inputs to dI3 INs was only increased in the mice that didn't receive treadmill training at 3 weeks post injury. On the other hand, in the motoneurons, presynaptic inhibition was initially increased at 6 days post injury but then was decreased in the following weeks post injury both with and without training. In the central excitatory synapses, a reduction in inputs to both dI3 INs and motoneurons was observed. This reduction was prevented by training. These results suggest remodelling of spinal circuits during training as a form of adaption to promote locomotor recovery.

Disclosures: S. Goltash: None. **T.V. Bui:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation SCIRTS 651093
CIHR PJT 180556

Title: Spinal cord dI3 interneurons modulate stretch reflexes and hindlimb motor tone in spinalized mice

Authors: *A. LALIBERTE, R. KHODR, T. V. BUI;
Biol., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: The dI3 population of spinal cord interneurons (INs) is a group of excitatory INs that receive varied sensory afferent input and project ipsilaterally to motoneurons. Prior experiments have discovered that the permanent silencing of dI3 INs has a minimal impact on locomotor function in intact animals, but substantially interferes with locomotor rehabilitation and recovery after spinal cord injury. To gain insight into how dI3 INs are involved with the recovery of

locomotor function, the inhibitory or excitatory DREADD receptor (hM4Di or hM3Dq) was expressed in dI3 INs using a hybrid transgenic mouse line (Isl1-Cre:Vglut2-Flp) combined with an hM4Di reporter line, or an intraspinally-injected adeno-associated virus expressing hM3Dq. Consistent with prior experiments, transient inhibition of hM4Di-expressing dI3 neurons with the DREADD agonist JHU37160 (0.5mg/kg) did not significantly impact locomotor function in intact animals. However, following T9-T10 transection, transient silencing of dI3 INs resulted in a significant loss of flexor motor tone, demonstrated by an increase in ankle joint angle during treadmill locomotor assessment (+43.9° ± 11.6°, n=7, paired t-test, p=0.0065). This coincided with hindlimb flaccidity during a treadmill locomotor task. Conversely, activation of dI3 interneurons in $dI3^{hM3Dq}$ mice resulted in a tonic increase in tibialis anterior EMG activity that was partially modulated by passive stretch or relaxation. Disinhibition of locomotor rhythmogenesis via the administration of quipazine was also performed to determine whether dI3 stimulation could improve the quality of locomotion elicited through serotonergic agonism. Subsequent viral anterograde tracing of dI3 projections (GFP+) to fluorogold-labeled gastrocnemius or tibialis anterior motoneurons identified substantial innervation of gamma motoneurons (ERR3+) in addition to alpha motoneurons. Based on these results, dI3 INs appear to regulate hindlimb motor tone, possibly through the modulation of fusimotor drive and stretch reflexes.

Disclosures: **A. Laliberte:** None. **R. Khodr:** None. **T.V. Bui:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 289.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation SCIRTS 651093
CIHR PJT 180556

Title: A novel optogenetic mouse model of hindlimb spasticity after spinal cord injury

Authors: A. M. LALIBERTE[1], S. GOLTASH[1], ***T. V. BUI**[2];
[2]Univ. of Ottawa, [1]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Spasticity is a common consequence of spinal cord injury, disrupting motor function and resulting in significant discomfort. While elements of post-SCI spasticity can be assessed using pre-clinical SCI models, the robust measurement of spasticity severity can be difficult due to its periodic and spontaneous appearance. Electrical stimulation of sensory afferents can be used to elicit hyperreflexia or bouts of clonus; however, the placement of surface electrodes on the hindlimbs of awake animals can induce stress or encumbrance that could influence the expression of spasticity behaviour. Therefore, we have generated a novel mouse model of SCI-related spasticity that utilizes optogenetics to activate a subset of cutaneous Vglut2+ sensory

afferents to produce reliable incidences of hindlimb spasticity. To examine the efficacy of this optogenetic spasticity model, a T9-T10 complete transection injury was performed in Islet1-Cre$^{+/-}$;Vglut2-Flp$^{+/-}$;CreON-FlpON-CatCh$^{+/-}$ mice, followed by the implantation of EMG electrodes into the left and right gastrocnemius and tibialis anterior muscles. Beginning at 9 days post injury, EMG recordings were performed during episodic optogenetic stimulation during 1-2 sessions per week until 5 weeks post injury (n=10 females, n=5 males). Each mouse was placed on a transparent surface while an optic fiber coupled to a 470nm wavelength LED was positioned under the palmar surface of either mouse hindpaw. Each recording session delivered 100 ms light pulses 9 times to each hindpaw followed by 10 seconds of EMG recording. The results of these recordings demonstrated significant increases in the amplitude of EMG response to the light stimulus from 2 to 5 weeks post injury, indicating hyperreflexia. Interestingly, this hyperreflexia was significantly greater in the female cohort in comparison to the males. Numerous incidences of clonus were also detected through EMG and visual observation during the testing period, supporting the presence of spasticity. As such, the optogenetic mouse model developed for this study appears to reliably elicit spasticity in SCI mice and may be valuable for the study of SCI-related limb spasticity mechanisms and therapeutic interventions.

Disclosures: **A.M. Laliberte:** None. **S. Goltash:** None. **T.V. Bui:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS
VA
Craig H. Neilsen Foundation

Title: Reticulospinal Contributions to Spasticity in Humans with Subacute and Chronic Spinal Cord Injury

Authors: *D. DE SANTIS[1], S. SANGARI[1,2], M. A. PEREZ[1,2,3];
[1]Shirley Ryan AbilityLab, Chicago, IL; [2]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Edward Hines Jr., VA Hosp., Hines, IL

Abstract: Evidence showed that the majority of individuals with subacute and chronic incomplete spinal cord injury (SCI) develop symptoms of spasticity (Sangari and Perez, 2022). Spasticity has been associated to an aberrant facilitation from reticulospinal inputs in individuals with chronic SCI (Sangari et al., 2019). However, the extent to which reticulospinal input contribute to spasticity in humans with subacute and chronic SCI remains unknown. We tested spasticity in the quadriceps femoris muscle in individuals with subacute (~1-month post-injury during inpatient rehabilitation) and chronic (>1 year post-injury, ~12 years) SCI using the

Modified Ashworth Scale (MAS) and the Pendulum test (first swing angle, FSA) (subacute: MAS=2.8±0.5, FSA=63.4±11.4; chronic: MAS = 3.2±0.8). We examined the StartReact response, an involuntary release of a planned movement via a startling acustic stimulus that engages the reticulospinal tract, by measuring reaction times from electromyographic activity in the quadriceps femoris muscle during isometric knee extension. The StartReact response was measured as difference between the reaction time to visual versus a loud acoustic (120 dB) stimuli (Δ RT) and the reticulospinal gain (RSG; Baker and Perez, 2017). Maximal voluntary contraction (MVC) in the quadriceps muscle was measured during isometric knee extension. Measurements were also acquired in a group of age-matched control subjects. We found that MVC was reduced to a similar extent in individuals with subacute and chronic SCI compared to control subjects (controls=0.3±0.1 mV; subacute=0.1±0.03 mV chronic=0.07±0.04 mV). However, the StartReact response was larger in individuals with chronic (Δ RT=111.3±28.7 ms; RSG=2.3±0.9) compared with subacute (Δ RT=87.3±3.6 ms; RSG=1.5±0.1) SCI and control subjects (Δ RT=85.2±16.9 ms; RSG=1.6±0.4). Our results indicate a differential contribution of reticulospinal inputs to spasticity in the subacute vs chronic phase of SCI, suggesting the time-dependent presence of compensatory mechanisms in spasticity.

Disclosures: D. De Santis: None. **S. Sangari:** None. **M.A. Perez:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.11

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS
VA
Craig H. Neilsen Foundation

Title: Prevalence of Spasticity in Humans with Spinal Cord Injury with Different Injury Severity

Authors: *S. SANGARI[1,2], M. A. PEREZ[1,2,3];
[1]Shirley Ryan AbilityLab, Chicago, IL; [2]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Edward Hines Jr., VA Hosp., Hines, IL

Abstract: Spasticity is one of the most common symptoms manifested following spinal cord injury (SCI). The aim of the study was to assess spasticity in people with subacute and chronic SCI with different severity injury standardizing the time and assessments of spasticity. We tested 110 individuals with SCI classified by the American Spinal Injury Association Impairment Scale (AIS) as motor complete (AIS A and B; subacute, n=25; chronic, n=33) or motor incomplete (AIS C and D; subacute, n=23; chronic, n=29) at a similar time post-injury (subacute ~1-month post-injury during inpatient rehabilitation and chronic ≥1-year post-injury) using a clinical (Modified Ashworth Scale) and kinematic (Pendulum test) outcomes to assess spasticity in the

quadriceps femoris muscle. Using both methodologies, we found that among participants with subacute motor complete injuries, a small percentage of them showed spasticity while the majority showed no spasticity. In contrast, among people with subacute motor incomplete injury, both methodologies revealed that most individuals showed spasticity while only a small percentage showed no spasticity. In chronic injuries, most participants showed spasticity regardless of injury severity. Notably, when spasticity was present, its magnitude was similar across injury severity in subacute and chronic injuries. Our results suggest that the prevalence, but not the magnitude, of spasticity differs between individuals with motor complete and incomplete SCI in the subacute and chronic stage of the injury. We argue that considering the 'presence of spasticity' might help the stratification of participants with motor complete injuries for clinical trials.

Disclosures: S. Sangari: None. **M.A. Perez:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Program #/Poster #: 289.12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR

Title: The role of inhibited oligodendrocyte remyelination on locomotor, cognitive, and anxiety-related behaviors in young and aged spinal cord injured mice.

Authors: *S. WHEELER[1,2], B. KONDILES[1,3], S. B. MANESH[1,4], J. LIU[1], W. TETZLAFF[1,3]; [1]Intl. Collaboration on Repair Discoveries (ICORD), Univ. of British Columbia, Vancouver, BC, Canada; [2]Cell and Developmental Biol., [3]Zoology, [4]Neurosci., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Spinal cord injury (SCI) can be a costly and debilitating affliction that worsens with age. In western nations, the average age of injury has increased and is equally spread out between 20-65 years, reflecting an increase in the older population. Spinal cord injury (SCI) typically causes focal demyelination of spared axons near the site of injury. Surprisingly, we did not find an impairment of locomotor recovery after spinal cord injury when remyelination was inhibited in young adult mice (Duncan et al. 2018, Nat. Comm. PMID: 30076300). However, the functional impact of remyelination after SCI at a higher age is unknown. Here, we aim to assess the importance of remyelination for locomotor recovery and cognitive function comparing injury in young (3-5 month) versus older (15-18 month) transgenic mice of both sexes. We used an inducible knockout (iKO)of *Myrf,* a key transcription factor for myelination expressed in oligodendrocyte progenitor cells (OPCs), by crossing a PDGFRαCreERT2 driver line with mice carrying a floxed Exon8 in their Myrf gene ($Myrf^{fl/fl}$). This inhibits their maturation into oligodendrocytes (OLs) and prevents new myelination. These mice are compared to littermate

controls, who have a functional *Myrf gene.* Young and older animals underwent a moderate thoracic (T9/10) level contusion, or a sham injury (laminectomy), and are currently observed for three months. To examine the influence of age and remyelination inhibition on locomotion and cognition in injured or sham mice we will complete a variety of behavioral tests including an open field locomotion test (Basso mouse score) and a horizontal ladder test to assess the differences in locomotor ability. We assess cognition through the Y-maze and the object relocation task. Finally, using the marble burying task, elevated plus maze and fecal boli counts during open field locomotion, we will assess anxiety-related behaviors. Preliminary data show that older remyelination incompetent injured mice with Myrf iKO, compared to older SCI mice with intact Myrf, show deficits within locomotor and cognitive tests, but no difference in anxiety related behaviors. These data suggest a difference of younger versus older mice in their compensation of remyelination failure after SCI.Funded by CIHR.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Program #/Poster #: 289.13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Daniel & Ada Rice Foundation

Title: Age-related differences in inflammatory and behavioral responses following spinal cord contusion in rats

Authors: *B. T. DAVID, S. Y. OH, Q. SHEN, Y. LOPEZ-RAMIREZ, D. TUCKER, N. WROBEL, B. AVONTS, J. ALBERTS, J. MOSS, K. RAUE, R. G. FESSLER;
Rush Univ. Med. Ctr., Rush Univ. Med. Ctr., Chicago, IL

Abstract: The damage that results from spinal cord injury (SCI) is caused by both the initial mechanical trauma and the secondary injury that exacerbates the inflammatory reaction at the lesion site. As the average age at which an individual suffers an SCI has increased over the past decades, it has become increasingly relevant to determine what role age has on that inflammatory response. The aging process has been thought to be coincident with a state of nonresolving activation of inflammatory pathways, but many studies that have investigated the role of aging and SCI have found mixed results. Here, using the Infinite Horizon Impactor, we administered moderate contusive injuries to rats at a variety of ages between 3 and 12 months. We found that the older animals had a trend towards a worse recovery of both hindlimb locomotion and bladder function, despite having a lower microglia/macrophage population at the injury epicenter. Moreover, the reduced inflammatory response in older rats was not detected during the acute phase. This inflammation data is contrary to what some have reported in significantly older

animals (18 months), suggesting a possible shift in the inflammatory response with age, as opposed to a gradual increase in overall systemic inflammation.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Program #/Poster #: 289.14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS
VA

Title: Altered Regulation of Ia Afferent Input during Voluntary Activity after Human Spinal Cord Injury

Authors: *B. CHEN[1], M. A. PEREZ[1,2,3];
[1]Shirley Ryan AbilityLab, Chicago, IL; [2]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Edward Hines Jr. VA Med. Ctr., Chicago, IL

Abstract: Altered Regulation of Ia Afferent Input during Voluntary Activity after Human Spinal Cord Injury
Bing Chen[1] and Monica A. Perez[1,2]
[1]Shirley Ryan AbilityLab and Northwestern University, Chicago, US and [2]Edward Hines Jr., VA Medical Center, Chicago, US
Sensory input converging on the spinal cord contributes to the control of voluntary activity. Although sensory pathways reorganize following spinal cord injury (SCI), the extent to which sensory input from Ia afferents is regulated during voluntary activity after the injury remains largely unknown. To address this question, we examined the soleus H-reflex and conditioning of the H-reflex by stimulating homonymous and heteronymous nerves [depression of the soleus H-reflex evoked by common peroneal nerve stimulation (D1 inhibition) and the monosynaptic Ia facilitation of the soleus H-reflex evoked by femoral nerve stimulation (FN facilitation)] at rest, and during tonic voluntary activity in humans with and without chronic incomplete SCI. We found that during voluntary activity the soleus H-reflex size increased in both groups compared with rest, but to a lesser extent in SCI participants. Compared with rest, the D1 inhibition decreased during voluntary activity in controls but it was still present in SCI participants. Further, the FN facilitation increased in controls but remained unchanged in SCI participants during voluntary activity compared with rest. Changes in the D1 inhibition and FN facilitation were correlated with changes in the H-reflex during voluntary activity, suggesting an association between outcomes. These findings provide the first demonstration that the regulation of Ia

afferent input from homonymous and heteronymous nerves is altered during voluntary in humans with SCI, resulting in lesser facilitatory effect on motor neurons.

Disclosures: B. Chen: None. **M.A. Perez:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Topic: C.11. Spinal Cord Injury and Plasticity

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Department of Defense W81XWH1810250
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Dr. Miriam and Sheldon G Adelson Medical Research Foundation

Title: Monitoring of Autonomic Dysreflexia in Rhesus Macaques after Cervical Spinal Cord Injury and Delayed Intramedullary Grafting of Human Neural Stem Cells

Authors: P. M. BARTMEYER[1], N. P. BISCOLA[2], M. M. RAIMUNDO[4], E. S. ROSENZWEIG[5], J. HYDE[6], C. MANNION[6], S. HAWBECKER[6], R. WURR[6], R. MACON[6], Y. S. NOUT-LOMAS[7], C. J. SPARREY[8], M. S. BEATTIE[9,10], J. C. BRESNAHAN[9,10], ***L. A. HAVTON**[3,11];
[2]Neurol., [3]Neurol. and Neurosci., [1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Univ. of Campinas, UNICAMP, Campinas, Brazil; [5]Neurosciences, UCSD Dept. of Neurosciences, La Jolla, CA; [6]California Natl. Primate Res. Center, UC Davis, Davis, CA; [7]Col. of Vet. Med. and Biomed. Sciences, Colorado State Univ., Fort Collins, CO; [8]Mechatronic Systems Engineering, Simon Fraser Univ., Surrey, BC, Canada; [9]Dept. of Neurosurgery, UCSF, San Francisco, CA; [10]VA Med. Ctr., San Francisco, CA; [11]James J. Peters Veterans Affairs Med. Ctr., Bronx, NY

Abstract: Spinal cord injury (SCI) may result in autonomic impairments, including autonomic dysreflexia (AD) with episodic blood pressure elevations. AD most commonly presents after an injury above the T6 segmental level and may be accompanied by reflex bradycardia. Significant progress has been made with regards to improvement in motor function when a cervical SCI is followed by intramedullary grafting with human neural stem cells in rhesus macaques (Rosenzweig et al., *Nat Med*, 2018). However, the effects of SCI and human NSC grafts on autonomic function are not known. We performed comprehensive autonomic recordings, including collection of cardiovascular vital signs in response to bladder filling, rectal probe activation of external anal sphincter guarding reflex, and colorectal distension in rhesus macaques. Animals underwent a cervical spinal cord hemisection or hemicontusion followed by delayed intramedullary delivery of human neural stem cells in a subset of primates. We show a significant increase SBP in the SCI group of animals in response to bladder filling and sustained

colorectal distension. A significant number of 8/16 animals in the injured group, with or without a human NSC graft, compared to 1/12 animals in the control group, met criteria for clinical AD with an increase in SBP of over 20 mmHg in response to bladder filling. Perfusion index (PI) is a non-invasive measure of peripheral blood flow and was introduced as a new biomarker for cardiovascular function. PI was measured at the first digit of the hand and foot. PI(hand) was increased and PI(foot) was decreased in the SCI group, suggestive of increased and decreased peripheral blood flow, respectively. In both the SCI and control groups, the PI measurements were highly correlated with changes in SBP. In attempts to evaluate the potential utility of PI as a non-invasive biomarker for SBP, a machine learning strategy showed PI measurements as the preferred outcome measure over other vital signs, including heart rate, in both the SCI and control groups for SBP predictions. We conclude that AD may present in rhesus macaques after SCI. In addition, PI recordings from the upper and lower body show utility for longitudinal monitoring of autonomic functions and promise as a surrogate biomarker for SBP.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Program #/Poster #: 289.16

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation CHNF#596764
NIH Grant R01NS118200

Title: Spinal cord injury directly impairs resolution of inflammation and pressure ulcer healing

Authors: *C. P. VADALA[1], F. O. NOVAIS[2], A. R. FILOUS[1], J. M. SCHWAB[1];
[2]Microbial Infection & Immunity, [1]The Ohio State Univ., Columbus, OH

Abstract: Background: SCI disrupts the communication between the central nervous & immune systems and results in systemic immune dysregulation. Resolution of inflammation is an active process that stimulates the removal of inflammatory cells (efferocytosis) from the injury site and is required for effective wound healing, a process impaired by SCI. A prevalent complication following SCI are PU of the skin. PU are highly preventable but often do not heal on their own and are origins for further infection or septic conversions.**Hypothesis**: SCI directly blunts PU healing via dysregulated immune responses, resulting in impaired resolution of inflammation and skewed wound healing.**Methods**: We assessed PU healing and immune responses using severe SCI (T3 crush) model and sham controls. The mouse PU model utilizes three 24-hour ischemia/reperfusion cycles (12hx12h) using magnets (1012G) applied to the

dorsal skin beginning three days after SCI. This creates two distinct skin lesions caudal to the SCI (lower back). Wound healing and inflammatory responses are assessed using histology and FACS analysis of the PU skin up to 33 days post-SCI.**Results:** FACS analysis demonstrated that inflammatory responses at the PU site are skewed in animals with SCI. Histologically, we determined differences in wound composition between SCI and sham groups.**Conclusions:** Our data demonstrates that SCI directly interferes with wound healing using a controlled PU model. Impaired resolution is a candidate mechanism contributing to the maladaptive systemic immune response. The application of resolution agonists may rescue a SCI-induced resolution deficit and propagate wound healing.

Disclosures: C.P. Vadala: None. **A.R. Filous:** None. **J.M. Schwab:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.17

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation

Title: Depression-like behavior, cognitive performance, and hippocampal neurogenesis following thoracic spinal contusion in male and female rats

Authors: *A. STEFANOV[1,2], R. M. JOSEPH[1], K. ARAGUZ[1], C. GUICE[1], A. HEMPHILL[1], K. BRAKEL[1,2], J. RAU[1,2], K. S. MONTGOMERY[1], M. A. HOOK[1];
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Abstract: In addition to the loss of physical and physiological function after a spinal cord injury (SCI), 26% of people living with spinal injury suffer from major depressive disorder (MDD) and 60% experience impairments in cognition. These comorbidities are an additional and staggering burden during recovery, necessitating a better understanding of their underlying mechanisms. To address this, we examined depression-like behavior and cognitive deficits in a rat model of a lower-thoracic contusion SCI. Young (3 months old at injury) and middle-aged (9 months old at injury) male and female Sprague-Dawley rats served as subjects. Using our behavior ethogram, we tested components of depression-like behavior such as anhedonia, decreased sociability, and learned helplessness, in acute and chronic phases of recovery compared to pre-injury baseline. We found that depression-like behavior manifests in a sex- and age-dependent manner in 30-45% of subjects, commensurate with the clinical incidence, within a month post-SCI, and that it persists into the chronic phase of recovery. To examine cognition, we tested the same rats on the Barnes Maze test of spatial learning at 170 days post injury. We found that the subset of SCI males that display depression-like behavior also demonstrated poor spatial learning strategies and memory retention. Intriguingly, however, cognitive deficits were not observed in female rats,

either as a consequence of SCI or comorbid with depression. Assessment of recovery of locomotor function and the histological analysis of surviving gray and white matter around the spinal cord lesion site confirmed that depression and cognitive impairments were not due to differences in motor function or injury severity. The systemic expression of pro- or anti-inflammatory cytokines also did not differ across affect or injury groups. We are now focused on how these outcomes are related to hippocampal neurogenesis. Overall, the findings in our rodent SCI model suggest an association between acute depression and chronic cognitive decline that may be linked to disrupted hippocampal function after spinal cord injury.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Conacyt CF1311312
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Title: Effect of unilateral lumbar 6 avulsion on nerve conduction of five peripheral nerves of the lower extremity in the domestic rabbit (Oryctolagus cuniculus).

Authors: Z. FLORES-LOZADA[1], A. FLORES-HERNANDEZ[1], R. ZEMPOALTECA[3], I. JIMENEZ-ESTRADA[4], F. CASTELÁN[5], M. MARTINEZ-GOMEZ[6], ***D. L. CORONA-QUINTANILLA**[2];
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Abstract: Skeletal muscle is involved in vital behaviors such as locomotion and posture. Locomotion is a fundamental behavior for bipedal and quadrupedal individuals. In the spinal cord there are sensory-motor regulation nerve circuits that actively participate in maintaining posture or performing locomotion. Specifically, at the lumbar level (L6) is integrated somatic and visceral reflexes to regulate lower urinary tract, posture and locomotion functions. But, with the spinal cord injury produced a muscular deterioration and the unrest in the sensation of balance. The separation of the spinal cord from the anterior roots is nominated ventral root avulsion (VRA), this type of injury commonly occurs at the L6, and this produces a low survival of motor neurons. But, the effect of VRA on nerve conduction of nerves affected by injury is lacking. The objective is to determine what happens with the nerve conduction of peripheral

nerves of the lower extremity after L6 VRA. Young female rabbits (aged 8 ± 2 months, n=14) were used for this study. The rabbits were anesthetized and divided into three groups: 1) Control, to characterize the compound action potentials at the L6 dorsal root level during electrostimulation of the quadratus femoris muscle (nCuaF), sciatic nerve (nC), lateral femoral cutaneous nerve (nCutF) and branches 1 and 2. (n=4). Other 10 females were randomly divided into two groups, 2) sham (n=5) and 3) L6 VRA (n=5). In the sham and L6 VRA groups. After of 30 days in both groups were recordings of action potentials compound on the dorsal root during electrical stimulation of the five peripheral nerves of the lower extremity were performed. The results show that there are differences with respect to the parameters of the action potentials compound from the five lower extremity nerves. Specifically, the nC shows significant differences with respect to the other nerves in latency, amplitude and number of components. These differences are possibly due to the type of axons that form the nC (sensory and motor), whereas the axons of the nCuaF, R1 and R2 nerves are motor and the nCutF axons are only sensory. AVR L6 lesions significantly and differentially affect compound action potentials at the dorsal root level of all five nerves. After injury the composite action potentials that could be recorded at the dorsal root are those of the nCuaF, nC and nCutF nerves. Thus, L6 VRA differentially affects nerve conduction in the nerves of the lower extremity. Nerves considered motor (nCuaF, R1 and R2) are more susceptible to degeneration by injury VRA L6 than nerves with sensory components (nC and nCutF).

Disclosures: Z. Flores-Lozada: None. **A. Flores-Hernandez:** None. **R. Zempoalteca:** None. **I. Jimenez-Estrada:** None. **F. Castelán:** None. **M. Martinez-Gomez:** None. **D.L. Corona-Quintanilla:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.19

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CONACYT Grant 1089021

Title: Standardization of a model of depressive behaviors associated with chronic neuropathic pain induced by traumatic spinal cord injury in rats

Authors: *A. RANGEL-HERNÁNDEZ[1,2], C. RÍOS-CASTAÑEDA[1,2], A. MATA-BERMÚDEZ[1], H. A. ROMERO-SÁNCHEZ[1,2], G. JARDON-GUADARRAMA[1], M. MARTÍNEZ-CÁRDENAS[1], A. DÍAZ-RUÍZ[2];
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Abstract: Traumatic spinal cord injury (LTME) is characterized by direct mechanical damage to nerve tissue that is usually associated with devastating consequences such as damage to motor,

sensitive and autonomic functions, as well as an impaired psychological state and a decrease in the patient's quality of life. People with LTME may experience psychological consequences such as an increased risk of depression and anxiety, as well as physical consequences such as neuropathic pain as the main consequence, up to loss of limb mobility or urinary incontinence. Neuropathic pain generated by LTME is chronic pain that is a direct consequence of LTME as it is caused by direct cell damage and ischemia and is defined as an unpleasant sensory and emotional experience associated with, or like that associated with, actual or potential tissue damage, according to the International Association for the Study of Pain (IASP). Unlike other chronic pain conditions, LTME pain is a complex phenomenon involving biological, behavioral, emotional, and environmental factors that often play a role. Depression is a phenomenon that is associated with LTME due to the alteration of multiple neurotransmitters that are involved in the generation of chronic neuropathic pain. This project focuses on studying the development of depressive behaviors associated with neuropathic pain caused by LTME. Female Wistar rats weighing approximately 200g to 250g (n=21) were evaluated according to Policies on the Use of Animals in Neuroscience Research and the Guide for the Care and Use of Laboratory Animals, for 35 days. After surgical preparation and exposure of the dorsal vertebral column, the animals were dropped a metal cylinder of 10g of weight at a height of 6.25 mm (T10) to produce tactile allodynia and mechanical hyperalgesia, which is a characteristic behavior of neuropathic pain. Tactile allodynia and mechanical hyperalgesia were evaluated by measuring paw withdrawal in response to probing with a series of calibrated von Frey filaments. Withdrawal threshold was tested by increasing and decreasing stimulus strength eliciting paw withdrawal. Depressive behaviors were evaluated with the sucrose preference and forced swimming test. Sucrose preference was assessed by measuring the amount of sucrose water ingested per rat over a period of 2 hours, while forced swimming was assessed by measuring the rat's immobility index over a period of 10 minutes. Traumatic spinal cord injury produced tactile allodynia and mechanical hyperalgesia in female rats observed from day 11 to day 35. Tests of sucrose preference and forced swimming showed an onset in depressive behaviors around day 21 and remaining until day 35.

Disclosures: **A. Rangel-Hernández:** None. **C. Ríos-Castañeda:** None. **A. Mata-Bermúdez:** None. **H.A. Romero-Sánchez:** None. **G. Jardon-Guadarrama:** None. **M. Martínez-Cárdenas:** None. **A. Díaz-Ruíz:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.20

Topic: C.11. Spinal Cord Injury and Plasticity

Support: the Basic Science Research Program (2019R1I1A2A01060115) through the National research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning
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Korea Health Industry Development Institute (KHIDI) funded by the Ministry of Health & Welfare, Republic of Korea.

Title: Analysis of functional and behavioral changes after force-depended spinal cord injury in a rat.

Authors: *J. LEE[1,2,3], J. KIM[1,2,3,4];
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Abstract: A traumatic spinal cord injury (SCI) is a devastating event with negative physiological, psychological, and social consequences for individuals, and an economic burden on society. The injury severity of SCI is depending on many factors such as injury force, displacement of spinal cord caused by mechanical crash, compressing duration, and impulse. In this study, the functional and behavioral changes depending on the injury severity were investigated. In this study, a contusive and graded SCI was made at T11 segment in adult male SD-rats using Infinite Horizons (IH) impactor to investigate the correlation between injury severity factors (force, displacement, impulse, and duration) and motor and sensory functions. Experimental rats were divided into three groups into 100, 150, and 200 kdyn impact force, respectively. After contusive SCI, the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale and combined behavioral score (CBS) for motor functions, mechanical and thermal paw withdrawal threshold (PWT) for sensory functions, and Modified Ashworth Score (MAS) for the spasticity were performed (before and 1, 3, 5, 7, 10, 14, 21, 28, 35, 42, 49, 56, 63, and 70 d after SCI). In the present results, motor recovery was delayed as applied force increased, but in sensory tests and MAS, only 100 kdyn group showed significant difference with other two groups. In BBB and CBS, 100 and 150 kdyn groups showed plateau around 3 weeks after SCI. Motor functions in 200 kdyn group continuously recovered until the end of experiment. The change of mechanical PWT in 200 kdyn was delayed compared to other two groups. BBB, CBS, and MAS showed high relation with actual force, displacement, impulse and duration (r^2>0.6) in both periods. However, mechanical PWT showed low relation with actual force, displacement, impulse and duration (r^2<0.4). In mechanical and thermal PWT, there were no relations with injury severity factors in early period. But, in late period, the variation was high only in low actual force, displacement, duration, and impulse in mechanical PWT. The present results demonstrated that changes of motor and sensory functions after force-depended SCI and the high correlation of motor function and injury severity factors, but there was no relation of sensory function and injury severity factors.

Disclosures: **J. Lee:** None. **J. Kim:** None.

Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.21

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS122888

Title: Role of kidney dysfunction after spinal cord injury

Authors: *J. H. GUMBEL[1], J. R. HUIE[1], J. SACREMENTO[1], A. R. FERGUSON[2]; [1]Univ. of California, San Francisco, San Francisco, CA; [2]Neurolog. Surgery, UCSF, San Francisco, CA

Abstract: Spinal cord injury (SCI) results in significant deficits with urinary function, which includes detrusor overactivity, urinary incontinence, detrusor-sphincter dyssynergia, and polyuria. Further, disorders with the cardiovascular system also arise after SCI, including coronary artery disease and dysregulation of blood pressure due to autonomic dysregulation. Together, these are among the leading issues that affect quality of life in the SCI population. However, little is known about how SCI effects the kidneys, which regulate blood pressure through hemodynamics and water/solute balance. It has been previously established that SCI impacts kidney function through the alteration of key water/solute balance biomarkers, such as vasopressin (AVP), vasopressin receptor 2 (V2R), atrial natriuretic peptide (ANP), and natriuretic peptide receptor A (NPRA) to name a few. Given that these receptors are altered after SCI, combined with the inability to regulate blood pressure, it is likely that there are more biomarkers altered after SCI, thus causing a cyclical autonomic dysfunction. This current study aimed to investigate the effect of SCI on kidney dysfunction, targeting acute kidney injury biomarkers kidney injury molecule 1 (KIM1) and neutrophil gelatinase-associated lipocalin (NGAL). This project has strong translational potential since the kidneys are significantly impacted after human SCI, as shown by the increased incidence in chronic kidney disease in the SCI population and play a key role in cardiovascular and hemodynamic regulation and homeostasis.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.22

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Comparing immunosuppressed spinal cord injury rat models for human neural progenitor cell xenotransplant studies

Authors: *Z. LOU[1], A. POST[1], N. NAGOSHI[2], N. HEJRATI[1], J. CHIO[3], M. KHAZAEI[4], M. G. FEHLINGS[5];
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Abstract: Research rationale: Current preclinical research on neural progenitor cell (NPC) transplantation to treat spinal cord injury (SCI) relies on clinically relevant animal models to test efficacy and safety of prospective treatments. Given their potential role as an autologous cell source, human-derived induced pluripotent stem cell (iPSC) NPCs are of particular relevance when designing studies with clinical applicability. However, immune system mediated rejection of grafts is one of the challenges when studying human cells in animal models. **Objective:** In this study, we aimed to determine the optimal immunosuppression method in terms of viability of the grafted cells and health of the animals when studying effects of grafted human NPCs in the injured spinal cord of rats. **Methods:** We compared the viability of grafted human cells in rats that were either treated with an immunosuppressive agent (Tacrolimus, Cyclosporin A, or Mycophenolate Mofetil), or of an immunodeficient strain (athymic nude rats, and X-SCID rats), in the context of a clinically relevant cervical SCI model. **Results:** Immune cells were shown to have infiltrated the injured spinal cord and persist for up to 2 weeks post- injury. We found that FK506, which had been reported to have a higher potency than CsA in binding calcineurin and inhibiting T cell proliferation, resulted in the highest level of cell survival among the immunosuppressants tested. NPCs transplanted into athymic nude rats displayed similar amounts of cell survival as the most effective immunosuppressant options, with better overall health for the animals. **Conclusions:** The athymic nude rat breed is the best balance of risk and benefits for *in vivo* studies involving NPC transplantation in SCI, as they require less skilled labour to maintain the immunosuppression and result in a better quality of life for the animals. Moreover, without confounding influences from immunosuppressant co-administration, it is easier to elucidate the effects of treatments.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

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Program #/Poster #: 289.23

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH R01NS099076
NIH R01DA03190
NIH R01NS085426
NIH F31DK123840

Title: Intraspinal dopamine synthesis, release, and regulation of micturition reflexes after spinal cord injury

Authors: *E. OATMAN, J. H. DEFINIS, Z. D. BRODNIK, R. A. ESPAÑA, S. HOU; Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Spinal cord injury (SCI) often leads to urinary dysfunction. While a spontaneous micturition reflex can be established over time, the emergence of bladder hyperreflexia and detrusor-sphincter-dyssynergia cause inefficient voiding and urinary retention, leading to repeated urinary tract infections and kidney damage. The mechanisms underlying these urinary disorders and this recovered reflex are not well understood. We recently discovered a subpopulation of tyrosine hydroxylase (TH) cells in the lower spinal cord that may contribute to dopamine (DA) production and facilitate the development of this spontaneous micturition reflex following SCI. To identify if these TH^+ cells originate from neurogenesis, Bromodeoxyuridine (BrdU) was injected i.p. three weeks after a complete transection of the 10th thoracic (T10) spinal cord in female rats. Ensuing immunostaining showed no BrdU-labeled TH^+ cells in the cord. Notably, a portion of TH^+ cells express the inhibitory transcription marker PAX2. To further elucidate their characteristics, western blots and qPCR were performed to examine the expression of DA related proteins and genes. DA release was tested in injured spinal cord slices using fast scan cyclic voltammetry (FSCV). Consequently, there was decreased but detectable gene and protein expression of TH, with no changes in expression of either dopamine decarboxylase (DDC) or dopamine transporter (DAT) in the lumbosacral spinal cord. Focal electrical stimulation of the spinal region where TH^+ cells reside elicits DA release. Metabolic cage assays for spontaneous micturition function are ongoing with central drug administrations of the DA precursor L-DOPA or the non-selective DA receptor agonist apomorphine despite no robust effects with peripheral drug delivery. In conclusion, spinal TH^+ cells undergo injury-induced plasticity and are capable of synthesizing and releasing DA following SCI. Newly emerged TH^+ cells may develop from a phenotypic switch rather than neurogenesis. Ultimately, increasing DA signaling in the lower cord enhances recovered micturition function after SCI.

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Poster

289. Spinal Cord Injury: Neuromodulation, Circuit, and Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 289.24

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Daniel & Ada Rice Foundation

Title: Three-dimensional painted micro-scaffold materials enhance immunomodulatory ability of transplanted cells

Authors: *J. MOSS[1], Q. SHEN[2], Y. LOPEZ-RAMIREZ[2], D. TUCKER[2], M. SANDHYAPAGU[2], K. RAUE[1], A. JAKUS[3], R. SHAH[3], R. G. FESSLER[2], B. T. DAVID[2];
[1]Rush Univ. Med. Col., Chicago, IL; [2]Rush Univ. Med. Ctr., Chicago, IL; [3]Dimension Inx, Chicago, IL

Abstract: Spinal cord injuries (SCIs) are known to produce a myriad of effects over the acute, sub-acute, and chronic period following injury, including paralysis, altered sensation below the level of the injury, as well as aberrant autonomic responses. To date, the only FDA-approved treatment for acute SCI has been methylprednisolone, but that has fallen out of favor both domestically and internationally over the past decade. We have begun developing a cell-free, self-contained, acute-care, minimally invasive biomaterial treatment for both contusive and open SCIs that diminishes the onset and severity of secondary injury while simultaneously supporting and improving subsequent treatments (e.g., cell transplantation). Specifically, we investigated micro-scaffolds comprised of 3D-Painted Fluffy-PLG, an electrically conductive 3D-Graphene, and a blend of the two (Fluffy-Graphene). We have established the preliminary safety and lack of immunogenicity of micro-meshes made of 3D-printed Fluffy-PLG, 3D-Graphene, and Fluffy-Graphene when implanted in the spinal cords of both sham and injured rats, when sacrificed either sub-acutely or chronically. Further, we have shown that by acutely implanting the Fluffy-PLG into the injury site (via acute myelotomy), and then transplanting bone marrow mesenchymal stem cells (MSCs) directly into the biomaterial implant one day later, the immunomodulatory ability of MSCs is improved in comparison to those injured rats that received MSC transplants directly into the parenchyma at the same time point.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.01

Topic: D.01. Somatosensation

Title: Characterizing the diversity of cell types in the Dorsal Root Ganglia (DRG) by Integrated Constellation Pharmacology approach

Authors: *M. GIGLIO, J. CARTER, J. TUN, V. CHUA, K. CHASE, L. S. LEAVITT, M. J. GIACOBASSI, M. WATKINS, R. K. ALLURI, K. TSOURMAS, R. TEICHERT, S. RAGHURAMAN, B. M. OLIVERA;
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Abstract: The dorsal root ganglia (DRGs) consist of different neuronal and non-neuronal cell types that are involved in transmitting sensory information from the periphery to the central

nervous system. There is a vast diversity of neuronal cell types that detect and respond to a variety of internal and external stimuli. In contrast, the diversity of non-neuronal cells in the DRG remains to be defined. In this work, we used Constellation Pharmacology (CP) approach to identify and characterize the functional heterogeneity of neuronal and non-neuronal cell types in the DRG. CP is a cell-based high-content phenotypic-screening platform that utilizes pharmacological agents to uncover the combinations of ion channels and receptors that are expressed in the plasma membrane and can define specific cell types. By combining this platform with downstream assays such as immunofluorescence, whole-cell electrophysiology and single-cell transcriptomics, we uncovered ~19 different neuronal cell types and 5 non-neuronal cell types. As a proof-of-concept, four neuronal cell types are discussed in greater detail: proprioceptors, Aδ RA-LTMRs, and two subtypes of cold sensors. CP guided transcriptomic analyses of representative cells revealed unique expression of ion channels and receptors that were functionally verified by whole-cell electrophysiology. This approach provides a unique opportunity to characterize the key signaling proteins important for cellular physiology as well as identify molecules essential for the cross-talk between different neuronal and non-neuronal cell types. The communication between different cell types can influence several physiological and pathophysiological processes and the study lays the foundation for investigating molecular targets that are relevant to the progression of neurological diseases such as chronic pain.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.02

Topic: D.01. Somatosensation

Support: MRC grant MR/V012738/1

Title: Effects of non-uniform distributions of voltage-gated Na^+ channels on spike propagation through the dorsal root ganglion in non-myelinated axons: a computational modeling study.

Authors: *D. B. JAFFE[1], N. GAMPER[2];
[1]Univ. of Texas at San Antonio, Univ. of Texas at San Antonio, San Antonio, TX; [2]Nikita Gamper, Univ. Leeds, Leeds, United Kingdom

Abstract: Within dorsal root ganglia action potential failure at the axonal bifurcation of sensory neurons, generally referred to as the T-junction (TJ), is well-described. However, the relative geometry of parent and child axons of non-myelinated fibers alone cannot account for the low-safety factor of spike throughput. Indeed, using computer modeling we find that a repertoire of

Na_V channels (TTXs, Na1.8, and $Na_V1.9$) based on a model of spike conduction in the spinal nerve (Tigerholm et al., *J. Neurophys*. 111:1721-1735, 2014) and somatic electrogenesis generally do not fail at the TJ. Our simulations predict that only when $Na_V1.8$ densities are lowered below 10% of the spinal nerve or soma does the following frequency approach experimental observations. Likewise, to observe $GABA_A$ receptor-dependent inhibition of spike throughput, as observed experimentally, a similar distribution of $Na_V1.8$ conductance is necessary. It is likely that reduced axonal excitability proximal to the TJ through a combination of reduced Na channel density and expression of K_V7 channels (with a significant fractional activation near the resting potential) contribute to the low safety factor for spike propagation and possible gating of signaling through the DRG.

Disclosures: D.B. Jaffe: None. **N. Gamper:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.03

Topic: D.01. Somatosensation

Title: High throughput screening of TRPV1 identifies novel antagonists from Theraphosidae venoms

Authors: *S. TRIM[1], D. MCCULLOUGH[1], A. COOK[2], C. KILPACK[2], J. WESLEY[2], B. KOCI[2];
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Abstract: TRPV1 is a well-studied target for pain therapeutics which is activated by noxious heat, protons and most widely known, the vanilloid capsaicin. Agonism of this ligand gated ion channel is firmly linked to the generation of nociceptive signals and pain. However, blockade of these noxious signals with small molecules antagonists have been challenging as they lead to febrile responses and hyperthermia, so TRPV1 therapeutics have ended up being desensitizing agonists that need to be given with analgesics to counter the initial signals before receptor internalization leads to desensitization. The likely cause of the failure of antagonists is due to the link between noxious thermal pain and thermal homeostasis signals from this channel. Therefore there is a need for new classes of TRPV1 antagonist that may be able to dampen the nociceptive response avoiding the homeostatic signaling. Many venomous animals have evolved venoms that contain TRPV1 agonists as defensive venoms, however little is known about the TRPV1 antagonists in venoms and how they work. This study used a venom peptide library designed to maximise the chances of discovering TRPV1 antagonists using a high throughput screening platform. We successfully collected and standardised venoms from 15 Theraphosidae species, these were then fractionated by in-line 2D HPLC to produce a Targeted Venom Discovery Array (T-VDA). This T-VDA, contained 915 venom fractions that were screened at 2ug/ml against HEK 293 cells expressing TRPV1 in duplicate using the Sophion Qube high-throughput

automated patch clamp in 384 well. From this screen 18 fractions were identified as hits with inhibition of Capsaicin induced TRPV1 signal of >25%. These 18 hits were all from new world Theraphosidae species of three genera - *Lasiodora, Chromatopelma and Phormictopus*. All species are not known for painful bites and no previously known TRPV1 ligands have been identified from these Genera. Hits were confirmed in concentration response and identified by mass spectrometry. Through this work we have identified novel peptide antagonists of TRPV1 from Theraphosidae venoms and added further evidence of the successful utility of high-throughput automated patch clamp in drug discovery.

Disclosures: **S. Trim:** A. Employment/Salary (full or part-time):; Venomtech Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Venomtech Ltd. **D. McCullough:** A. Employment/Salary (full or part-time):; Venomtech Ltd. **A. Cook:** A. Employment/Salary (full or part-time):; Eurofins Panlabs Inc. **C. Kilpack:** A. Employment/Salary (full or part-time):; Eurofins Panlabs Inc. **J. Wesley:** A. Employment/Salary (full or part-time):; Eurofins Panlabs Inc. **B. Koci:** A. Employment/Salary (full or part-time):; Eurofins Panlabs Inc.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.04

Topic: D.01. Somatosensation

Support: Thompson Family Foundation Initiative in CIPN & Sensory Neuroscience
NIGMS T32 Training grant
Howard Hughes Medical Institute

Title: Genetic Identification of a novel molecular player Scn1b underlying individual susceptibility to Chemotherapy Induced Peripheral Neuropathy.

Authors: *K. NGUYEN, S. K. CHEUNG, C. VUONG, D. BAUTISTA, E. A. LUMPKIN; Mol. and Cell Biol., UC Berkeley, Berkeley, CA

Abstract: Chemotherapy-induced peripheral neuropathy (CIPN) is a common adverse side effect of chemotherapy drugs, such as paclitaxel (PTX). Some of the most debilitating symptoms of CIPN include spontaneous pain and mechanical allodynia, a condition where light touch becomes painful. CIPN susceptibility varies widely among patients, but it is unclear what biological factors determine the risk of CIPN and how these factors give rise to the range of sensory symptoms. In this study, we performed an unbiased screen to identify genetic factors that predispose individuals to CIPN by examining the correlation between PTX-evoked changes in mechanical sensitivity, and sensory-neuron transcript expression across genetically distinct mouse strains. From this screen, we identified *Scn1b* as one of the top positively-correlated genes with PTX-evoked changes in mechanical sensitivity (r=0.57). SCN1B is an auxiliary subunit of

voltage-gated Na+ channels known to regulate the excitability of neurons and cardiac myocytes. Altered SCN1B function is known to cause severe diseases in humans. Our goal is to determine the role of *Scn1b* in sensory neurons and whether *Scn1b* contributes to changes in sensory neuron excitability and/or mechanical sensitivity under both naïve and CIPN-evoked conditions. To determine whether *Scn1b* is expressed in sensory neurons, we analyzed a single-cell RNA-seq public dataset for dorsal root ganglion (DRG) neurons in combination with fluorescent in situ hybridization (RNAscope) and found that *Scn1b* transcripts are highly expressed in the DRG and enriched in touch receptors. Our next goal is to examine whether loss of *Scn1b* alters the excitability of sensory neurons using Fura-2 calcium microfluorimetry. Interestingly, a comparison of un-normalized baseline fura-2 ratios showed a slight elevation in baseline calcium for $Scn1b^{-/-}$ sensory neurons compared with $Scn1b^{+/+}$ sensory neurons. Given these data, our current working model is that changes *Scn1b gene* expression leads to differences in sensory neuronal excitability which could contribute to mechanical allodynia.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: D.01. Somatosensation

Support: NIH Grant 1R01NS115209

Title: Distinct functional roles of TRP channels in cold nociception of Drosophila larva

Authors: *A. SAKURAI, N. MAKSYMCHUK, J. M. LETCHER, D. N. COX, G. S. CYMBALYUK;
Georgia State Univ., Georgia State Univ., Atlanta, GA

Abstract: Transient receptor potential (TRP) channels are a large and functionally diverse family of cation-permeable channels that provide cells with different modalities of sensitivity by opening upon various physical and chemical stimuli. In *Drosophila*, a specific combination of TRP channels enables the class III (CIII) primary sensory neurons to sense cold. Knockdown of one of the TRP channels (TrpA1, Pkd2, Trpm) severely impairs the animal's cold-evoked behavioral responses (Turner et al., 2016). In this study, we examined the roles of the three TRP channels in the spiking responses of CIII neurons using animals in which one of the channels was knocked down by the CIII-specific expression of RNAi. Furthermore, we investigated their roles by constructing a series of computational models of CIII neurons representing each knockdown case.
CIII neurons exhibited a characteristic spiking response to a fast temperature drop from 24°C to 10°C at the rate of 2-6°C/sec, with approximately 40% of them showing a peak spike frequency

mostly due to bursts of spikes during the temperature drop, after which the spike frequency decreased and settled to a relatively steady value (Maksymchuk et al. 2022). In TrpA1- and Trpm-knockdown animals, there was approximately a 15% reduction to control animals in the number of neurons exhibiting an initial peak in response to a fast temperature drop. In particular, the initial bursting activity was almost absent in TrpA1-knockdown neurons. In contrast, Pkd2-knockdown showed that about 10% more neurons exhibited the initial peak response while showing significantly reduced tonic spiking activity during the steady-phase of the temperature protocol. When stimulated with a slow temperature decrease (0.12°C/s), CIII neurons showed significantly lower spike frequencies in Pkd2-knockdown animals than in control. Interestingly, Trpm-knockdown neurons showed more frequent burst discharges during the slow temperature decrease than controls.
Based on the electrophysiological observations, we implemented a phenomenological representation of three TRP currents into a model CIII neuron with temperature-dependent activation and Ca^{2+}-dependent inactivation. The model reproduced CIII responses to a variety of temperature change protocols revealing coding of temperature rate of change and magnitude of temperature.
Together, our results suggest distinct roles of TRP currents: TrpA1 codes the rate of temperature change, and Pkd2 codes the magnitude of temperature change. Our results may also indicate that Trpm-knockdown may result in more calcium influx than controls due to more frequent burst discharges during a slow temperature decrease.

Disclosures: **A. Sakurai:** None. **N. Maksymchuk:** None. **J.M. Letcher:** None. **D.N. Cox:** None. **G.S. Cymbalyuk:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.06

Topic: D.01. Somatosensation

Support: DOD Grant W81XWH1710413

Title: Functional assessment of nicotinic receptor subtypes in the dorsal root ganglion

Authors: *J. TUN[1], L. LEAVITT[2], K. CHASE[1], S. RAGHURAMAN[1], B. OLIVERA[1];
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Abstract: Dorsal root ganglion (DRG) neurons express a diverse repertoire of ion channels and receptors; in general, the role of an individual receptor in shaping the function of a specific sensory neuron is not well understood. A major complexity in addressing the function of individual receptors is the existence of complex heteromeric isoforms, each with its own distinctive properties. For example, nicotinic acetylcholine receptors (nAChR) exist in many hetero-pentameric subtypes, with different functional roles in sensory signaling. In this

presentation, data on the cell-type specific functional expression of different nAChRs receptor subtypes in DRG neurons will be presented. We are presently characterizing how the expression of each of these receptor subtypes changes with time after chronic pain is triggered by nerve injury in the chronic constriction injury (CCI) mouse model. In addition, we have monitored the effects of a specific inhibitor of the alpha-9 alpha-10 nAChR, on the molecular changes that accompany disease progression in the CCI animal model.

Disclosures: J. Tun: None. **L. Leavitt:** None. **K. Chase:** None. **S. Raghuraman:** None. **B. Olivera:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.07

Topic: D.01. Somatosensation

Support: NIH grant AR057194

Title: Role of thermosensitive TRP channels in thermal preference of mice

Authors: M. IODI CARSTENS[1], A. MAHROKE[2], T. SELESCU[3], ***E. CARSTENS**[1]; [1]Univ. of California Davis, [2]NPB, Univ. of California Davis, Davis, CA; [3]Fac. of Biol., Univ. of Bucharest, Bucharest, Romania

Abstract: Transient Receptor Potential (TRP) ion channels are important for sensing environmental temperature. TRPV4 senses warmth (25-34ºC), TRPV1 senses heat (>42ºC), TRPA1 senses cold (<17ºC), and TRPM8 senses cool-cold (21-26ºC). We investigated if knockout (KO) mice lacking these TRP channels exhibited changes in thermal preference. Thermal preference was tested using a dual hot-cold plate with one thermoelectric surface at 30ºC and the adjacent surface at a temperature of 15-45ºC in 5 ºC increments. Blinded observers counted the number of times mice crossed through an opening between plates and the percentage of time spent on the 30ºC plate. In a separate experiment, observers blinded as to genotype also assessed the temperature at the location on a thermal gradiant (1.83 m, 0-50ºC) occupied by the mouse at 5-min intervals over 2 hr. Male and female wildtype mice preferred 30°C and significantly avoided colder (15-20°C) and hotter (40-45°C) temperatures. TRPA1KO, TRPV4KO and male TRPV1KO mice were similar, while female TRPV1KOs did not show significant thermal preferences across the temperature range. Male and female TRPM8KOs did not significantly avoid 15-35ºC temperatures. Most mice exhibited fewer plate crossings at hot and cold temperatures and more crossings at thermoneutral temperatures, except for female TRPV1KOs and male and female TRPM8KOs. Occupancy temperatures along the thermal gradiant exhibited a broad distribution that decreased somewhat over time. Mean occupancy temperatures (recorded at 90-120 min) were significantly higher for females (30-34°C) compared to males (26-27°C) of all genotypes, except for TRPA1KOs which exhibited no sex difference.

The results indicate (1) sex differences with females (except TRPA1KOs) preferring warmer temperatures, (3) reduced thermosensitivity in female TRPV1KOs, and (3) reduced sensitivity to cold and innocuous warmth in male and female TRPM8KOs consistent with previous studies.

Disclosures: M. Iodi Carstens: None. **A. Mahroke:** None. **E. Carstens:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.08

Topic: D.01. Somatosensation

Title: Mechanosensitive Piezo Channel, PEZO-1, regulates food deglutition in C. elegans.

Authors: *Y.-J. PARK[1], J. YEON[3], D.-Y. KIM[2], W. HEO[1], H. HWANG[1], S. JUN[4], K. LEE[5], K. KIM[1];
[1]Brain sciences, [2]Daegu gyeongbuk institute of science & technology, Daegu gyeongbuk institute of science & technology, Daegu, Korea, Republic of; [3]Brandeis Univ., Waltham, MA; [4]Univ. of Pennsylvania, Philadelphia, PA; [5]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: The PIEZO ion channel is an evolutionarily conserved mechanosensitive channel (Coste et al., 2010). Mammalian two PIEZO genes, Piezo1 and Piezo2, of which functions have been shown to be involved in mechanosensation (Coste et al., 2010, Woo et al., 2014, Nonomura et al., 2017). C. elegans genome has a single PIEZO gene, pezo-1, which encodes 14 isoforms (Bai et al., 2020). The molecular function of PEZO-1 in C. elegans has yet to be fully determined. To examine pezo-1 function, we grouped 14 isoforms depending on the mRNA length and observed their expression patterns. The long isoforms are specifically expressed in the pharyngeal-intestinal valve (piv), which is predicted to mediate food swallowing (Avery and Thomas, 1997). To examine PEZO-1 function in piv-mediated food swallowing, we fed animals with bacterial-sized GFP-microsphere and found that pezo-1 mutant animals show excess accumulation of GFP in the intestine lumen. Defects in pezo-1 mutants were restored by expression of long isoform PEZO-1 or mouse PIEZO1. We also observed that when GFP-microspheres are fully accumulated, the pharynx is pulled posteriorly to push GFP-microspheres down into the posterior intestine. We named this as a pharyngeal plunge. We next found that the piv exhibits calcium transient during pharyngeal plunge and the optogenetic activation of piv induces the pharyngeal plunge. Furthermore, Ectopic expression of PEZO-1 in C. elegans chemosensory neuron confers head bending-dependent responses. These results demonstrate that the C. elegans PIEZO channel regulates pharyngeal plunge and provides insights to understand the function of the mammalian PIEZO channel shown to be expressed in the esophagus.

Disclosures: Y. Park: None. **J. Yeon:** None. **D. Kim:** None. **W. Heo:** None. **H. Hwang:** None. **S. Jun:** None. **K. Lee:** None. **K. Kim:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.09

Topic: D.01. Somatosensation

Support: NIH grant R01NS115209 awarded to Daniel Cox and Gennady Cymbalyuk

Title: The role of calcium dynamics in spiking responses of the cold-sensing neurons in Drosophila larva

Authors: *N. MAKSYMCHUK, A. SAKURAI, S. KOROGOD, N. J. HIMMEL, A. A. PATEL, J. M. LETCHER, D. N. COX, G. S. CYMBALYUK;
Georgia State Univ., Atlanta, GA

Abstract: The ability to sense low temperature is crucial to the survival of insects and other animals in habitats with fluctuating temperatures. Taking advantage of the wealth of neurogenetic, pharmacological, and electrophysiological tools available in the *Drosophila* model system, we investigated larval Class III (CIII) sensory neurons responsible for cold nociception. In response to temperature decrease, these neurons generate patterns assembled of two distinct elemental events, bursts and spikes. Bursts occur more frequently along with a fast temperature drop to noxious cold, and are then followed by spiking with frequency adaptation. Previously, our CIII neuron model with the phenomenological representation of cold-sensitive TRP currents, having temperature-dependent activation and calcium-dependent inactivation properties, captured these phenomena. Our recent experimental data further implicate complex Ca^{2+} dynamics, including Calcium-induced Calcium Release (CICR), and its interaction with Ca^{+}-activated ionic currents, *e.g.* Ca^{2+}-activated Cl^{-} channels and Ca^{2+}-activated K^{+} channels, and hyperpolarization-activated current. Application of $NiCl_2$, an inhibitor of T-type Ca^{2+} channels, and chelation of intracellular Ca^{2+} by BAPTA-AM both reduced the frequency of bursting induced by cold stimulation, whereas ryanodine application had an opposite effect. Apamin, an SK channel blocker, increased the number of spikes in each burst. The general spiking activity was also sensitive to the modification of the Cl^{-} gradient across the cell membrane; application of saline with reduced chloride content induced spontaneous bursting activity and enhanced cold-evoked responses of CIII neurons. We developed a suite of computational CIII models with the aforementioned currents and processes incorporated. We investigated how the TRPA1 and PKD2 TRP currents interact with the CICR mechanism to induce an amplified Ca^{2+} response appropriate for neural coding of cold nociception. In this model, intracellular Ca^{2+} dynamics include ER as the main Ca^{2+} store, mitochondrial Ca^{2+} store, and cytosolic Ca^{2+} binding proteins and describes the exchange of Ca^{2+} between them and two cytosolic micro-domains. Our model results show that Ca^{2+} dynamics, by affecting Cl^{-} ion homeostasis and interaction of the Ca^{+}-activated chloride and SK potassium currents, play the key roles in distinguishing between bursting and spiking components of the activity patterns generated by CIII neurons in response to cold temperature stimulation.

Disclosures: **N. Maksymchuk:** None. **A. Sakurai:** None. **S. Korogod:** None. **N.J. Himmel:** None. **A.A. Patel:** None. **J.M. Letcher:** None. **D.N. Cox:** None. **G.S. Cymbalyuk:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.10

Topic: D.02. Somatosensation – Pain

Support: 1R01NS121533-01A1
P20GM103643

Title: Modulation of Nociceptor Excitability by Targeting CUGBP Elav-Like Family Member 4 (CELF4) RNA-Binding Protein

Authors: M. MUETH[1], E. GRLICKOVA-DUZEVIK[2], P. NEUFELD[3], T. E. KING[4], P. J. WARD[5], C. STRAUB[6], *B. HARRISON[3];
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Abstract: Post-translational control of mRNA translation has recently emerged as a key neuron-intrinsic mechanism for modulation of nociceptor sensitivity. However, the identity of mRNAs and the RNA-binding proteins (RBPs) that regulate those mRNAs remains largely elusive. Our computational analyses of binding sites on “pro-nociceptive” transcripts expressed in nociceptors uncovered CUGBP Elav-Like Family Member 4 (CELF4) as a candidate RBP. Histological assessments of naïve rodent tissues revealed that CELF4 protein is concentrated in cell bodies (soma) of TrpV1-expressing nociceptors in dorsal root ganglia (DRG). Informatic analyses of ‘omics data from rodent pain models show differential expression of CELF4 and CELF4-interacting mRNAs during chronic pain. Genetically modified mice with Celf4 gene knocked down from adult sensory neurons, displayed robust mechanical and thermal hypersensitivity. Acutely dissociated, capsaicin sensitive DRG neurons were hyper excitable with strikingly reduced rheobase and increased action potential firing rate. Together, these observations strongly suggest that CELF4 is a tonic suppressor of nociceptor excitability, likely through limiting translation of pro-nociceptive mRNAs, and is therefore a highly promising target for therapeutic modulation of nociceptor excitability.

Disclosures: **M. Mueth:** None. **E. Grlickova-Duzevik:** None. **P. Neufeld:** None. **T.E. King:** None. **P.J. Ward:** None. **C. Straub:** None. **B. Harrison:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.11

Topic: D.02. Somatosensation – Pain

Support: R01NS121533
P20GM103643
U54AG062334

Title: Members of the CUGBP Elav-Like Family of RNA-Binding Proteins are Expressed in Distinct Populations of Primary Sensory Neurons

Authors: E. GRLICKOVA-DUZEVIK[1], T. M. REIMONN[2], M. MICHAEL[1], A. MCGRATH-CONWELL[2], *T. TIAN[3], J. OWYOUNG[3], P. NEUFELD[2], D. MOLLIVER[2], P. J. WARD[4], B. J. HARRISON[2];
[1]Dept. of Biomed. Sciences, Col. of Osteo. Med., [2]Univ. of New England, Biddeford, ME; [3]Emory Univ., Atlanta, GA; [4]Emory Univ. Dept. of Cell Biol., Atlanta, GA

Abstract: Primary sensory Dorsal Root Ganglia (DRG) neurons are diverse, with distinct populations that respond to specific stimuli. Previously, we observed that functionally distinct populations of DRG neurons express mRNA transcript variants with different 3' untranslated regions (3'UTR's). 3'UTRs harbor binding sites for interaction with RNA-binding proteins (RBPs) critical for targeting mRNAs to subcellular domains, modulating transcript stability and regulating the rate of translation. In the current study we sought to determine if 3'UTR-binding proteins are restricted to specific DRG neuron populations. Analysis of publicly available single-cell RNA-Sequencing (scRNA-Seq) data generated from adult mice revealed that 17 3'UTR-binding RBPs were enriched in specific populations of DRG neurons. This included 4 members of the CUGBP Elav-Like Family (CELF). CELF2 and CELF4 were enriched in peptidergic, CELF6 in both peptidergic and nonpeptidergic and CELF3 in tyrosine hydroxylase-expressing neurons. CELF4 is a known regulator of neural excitability, likely through modulation of protein synthesis via binding to interaction sites within the 3'UTRs of mRNAs. Immunofluorescence studies showed 60% of CELF4+ neurons are small diameter C fibers and 33% medium diameter myelinated (likely Ad) fibers. Co-expression analyses using transcriptomic data and quantitative immunofluorescence revealed that CELF4 is enriched in nociceptive neurons that express GFRA3, CGRP and the capsaicin receptor TRPV1. Finally, genes with CELF4 binding motifs expressed in CELF4+ neurons are significantly associated with gene ontology (GO) terms such as "RNA-binding" and "translation". We propose that CELF4 may therefore control a novel regulon that coordinates the translation of mRNAs encoding components of the protein translation apparatus in nociceptors.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.12

Topic: D.02. Somatosensation – Pain

Support: NS055251 (DL)

Title: Optogenetic activation of Cacna1h-expressing low threshold mechanoreceptors in skin mimics nocifensive behavior and hypersensitivity in mice

Authors: *R. MEIR[1], D. LIPSCOMBE[2];
[1]Neurosci., [2]Carney Inst. for Brain Sci., Brown Univ., Providence, RI

Abstract: Voltage-gated calcium (Ca_V) channels play a critical role in transmitting information about various sensory modalities including thermal and mechanical stimuli. Our recent work has demonstrated a selective role for peripheral $Ca_V2.2$ channels (*Cacna1b*) in capsaicin-induced heat but not mechanical hypersensitivity (DuBreuil et al., 2021). Here we investigate the role of $Ca_V3.2$ channels (*Cacna1h*), which are expressed in C and Aδ low threshold mechanoreceptors (LTMRs), in regulating mechanosensitivity. $Ca_V3.2$ channels are implicated in regulating mechanosensitivity thresholds in a pro-excitatory manner, but their precise mechanism of action is not fully understood. We generated a *Cacna1h*$^{Cre\ +/+}$ knock-in mouse model to express Cre-dependent reporters, including ChannelRhodopsin (ChR2), in C and Aδ LTMRs. LED light (465 nm) directed at glabrous skin of *Cacna1h*$^{ChR2\text{-}YFP+/-}$ hindpaws triggered robust, intensity-dependent plantar paw withdrawal responses, consistent with tactile-free activation of Aδ LTMRs. We assessed changes in the number of evoked responses following intraplantar administration of Complete Freund's Adjuvant (CFA). CFA triggers long-lasting hypersensitivity to tactile stimulation and shifts the dose response curve so that lower intensities trigger a greater number of responses for 5 days following CFA injection. Our findings show that optogenetic activation of *Cacna1h*-expressing Aδ LTMRs can model CFA-enhanced behavioral responses exhibited as a left and upward shift in the LED-intensity response curve. We have established intradermal AAV injection techniques to target delivery of ChR2, and other reporters, to *Cacna1h* neurons innervating the hindpaw of our *Cacna1h*$^{Cre\ +/+}$ mice for enhanced spatial and temporal resolution of genetic manipulations. We will compare LED-evoked responses of AAV injected mice with knock-in strains to determine if there are behavioral differences to LED stimulation in the different mouse models. To further elucidate the role of Ca_V channels in mechanosensation and the induction of sensory hypersensitivity, we will use pharmacological agents to inhibit different Ca_V channels selectively, during baseline and following CFA to determine their role in LED-evoked behaviors. We will also use 2-P calcium imaging to investigate how Ca_V channels contribute to calcium signals within *Cacna1h*-expressing nerve endings in skin in response to LED in normal and hypersensitive states. This will allow us to further establish an optogenetic model to define the key molecular players in sensory transduction from nerve endings in skin, including the steps involved in the development of hypersensitivity.

Disclosures: **R. Meir:** None. **D. Lipscombe:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.13

Topic: D.01. Somatosensation

Title: Distribution, morphology and functions of DRG neurons in the adult zebrafish

Authors: *W. CHANG, M. HALE;
Dept. of Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL

Abstract: The ability to sense and move are vital biological processes for all animal. This is partly accomplished through the activation of sensorimotor circuit in the spinal cord, which receive and filter peripheral information and determine which signals are transmitted to high order brain areas and to spinal motor pathway. Since the mammalian nervous system is comparatively complex and difficult to analyze, it has been important to develop experimental models in which the cellular bases of sensorimotor behavior can be addressed. The zebrafish (*Danio rerio*) is a small fresh-water species widely used as a vertebrate model for human physiology and pathologies. Despite significant progress in elucidating the structure, development and physiology of motor circuits in larval and adult zebrafish, little is known about the functional organization of the adult zebrafish sensory system. We investigated the distribution, morphology and functions of neurons in the dorsal root ganglion (DRG), principal neurons that receive/mediate somatosensory input. We found that the number of DRG neurons among 2-month-old adult zebrafish varied, smaller zebrafish have fewer DRG neurons than do bigger zebrafish. There was no significant difference in neuronal size distribution among 2-month-old zebrafish of different body lengths. To explore whether sensory innervation is indicative of topographic organization of neurons in the DRG, we simultaneously retrograde labelled the dorsal and anal fins. Dorsal fins are located dorsally while anal fins are located ventrally in the same region along the anterior-posterior body axis. The results showed that there is topographic organization in the DRG. Cell bodies of neurons innervating the dorsal fins are distributed relatively dorsally in the DRG compared to those innervate to the anal fins. Patch recording with current injection showed that there are at least two types of firing pattern, adapting and non-adapting. The two types are significantly different in cell size, rheobase, input resistance and spatial distribution. Extracellular recording of the DRG neurons showed that DRG neurons display fast and slowly adapting activities in response to fin mechano-stimulation. Our results here establish the foundation to further study somatosensation circuitry in adult zebrafish and, further, to explore how it changes in pathological conditions, such as in limb amputation and spinal cord injury.

Disclosures: **W. Chang:** None. **M. Hale:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.14

Topic: D.02. Somatosensation – Pain

Support: SEP-Cinvestav 127 to VG-S
SEP-Cinvestav 269 to JM

Title: Role of bestrophin-1 in spinal nerve transection-induced tactile allodynia in rats

Authors: *J. MURBARTIÁN[1], G. GARCÍA[1], V. A. MARTINEZ-ROJAS[1], N. OVIEDO[2], V. GRANADOS-SOTO[1];
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Abstract: Bestrophin-1 belongs to the family of calcium-activated chloride channels. It has been described that nerve axotomy in mice enhances bestrophin-1 expression. In contrast, spinal nerve ligation does not modify bestrophin-1, although bestrophin-1 contributes to neuropathic pain. The aim of this study was to determine the participation of bestrophin-1 in two models of neuropathic pain in rats: L5/L6 spinal nerve ligation (SNL) and L5 spinal nerve transection (SNT). We determined the protein expression of bestrophin-1, as well as the growth-associated protein-43 (GAP43), a nerve regeneration marker, in injured L5 and uninjured L4 DRG at 1, 3, 7, 14 and 21 days after lesion. In addition, we performed immunohistochemical assays to determine bestrophin-1 localization in DRG. Finally, we evaluated the effect of bestrophin-1 overexpression using an in vivo plasmid transfection method in naïve rats. SNT upregulated bestrophin-1 and GAP43 protein expression in injured L5 and uninjured L4 DRG, while SNL enhanced GAP43, but not bestrophin-1 expression in DRG. Accordingly, intrathecal injection of the selective bestrophin-1 blocker $CaCC_{inh-A01}$ (1-10 µg), but not vehicle (15% DMSO), reversed tactile allodynia induced by SNT or SNL in a dose dependent manner. In addition, $CaCC_{inh-A01}$ prevented SNT-induced upregulation of bestrophin-1 and GAP43, but it did not affect SNL-induced upregulation of GAP43. Bestrophin-1 was mainly expressed in small and medium size neurons. SNT increased the co-localization of bestrophin-1 in peptidergic neurons ($CGRP^+$), but not in neuronal cells ($IB4^+$) in injured L5 and in uninjured L4 DRG. Intrathecal injection of bestrophin-1 recombinant channel induced tactile allodynia in naïve rats. The allodynic effect of bestrophin-1 overexpression was reversed by the intrathecal injection of $CaCC_{inh-A01}$. These data suggest that spinal bestrophin-1 expressed in small and medium size peptidergic neurons is the main calcium-activated chloride channel involved in neuropathic pain induced spinal nerve transection in rats.

Disclosures: J. Murbartián: None. **G. García:** None. **V.A. Martinez-Rojas:** None. **N. Oviedo:** None. **V. Granados-Soto:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.15

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS106888

Title: Neurogenic inflammation induced cold allodynia is sexually dimorphic and dependent on CGRP release

Authors: *C. YANG, T. JUNG, D. MCKEMY;
USC, USC, LOS ANGELES, CA

Abstract: The cellular and molecular mechanisms whereby neurogenic inflammation induces cold allodynia are poorly understood. Here, we determine if endogenous neuroinflammatory mediators produced during tissue insult lead to cold allodynia, as well examine the signaling pathways that mediate this particular pain modality. Specifically, we tested cold sensitivity in wildtype mice after an intraplantar injection of two distinct endogenous proalgesics: 4-hydroxy-2-nonenal (4HNE) and lysophosphatidic acid (LPA), agonists for the irritant receptor TRPA1 and the capsaicin receptor TRPV1, respectively. Each is known to produce neurogenic inflammation and pain, and we find that these molecules also induce robust cold allodynia that persists for several hours post-injection. To determine the molecular mechanisms underlying this phenotype, we first asked if these agents induced cold allodynia via release of the neuropeptide calcitonin gene-related peptide (CGRP), finding that 4HNE-mediated cold allodynia was inhibited by CGRP receptor antagonism in a sexually dimorphic manner. Pursuing this result further, we find that an intraplantar injection of CGRP produces cold allodynia in female and not male mice, consistent with our antagonism results. Next, we asked if signaling through the neurotrophin receptor GFRα3, which is known to mediate injury induced cold allodynia via the menthol receptor TRPM8, was involved in these phenotypes. Consistent with the critical role of GFRα3 in cold pain, cold allodynia induced by 4HNE and CGRP was absent in GFRα3-null mice. These results suggest that neurogenic inflammation-induced cold allodynia is a result of release of CGRP, which in turn signal the release of artemin to locally to activate TRPM8 afferents in GFRα3-dependent manner, revealing new targets for potential treatment of cold allodynia.

Disclosures: C. Yang: None. **T. Jung:** None. **D. McKemy:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.16

Topic: D.02. Somatosensation – Pain

Support: AR068989
NS045594

Title: Conditional mineralocorticoid receptor knockout in mouse sensory neurons has opposing effects on A and C cells during inflammatory low back pain

Authors: *K. A. QUALLS, W. XIE, J.-T. ZHANG, J. A. STRONG, J.-M. ZHANG;
Univ. of Cincinnati, Univ. of Cincinnati, Cincinnati, OH

Abstract: Epidural steroid injections (ESIs) are often administered clinically for the treatment of inflammatory low back pain, based on their ability to bind the anti-inflammatory glucocorticoid receptor (GR). However, many commonly used steroids can also bind the mineralocorticoid receptor (MR) with similar or greater potency. MR is expressed in human and rodent dorsal root ganglia (DRG), and has a pronociceptive role during inflammatory pain in rodents. Thus, ESIs given for low back pain could have lower efficacy due to off-target effects at the MR. In this study we characterized the functional role of the MR in sensory neurons during inflammatory low back pain in mice. To knock out MR only in sensory neurons, we injected AAV9-hSyn-eGFP-Cre into the hind-paw of MR fl/fl mice between postnatal day 7-10. This reduced MR mRNA expression in L4 DRG by ~50%. We used the local inflammation of the DRG (LID) model, in which 3 uL of 2 mg/mL of zymosan is injected over the right L4 DRG, to model inflammatory low back pain. Males and females were used in all experiments and analyzed for possible sex differences before combining data. Nociceptive and spontaneous pain measures including von Frey test, cold plantar assay, and rearing behavior were exacerbated by the LID model. With intracellular sharp electrode recording in isolated whole DRG, we observed that MR KO mice have increased measures of neuronal excitability in C cells compared to control mice, both without and with LID (POD4). Additionally, MR KO A cells showed reduced excitability measures during LID, and no changes to excitability in control mice. MR KO mice showed reduced expression of GR mRNA on POD28 after LID, possibly driven by lower GR-β isoform expression. We conclude that MR has opposing roles between small nociceptive C cells and large myelinated A cells during the LID model. Additionally, MR is playing an important functional role in C cells at baseline. Finally, MR knockdown reduced GR expression.

Disclosures: K.A. Qualls: None. **W. Xie:** None. **J. Zhang:** None. **J.A. Strong:** None. **J. Zhang:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.17

Topic: D.02. Somatosensation – Pain

Support: NRF Korea Grant 2020R1C1C101024513
NRF Korea Grant 2020R1A3A300192913
NRF Korea Grant 2022M3E5E801739511
KIST Grant 2E31502

Title: Activation of TRPV1 by PN (Plumula nelumbinis) and UR (Uncaria Rhyncophylla)

Authors: T. HA, G. HONG, *B. KANG;
Korea Inst. of Sci. and Technol., Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: This study investigated the activation of ion channels by Plumula Nelumbinis (PN) extract and Uncaria rhynchophylla(UR) extract at the cell level. Activation of TRPV1 was analyzed through a calcium imaging assay and through patch clamp experiments. The ion channels were overexpressed in HEK/293T cells using FuGENE ® HD Transfection Reagent. NNG Extract and UR extract activate Transient receptor potential vanilloid member 1(TRPV1) but not Transient receptor potential ankyrin 1(TRPA1) and TRPV2, TRPV3, TRPV4. TRPV1 was activated by several chemicals originating from extracts. Extracts activate TRPV1 of Dorsal Root ganglion(DRG) neurons. The extracts did not inhibit TRPV1. In conclusion, our study showed that several chemicals originating from herb extracts act as agonists of TRPV1. The findings of this study indicate the discovery of a new modulator of TRPV1, and further confirm their potential in pain therapy.

Disclosures: T. Ha: None. **G. Hong:** None. **B. Kang:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.18

Topic: D.02. Somatosensation – Pain

Support: LRAP

Title: Mapping the Functional Expression of Kv7 Subunits in Peripheral Sensory Neurons

Authors: *F. P. JONES[1], S. W. MILNE[2], P. MULLEN[3], K. G. PHILLIPS[4], E. SHER[5], N. GAMPER[6];
[1]Univ. of Leeds, [3]Fac. of Biol. Sci., [2]Univ. of Leeds, Leeds, United Kingdom; [4]Eli Lilly & Co, Indianapolis, IN; [5]Eli Lilly & Co, Windlesham, Surrey, United Kingdom; [6]Fac. of Biol. Sci., Univ. Leeds, Leeds, United Kingdom

Abstract: Pathological pain is an unmet clinical and societal problem; available treatments are often inadequate, expensive or invasive, thus, despite advances, new therapeutic targets are needed. We focus on a family of voltage-gated potassium channels, Kv7 (‘M’) channels, which

may represent such a target. Kv7 channels impose strong control over activity of excitable cells, including peripheral somatosensory neurons. The Kv7 channel family is comprised of five members (Kv7.1-Kv7.5) which are differentially expressed in many neurons, muscle and epithelial cells. Functional M channels are present in peripheral somatosensory neurons but exact subunit expression profile is debated. In order to develop a therapeutic strategy for targeting M current in nociceptors pacifically, it is necessary to close this knowledge gap. Here we combine measures of gene and protein expression with functional patch clamp analysis and behavioural testing to determine functionally dominant K_v7 subunit(s) in the peripheral nociceptors. We demonstrated that all but Kv7.1 are expressed in sensory neurons of rats and that Kv7.2, Kv7.3 and Kv7.5 are abundantly expressed. Kv7.2 immunoreactivity showed a bias to small-diameter neurons and Kv7.5 toward larger neurons with significant overlap between the various subunits in rats. Effects of Kv7.2/Kv7.3 selective agonist, ICA-27243, and *Kcnq2* knockdown suggest Kv7.2 is the dominant subunit controlling M current in TRPV1 expressing nociceptors, while in TRPV1-negative cells the functional contribution of other subunits (most likely Kv7.3 and Kv7.5) was more significant. ICA-27243 also significantly attenuated acute and inflammatory pain with comparable efficacy to retigabine (a less selective activator). *Kcnq2* knockdown significantly reduced the M current and rheobase in TRPV1 positive neurons and significantly increased the excitability of these neurons. Moreover, an *in vivo* knockdown of *Kcnq2* by intrathecal delivery of cholesterol-conjugated siRNA in rats produced a significant reduction in thermal and mechanical pain thresholds and an increased response to algogens. This was not the case with *Kcnq5* knockdown. The effects of *Kcnq3* knockdown is currently being investigated. These findings identify Kv7.2 as the functionally dominant K_v7 subunit in nociceptors.

Disclosures: F.P. Jones: None. **S.W. Milne:** None. **P. Mullen:** None. **K.G. Phillips:** None. **E. Sher:** None. **N. Gamper:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.19

Topic: D.02. Somatosensation – Pain

Support: NIH Grant DE023090

Title: Light and mechanical sensitivity of Nav1.8-ChR2eYFP afferent fibers that innervate the hindpaw glabrous skin of mice

Authors: *A. YAMADA[1], A. YAMADA[1], J. LING[1], J. DEBERRY[1], H. FURUE[2], J. GU[1];
[1]Univ. of Alabama At Birmingham, Univ. of Alabama At Birmingham, Birmingham, AL;
[2]Neurophysiol., Hyogo Med. Univ., Nishinomiya, Japan

Abstract: Nav1.8 channels are expressed on nociceptive fibers to play a role in generating nociceptive impulses. However, the properties of Nav1.8-expressing fibers have not been fully

studied. Here we used Scn10a^{Cre};Ai32 transgenic mice and characterized Nav1.8-ChR2eYFP fibers innervating the hindpaw glabrous skin of mice. Light and mechanical sensitivity of Nav1.8-ChR2eYFP fibers were recorded using skin-nerve preparations and the pressure-clamped single-fiber recordings. Impulses were evoked by stimulation of receptive fields with LED light or a mechanical force indenter. All C-fibers (conduction velocity, CV ≤ 1 m/s) responded to light stimulation pulse with multiple impulse firing. Majority (67%) of Aδ-fibers (1 m/s < CV < 10 m/s) responded to light stimulation, and most of the light-sensitive Aδ-fibers displayed single impulse firing and the remaining showed multiple impulse firing. One third (33%) of Aβ-fibers (CV ≥ 10 m/s) responded to light stimulation, and all of them displayed single impulse firing. Mechanical sensitivity of light-sensitive fibers was tested. Almost all light-sensitive C-fibers displayed slowly adapting (SA) impulses, and most of them had high mechanical thresholds but a small portion of them had low mechanical thresholds. For light-sensitive Aδ-fibers, almost all of them showed SA impulses in response to mechanical stimulation. In contrast, in light-insensitive Aδ-fibers, the majority of them displayed rapidly adapting (RA) impulses in response to mechanical stimulation. Mechanical thresholds were significantly higher in light-sensitive Aδ-fibers than in light-insensitive Aδ-fibers. For Aβ-fibers, both light-sensitive and light-insensitive fibers mostly displayed RA impulses. However, most light-sensitive Aβ-fibers had high mechanical threshold while most light-insensitive Aβ-fiber had low mechanical thresholds. We investigated behavioral responses to light stimulation of Nav1.8-ChR2eYFP fibers applied to the hindpaw plantar skin. With progressive increases of laser light intensity from 1 to 100 mW/mm^2 (50 ms), mice responded with paw lifting, flinching and fluttering, holding, licking, jumping, and vocalization. Collectively, Nav1.8-ChR2eYFP fibers innervating the hindpaw glabrous skin not only include nociceptive C-fibers and many Aδ-fibers, but also include a sizable number of Aβ-fibers that are potentially nociceptors. The activation of these three types of Nav1.8-ChR2eYFP fibers may account for the nociceptive behavioral responses induced by light stimulation. The study is supported by NIH R01 DE023090 to JGG.

Disclosures: A. Yamada: None. **A. Yamada:** None. **J. Ling:** None. **J. DeBerry:** None. **H. Furue:** None. **J. Gu:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.20

Topic: D.02. Somatosensation – Pain

Support: Royal Commission for the Exhibition of 1851 Industrial Fellowship Award

Title: A rare missense mutation causing functional alterations of the potassium ion-channel TREK2 is nominally associated with pain

Authors: *S. BOURNE[1], R. FORFAR[1], E. L. VEALE[2], P. D. WRIGHT[1], J. M. LARGE[1], C. MPAMHANGA[1], J. JERMAN[1], A. MATHIE[2];

[1]LifeArc, Stevenage, United Kingdom; [2]Medway Sch. of Pharm., Univ. of Kent, Chatham Maritime, United Kingdom

Abstract: TREK2 is a member of the two-pore domain potassium ion-channel family, that regulates the resting membrane potential of nociceptive sensory neurons. These promising therapeutic targets for the treatment of pain have yet to be exploited, however poor clinical translation of novel analgesics, with many failing at late stages, can impede therapeutic advances. One way to accelerate and improve drug translation is to use human functional genomics and naturally occurring genetic variations to validate targets. We highlight a TREK2 variant, Thr254Met, identified from the UK and Finnish Biobank GWAS results, which has a nominal association ($P < 0.05$) with 7 pain phenotypes (precordial, hip, throat, chest, temporomandibular, mononeuropathy and drug induced migraine pain). We cloned this mutant, located in the 3rd transmembrane domain of TREK2, into pcDNA5™/FRT and transiently expressed it in human tsA201 and CHO cells. Whole-cell voltage-clamp recordings from TREK2 Thr254Met expressed in tsA201 cells (n33) gave outward currents that were significantly reduced ($P < 0.001$, one way ANOVA Fisher's LSD) compared to WT (n13). Reduced basal activity was also observed in CHO cells using a high-throughput thallium flux assay on the FLIPR tetra (n3, $P < 0.001$, one way ANOVA Fisher's LSD). However, current passing through the Thr254Met variant could be rescued using small molecule agonists. To profile the pharmacology further, we screened 10 literature and in-house small molecule candidates in duplicate using a 10-point half-log serial dilution in thallium flux. There was no significant difference between Thr254Met and WT TREK2 responses in terms of potency (n3, $logEC_{50}$ +/- 0.5, nonlinear regression), however the percentage enhancement of current for some compounds was greater for Thr254Met ($P < 0.05$, two-way ANOVA Fisher's LSD). We confirmed that functional differences were not due to changes in mRNA expression using TaqMan RT-qPCR on CHO cell lysates (n=3, one way ANOVA Fisher's LSD) but likely due to disrupted hydrogen bonding within transmembrane domain 3, confirmed by probing the available homology models for TREK2 (PDB: 4BW5). Here we demonstrate a novel clinical Thr254Met variant associated with pain phenotypes that reduces TREK2 function, and which may impinge upon nociceptive signalling through altering neuronal firing. Furthermore, this data provides functional translational validation of TREK2 as a promising pain therapeutic target and highlights the potential of small molecule agonists to rescue function and provide therapeutic benefit.

Disclosures: S. Bourne: None. **R. Forfar:** None. **E.L. Veale:** None. **P.D. Wright:** None. **J.M. Large:** None. **C. Mpamhanga:** None. **J. Jerman:** None. **A. Mathie:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.21

Topic: D.02. Somatosensation – Pain

Support: Wellcome Trust Investigator award 212302/Z/18/Z

Title: In vivo spike propagation through the dorsal root ganglion reveals a potential peripheral somatosensory gate.

Authors: *V. K. LALL[1], P. MULLEN[2], H. HAN[3], X. DU[4], N. GAMPER[5];
[1]Univ. of Leeds, Univ. of Leeds, Leeds, United Kingdom; [2]Sch. of Psychology and Neurosci., St Andrews, United Kingdom; [4]Hebei Med. Univ., [3]Hebei Med. Univ., Hebei, China; [5]Nikita Gamper, Univ. Leeds, Leeds, United Kingdom

Abstract: The dorsal root ganglion (DRG) contains the cell bodies of peripheral somatosensory nerves, which detect and transmit signals to the CNS related to physical changes in the body's immediate environment or to the state of internal organs. It is often assumed that the peripheral somatosensory signals are first integrated in the spinal cord. Here we show that the DRG is able to modulate nociceptive signals before they enter the CNS. Simultaneous whole nerve recordings were obtained from the C8 dorsal root (DR) and spinal nerve (SN) in the decerebrate, arterially perfused preparation of the rat (18-22 day, Wistar rats of either sex) in the absence of anaesthetic. These nerves innervate the median nerve supplying the forepaw. C8 ventral roots were transected to avoid motor, efferent contamination. Using in-house custom written scripts to enable spike sorting, we were able to identify spikes in the SN and temporally match these to spikes propagated to the DR. Baseline firing in the SN was consistently higher than the DR, an effect we termed 'tonic' filtering. Direct application of GABA (200 μM) into the DRG did not alter tonic firing frequency in either aspect of the nerve (DR: 14.1 ± 1.2Hz Vs 14.2 ± 1.2Hz ($p= 0.95$), SN: 34.2 ± 2.5Hz Vs 35.1 ± 2.6Hz ($p= 0.8$); $n= 19$). Noxious stimuli (Randall Selitto) applied to the forepaw increased firing in both SN (31.4 ± 1.6Hz to 45.4 ± 2Hz, $p< 0.001$; $n= 20$) and DR (12.7 ± 0.9Hz to 26.8 ± 1.3Hz, $p< 0.001$). DRG application of GABA significantly reduced firing frequency in the DR (18.6 ± 1.1Hz, $p< 0.001$) but not in the SN (48.6 ± 2.3Hz, $p< 0.2$; $n= 13$), an effect we are interpreting as GABA-induced filtering of the conduction through the DRG. Spike sorting revealed that units with the slowest conduction latencies (likely the C fibers) were more likely to be filtered, suggesting that the DRG is able to selectively filter painful stimuli entering the spinal cord via GABAergic mechanisms. This notion was further confirmed in thermal stimulation experiments. Innocuous stimulation (cotton bud strokes, air puff, tactile brushstrokes) and proprioceptive stimulation also increased firing in the SN and DR however, GABA was unable to attenuate DR firing rates. Taken together, we report an in-vivo-like method for studying spike propagation through the DRG which does not require anaesthesia. Moreover our findings indicate that peripheral somatosensory ganglia may represent an intrinsic filter or even a peripheral 'gate' within the somatosensory system. This may serve as a novel therapeutic target for pain relief offering a unique opportunity to target the DRG with drugs with low CNS bioavailability, thereby reducing CNS related side effects.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.22

Topic: D.02. Somatosensation – Pain

Support: University of Leeds

Title: Anoctamin 6 functions as a phospholipid scramblase in the dorsal root ganglion

Authors: *A. GREGORIE, V. PRATO, S. RASOOLI-NEJAD, N. GAMPER;
Univ. of Leeds, Leeds, United Kingdom

Abstract: Anoctamin 6 (ANO6, TMEM16F) has been proposed to be both an ion channel and a phospholipid scramblase (PLS), with each function being calcium-activated. Many discrepancies arise as to the nature of ion ANO6 conducts, to the extent that the very identity of ANO6 as an ion channel has been disputed with no clear conclusion. It's role as a PLS has been more thoroughly investigated in several cell types; it was shown that ANO6 activity impacts the asymmetry of phospholipids within the membrane bilayer in a calcium-dependent manner; this, in turn, was linked to increases in membrane curvature and potentially exosome release. Here, using immunohistochemistry, we demonstrate robust expression of ANO6 within rat dorsal root ganglia (DRG) neurones. Additionally exosomal markers (CD63) were identified in the media of cultured DRG neurones, suggesting active exosome release. Calcium-activated phospholipid scrambling has been investigated using live-cell confocal imaging in conjunction with fluorescently tagged annexin-V, a protein which binds to exposed phosphatidylserine molecules on the outer membrane. Ionomycin and a putative ANO6 activator compound were shown to robustly increase annexin-V binding to DRG neurone membranes, as measured by the increase in fluorescence. The induced annexin V binding was inhibited by pre-incubation with the chloride channel inhibitor niflumic acid and by selective ANO6 knockdown using FANA antisense oligonucleotides, suggesting that ANO6 underlies a significant portion of the phospholipid scrambling within the membranes of DRG neurones. Thus far, whole-cell electrophysiology has been unable to demonstrate that ANO6 underlies any measurable calcium-activated chloride current within acutely dissociated rat DRG neurones, despite evidence that it is able to do so when overexpressed in heterologous expression systems. In summary, we show that ANO6 is functionally expressed in rat DRG neurones, where it functions as a PLS and may be involved in exosome release. These results uncover hitherto unknown communication mechanism between cells in the spinal ganglia.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.23

Topic: D.02. Somatosensation – Pain

Support: NINDS (R01 NS072446)
NINDS (R01 NS082331)
European Leukodystrophy Association (ELA 2019-012I2)
Arrivederci Foundation
Cure ALD Foundation
Leblang Charitable Foundation
Hammer Family Fund for ALD Research and Therapies for Women

Title: Peroxisome Metabolism Contributes to PIEZO2-Mediated Mechanical Allodynia

Authors: *A. QIAN[1], Y. GONG[1], F. LAHEJI[1], A. BERENSON[1], S.-O. PARK[1], R. KOK[2], M. SELIG[1], R. HAHN[1], R. SADJADI[1], S. KEMP[2], F. EICHLER[1];
[1]Dept. of Neurol., Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [2]Lab. Genet. Metabolic Diseases, Dept. of Clin. Chem., Amsterdam Univ. Med. Ctr., Amsterdam, Netherlands

Abstract: Mutations in the peroxisomal half-transporter ABCD1 cause X-linked adrenoleukodystrophy, resulting in elevated very long-chain fatty acids (VLCFA), progressive neurodegeneration and an associated pain syndrome that is poorly understood. In the nervous system of mice, we found ABCD1 expression to be highest in dorsal root ganglia (DRG), with satellite glial cells (SGCs) displaying higher expression than neurons. We subsequently examined sensory behavior and DRG pathophysiology in mice deficient in ABCD1 compared to wild-type mice. Beginning at 8 months of age, Abcd1−/y mice developed persistent mechanical allodynia. DRG had a greater number of IB4-positive nociceptive neurons expressing PIEZO2, the mechanosensitive ion channel. Blocking PIEZO2 partially rescued the mechanical allodynia. Beyond affecting neurons, ABCD1 deficiency impacted SGCs, as demonstrated by high levels of VLCFA, increased glial fibrillary acidic protein (GFAP), as well as genes disrupting neuron-SGC connectivity. These findings suggest that lack of the peroxisomal half-transporter ABCD1 leads to PIEZO2-mediated mechanical allodynia as well as SGC dysfunction. Given the known supportive role of SGCs to neurons, this elucidates a novel mechanism underlying pain in X-linked adrenoleukodystrophy.

Disclosures: A. Qian: None. **Y. Gong:** None. **F. Laheji:** None. **A. Berenson:** None. **S. Park:** None. **R. Kok:** None. **M. Selig:** None. **R. Hahn:** None. **R. Sadjadi:** None. **S. Kemp:** None. **F. Eichler:** None.

Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.24

Topic: D.02. Somatosensation – Pain

Support: CIHR
Alan Edwards Centre for Research on Pain

Title: Differential heterologous stimulation of primary somatosensory afferents in mice.

Authors: *Q. DEVAUX[1], H. ALKHANI[2], A. ASE[3], P. SEGUELA[3];
[1]Physiol., [2]Integrated program in Neurosciences, [3]Neurol. & Neurosurg., McGill Univ., Montreal, QC, Canada

Abstract: Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are now increasingly used in neuroscience research[1]. The functional study of sensory afferents relies increasingly on such genetic tools. However, the physiological and molecular mechanisms at play downstream of DREADD activation are still not fully understood.Optogenetic tools such as ChR2 have been validated as pain-eliciting transducers when expressed in several subtypes of dorsal root ganglia (DRGs) neurons expressing the voltage-gated sodium channel $Na_V1.8$[2]. However, while the excitatory opsin ChR2 is known to evoke an action potential through direct influx of positive ions, the excitatory DREADD hM3Dq works through metabotropic signaling. The outcome of activation of these heterologous actuators can therefore be quite different. We have reported that ionotropic activation using ChR2 versus metabotropic activation using the hM3Dq have radically distinct behavioral effects in the MrgprA3+ subtype of DRG neurons[3].Here we show that broad stimulation of Nav1.8-expressing neurons, commonly referred to as nociceptors, using the excitatory DREADD hM3Dq, does not elicit directly observable pain responses but instead sensitizes these afferents. Specifically, hM3Dq-driven activation of nociceptors enhances their sensitivity to thermal and mechanical stimuli. Electrophysiological evidence suggest that this is achieved through potentiation of the transient receptor vanilloid 1 (TRPV1) channels and the transient receptor potential canonical 3 (TRPC3) channels, respectively. Thermal sensitization is also observed while stimulating only the population of TRPV1+ neurons in vivo. Interestingly, inflammation is observed while stimulating the larger $Na_V1.8$+ neuronal population but is not induced when only stimulating the TRPV1+ subtype.Our aim is to further explore why and how DREADDs can elicit such different responses in DRG somatosensory afferents.**References**1.Roth BL. DREADDs for Neuroscientists. Neuron. 2016 Feb 17;89(4):683-94. doi: 10.1016/j.neuron.2016.01.040. PMID: 26889809; PMCID: PMC4759656.2.Uhelski ML, Bruce DJ, Séguéla P, Wilcox GL, Simone DA. In vivo optogenetic activation of Nav1.8+ cutaneous nociceptors and their responses to natural stimuli. J Neurophysiol. 2017 Jun 1;117(6):2218-2223. doi: 10.1152/jn.00083.2017. Epub 2017 Mar 15. PMID: 28298301; PMCID: PMC5454474.3.Sharif B, Ase AR, Ribeiro-da-Silva A, Séguéla P. Differential Coding of Itch and Pain by a Subpopulation of Primary Afferent Neurons. Neuron. 2020 Jun 17;106(6):940-951.e4. doi: 10.1016/j.neuron.2020.03.021. Epub 2020 Apr 15. PMID: 32298640.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 290.25

Topic: D.02. Somatosensation – Pain

Support: Ministerio de Educación Nacional, Ministerio de Industria, Comercio y Turismo, ICETEX and World Bank (792-2017 2a Convocatoria Ecosistema Científico—Colombia Científica para la financiación de proyectos de I+D+i).
Vicerrectoría de Investigación, Pontificia Universidad Javeriana, Grant ID 20278. Plant extracts with antinociceptive potential that modulate the Transient receptor potential ankyrin 1 (TRPA1).

Title: Bidens Pilosa derived metabolites as regulators of TRPA1 channels

Authors: *S. M. DUITAMA[1], X. M. RODRIGUEZ[2], S. L. ALBARRACIN[1], Z. Y. CASAS[1], Y. P. TORRES[1], P. SANTANDER[2], J. J. SUTACHAN[1];
[1]Pontificia Univ. Javeriana, Bogotá D.C, Colombia; [2]Fundación Juan N Corpas, Bogota D.C., Colombia

Abstract: The tumor microenvironment plays a crucial role in producing different signaling molecules that regulate metastasis, tumor growth, and pain. Transient receptor potential (TRP) channels transduce noxious stimuli associated with temperature, decreased pH, and pressure and are regulated by growth factors, neurotransmitters, and inflammatory mediators released from tumors. In particular, the only TRP family member directly activated by several factors associated with the tumor microenvironment is the transient receptor potential ankyrin 1 (TRPA1) that can undergo sensitization processes involving intracellular signaling pathways activated by inflammatory mediators released from the tumor. Therefore, TRPA1 channels expressed in sensory neurons have been seen as potential targets of antinociceptive compounds in treating cancer pain. Recently, a series of secondary metabolites obtained from Bidens Pilosa, a plant traditionally used in South America for pain management, such as terpenes, monoterpenes, and phenolic derivatives, have been shown to regulate the activity of TRPA1 channels. However, the signaling mechanisms by which the Bidens Pilosa-derivate metabolites regulate TRPA1 channels and pain have not been elucidated. Our results using cultures of rat dorsal ganglia neurons showed that extracts with high and medium polarity induce a significant decrease (more than 20%) in the viability of these neurons, in which it has been found that TRPA1 expression is present in 40% of the total neurons. In contrast, low polarity extracts did not significantly affect the viability of DRG neurons (less than 10%) in the range of concentrations evaluated (0 to 20ug/mL) for 24 hours. Likewise, we found that hydro and ethanolic polar extract did not affect the activity of the TRPA1 channel at a concentration of 5ug/mL, suggesting that Bidens Pilosa metabolites that regulate these channels are found in intermediate polarity and low extracts that could work through the regulation of signaling pathways involved in its sensitization process.

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Poster

290. Dorsal Root Ganglia and Afferents: Ion Channels and Receptors

Location: SDCC Halls B-H

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Program #/Poster #: 290.26

Topic: D.02. Somatosensation – Pain

Support: NIH R01 NS115209
Brains & Behavior, Georgia State University
2CI Neurogenomics, Georgia State University

Title: Prior cold experience alters cold-evoked electrophysiological responses and calcium dynamics in cold nociceptors

Authors: *K. J. DONALDSON, A. SAKURAI, A. A. PATEL, D. N. COX;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: To ensure proper growth, development and survival, organisms possess the ability to adapt to constantly changing sensory environments based on prior experience. *Drosophila* larvae predominantly respond to acute noxious cold (≤10°C) with a full body contraction (CT) behavior requiring activation of Class III multidendritic neurons (CIIIs) by cold stimuli. Previously, we've shown that prior cold experience (CE) causes cold-evoked behavioral plasticity, exhibited by increased magnitude of CT behavior. CE also induced an increase in C-bending behavior, a precursor to the rolling behavior typically associated with noxious heat stimulation and mediated by Class IV nociceptors (CIVs). In a set of parallel experiments, chronic "fictive" cold was achieved by targeted optogenetic stimulation of CIII neurons recapitulating the behavioral changes seen under CE and indicating that neuronal activity *sans* cold was sufficient to induce plasticity of the cold nociceptive circuitry. To better understand the cellular mechanism underlying this experience-dependent behavioral change, we recorded both calcium (Ca^{2+}) and electrophysiological responses to thermal stimuli in CIII cold nociceptors of larvae previously exposed to noxious cold temperatures. Third-instar larvae expressing GCaMP6 in CIIIs were reared at room temperature (22.5°C) before being placed in noxious cold (10° C) for various durations prior to being selected for live imaging or *in vivo* electrophysiological recordings. Cold stimulation of larvae which experienced 0 (control group), 24, or 48 hours of cold experience resulted in significantly increased Ca^{2+} signal above pre-stimulus levels, with 24-hour experience larvae showing a reduced peak response compared to controls and 48-hour experience groups. After 48 hours of cold experience, larval CIII neurons showed increased spontaneous spiking activity at room temperature and significantly more robust spiking responses to mild temperature drops at non-noxious temperatures (20°C and 15°C) than controls. At noxious cold temperatures, activity was similar between 48-hour CE and controls, mirroring the effects seen in Ca^{2+} experiments. Together these data indicate that prior CE differentially modifies the physiological activity of cold nociceptors to later thermal stimuli and begins to characterize cellular mechanisms underlying plasticity of the cold nociceptive system.

Disclosures: K.J. Donaldson: None. **A. Sakurai:** None. **A.A. Patel:** None. **D.N. Cox:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.01

Topic: D.02. Somatosensation – Pain

Support: NIH Grant P20GM103643

Title: The role of spinal cord processing of sensory input in maintaining movement-evoked breakthrough pain

Authors: *C. QUATTROCHIO, K. DRAGO, T. GUNTHER, V. EATON, T. KING;
Univ. of New England, Biddeford, ME

Abstract: Cancer-induced bone pain is characterized as moderate to severe ongoing pain and is commonly treated with opiates. However, approximately 80% of patients suffering from metastatic bone pain experience episodes of breakthrough pain (BTP) that occur despite medication controlling their background pain. BTP episodes last about 30 minutes and are often triggered by voluntary or involuntary movements. They can occur up to four to six times per day, and dramatically reduce the quality of life of those affected. Previous research demonstrated that movement of a tumor bearing bone induces BTP and blocking peripheral sensory input before movement prevents BTP. In contrast, peripheral nerve block after movement failed to reverse BTP. We hypothesize that peripheral input mediates initiation of movement induced BTP, whereas BTP is maintained centrally through a reverberating circuit within the spinal cord. Using a mouse model of cancer induced bone pain, we determined whether spinal lidocaine administration blocks movement induced suppression of rearing behaviors. Subjects were female C57BL/6 mice, weighing 20-25 grams. Under isoflurane anesthesia, Lewis Lung Carcinoma Cells were implanted and sealed into the femur. BTP was assessed 14 days later using movement-induced suppression of rearing behaviors in an automated open field chamber. Lidocaine (2%w/v) or saline (5 μl) was administered to the spinal cord five minutes following hindlimb movement and to non-movement controls. Data were analyzed using ANOVA with appropriate post-hoc analysis to determine group differences with significance set at $p<0.05$. Hindlimb movement significantly diminished rearing in tumor bearing animals compared to non-movement controls ($p<0.05$). Administration of spinal lidocaine five minutes post-movement blocked the movement-induced depression of rearing behaviors ($p<0.05$ vs movement-saline group). Importantly, spinal lidocaine did not alter rearing behaviors in animals that did not receive hindlimb movement. Our findings suggest that movement induced BTP is maintained at the level of the spinal cord. In conjunction with previous studies that demonstrated peripheral nerve block will prevent BTP, but not reverse movement induced BTP, we propose that the BTP is initiated by peripheral input but maintained within the spinal cord. This work has been supported by Kahn Family Foundation Research Fellowships to CQ and KD and by the NIH (National Institute of General Medical Sciences COBRE grant P20-GM-103643) supporting the UNE Behavior Core.

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Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.02

Topic: D.02. Somatosensation – Pain

Support: NIGMS Grant P20GM103643

Title: Comparison of paclitaxel formulation in rodent models of paclitaxel-induced peripheral neuropathy

Authors: *D. GIUVELIS, I. D. MENG, T. KING, D. BARLOW, K. L. HOUSEKNECHT, K. L. TUCKER;
Univ. of New England, Biddeford, ME

Abstract: Paclitaxel, commonly known as Taxol, is a chemotherapy medication used for a wide range of cancers, including pancreatic, cervical, breast, ovarian, and lung cancer. Paclitaxel-induced peripheral neuropathy (PIPN) is a common side effect of chemotherapy and has been shown to affect as many as 60-70 percent of chemotherapy patients. Rodent models are employed to model PIPN both for mechanistic and pre-clinical therapeutic studies, but the pain research community has often voiced a skepticism of the utility of PIPN models, citing high variability in behavioral outcomes. Here we report effects using two different paclitaxel formulations; Sigma-Aldrich #T7402 and Hospira Clinical USP. We tested these two formulations using a standard 4-dose injection (IP) every other day in mouse (4mg/kg) and rat (2mg/kg) models in males and females. Mechanoceptive and thermoceptive behavioral outcomes were measured using standard von Frey, Hargreaves, and cold plate assays. The development of PIPN was robust, reproducible, and observed in both sexes and species. We also observed radically-different behavioral time courses between formulations. PIPN was fully resolved by day 23 when induced using the Sigma-Aldrich formulation, whereas the hypersensitivity did not resolve until day 51 when using the clinical formulation. A pharmacokinetic study was run in the rat model to determine if drug exposure could help explain the differences we observed in behavioral response to the two formulations, but we did not observe any differences in the level of drug in the blood (plasma) following a single (2mg/kg, IP) dose administration. The C_{max} (nM) for the Sigma-Aldrich and clinical formulation was 35.4±6.77 and 29.3±5.32, respectively. Future pharmacokinetic studies are needed to look at the blood plasma levels after repeated drug administration. Paclitaxel produced PIPN in both rodent species and sexes, with formulation-dependent differences in the time course of mechanical and thermal hypersensitivity. Future studies are needed to better understand the mechanisms driving these differences.

Disclosures: D. Giuvelis: None. **I.D. Meng:** None. **T. King:** None. **D. Barlow:** None. **K.L. Houseknecht:** None. **K.L. Tucker:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

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Program #/Poster #: 291.03

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NIGMS P20GM103643

Title: Paclitaxel increases novel expression of major histocompatibility complex II (MHCII) and the MHCII transcription factor, Regulatory Factor X1 in dorsal root ganglion neurons from female mice

Authors: E. E. WHITAKER, N. E. MECUM, R. C. COTT, ***D. J. GOODE**;
Univ. of New England, Biddeford, ME

Abstract: Chemotherapeutic agents are often a life-saving cancer treatment but the development of intractable pain caused by chemotherapy-induced peripheral neuropathy (CIPN) is a major dose-limiting toxicity that restricts cancer survival rates. Recent reports demonstrate that the presence of T cells reduce the duration of chemotherapy-induced mechanical hypersensitivity in male and female mice. Furthermore, anti-inflammatory cytokines IL-10 and IL-4 are protective against CIPN. Recently, we have shown that paclitaxel (PTX) robustly increases anti-inflammatory IL-10 and IL-4 producing CD4+ T cells in the dorsal root ganglion (DRG); however, the increase only occurs in female mice. The mechanism by which CD4+ T cells are induced to secrete anti-inflammatory CD4+ T cells in female DRG is unknown. To explore the possibility of direct communication between CD4+ T cells and neurons, we screened published RNA-seq datasets of DRG neurons for immune molecules. We identified that DRG neurons express transcripts for major histocompatibility complex class II (MHCII) and the MHCII transcription factor, Regulatory Factor X1 (RFX1). Protein expression of MHCII and RFX1 was visualized in female and male C57BL6/J DRG tissue sections (n=4 mice/sex) and purified cultured DRG neurons (n=4 mice/sex) through immunohistochemistry and immunocytochemistry, respectively. Consistent with the RNA seq data, MHCII and RFX1 protein was found in all neurons independent of size in both male and female mice. Similarly, there was no difference in the percentage of $MHCII^+$ or $RFX1^+$ DRG neurons between naïve male and female mice. RFX1 protein increased in both male and female DRG neurons after PTX and returned to baseline levels during the resolution of CIPN. In addition, PTX increased the percentage of $MHCII^+$ DRG neurons; however, this only occurred in female mice with the greatest increase in <30μM diameter neurons 1- and 2- weeks post PTX (3- and 7-fold respectively). In contrast, the percentage of $MHCII^+$ neurons in male DRG decreased 1- and 2-weeks post-PTX. Our results suggest that DRG neurons from PTX-treated female mice have an increased capacity to directly activate anti-inflammatory CD4+ T cells to suppress CIPN.

Disclosures: E.E. Whitaker: None. **N.E. Mecum:** None. **R.C. Cott:** None. **D.J. Goode:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.04

Topic: D.02. Somatosensation – Pain

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FEDER

Title: Apparent lack of effect of oxaliplatin systemic treatment on the activity of unmyelinated nociceptors

Authors: *J. ALLARD[1], J. BARBIER[2], F. MARCHAND[2], J. BUSSEROLLES[2];
[1]E-Phys, Clermont Ferrand, France; [2]Univ. Clermont Auvergne, Clermont-Ferrand, France

Abstract: A better understanding of the neurological mechanisms responsible for the deleterious side effects of chemotherapeutic agents such as oxaliplatin is necessary. To this aim, we assessed the effect of a single oxaliplatin injection on the responses of unmyelinated DRG neurons in mice.C57BL6 male mice were treated with a single i.p. injection of 6 mg/kg oxaliplatin or vehicle. Experiments were performed under isoflurane anaesthesia after 3-4 days. The L4 DRG was exposed through a medio-lateral laminectomy of the corresponding vertebra. Single unit recording within the DRG was achieved with a conventional extracellular recording set up. The search of DRG neurons was exclusively based on orthodromic electrical stimulations from the hind paw. Evoked responses to brush, Von Frey 25-200 mN, pinch and water jet at 0, 24, 42, 46 and 50 °C were measured for each neuron. Blood gas, pH and haematocrit were measured at completion of the experiment. Sixteen vehicle and 20 oxaliplatin-treated mice were used. Oxaliplatin-treated mice showed a significant reduction of their 50 % mechanical threshold upon Von Frey assessment (mean, 3.3 mN versus 76 mN in vehicle-treated mice) and of the haematocrit (mean, 34.8 % versus 44.4 % in vehicle-treated mice). The median conduction velocities of the unmyelinated DRG neurons were similar between vehicle (0.91 m/s, n=40) and oxaliplatin-treated mice (0.92 m/s, n=50). All unmyelinated DRG neurons in the vehicle group were nociceptors but one C LTMR, and their modality distribution was not significantly different in vehicle and oxaliplatin-treated mice (polymodal, 37/34 %; mechano-specific, 22/26 %; heat-specific, 15/18 %; cold-specific, 7/16 %, respectively). Evoked responses were similar in both groups, but the low number of neurons for some modalities hampered the comparison. For example, cold-specific responses were obtained from only 3 vehicle neurons (range, 64-750 action potentials) and 8 oxaliplatin neurons (range, 11-660 action potentials). The present data suggest a lack of measurable effect of a single systemic oxaliplatin injection on the activity of unmyelinated DRG nociceptors. This is indirectly in line with data obtained with different methodologies suggesting that oxaliplatin triggers changes in the activity myelinated DRG neurons. From a technical point of view, the present study also demonstrates that single unit

recording within the DRG represents an alternative approach to the “teased-fibre technique” for the study of peripheral nociceptors in rodents.

Disclosures: J. Allard: None. **J. Barbier:** None. **F. Marchand:** None. **J. Busserolles:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: D.02. Somatosensation – Pain

Support: CRG/2020/002621/BHS
5/3/8/17/ITR-F/2020
SPARC/2018-2019/P435/SL

Title: Development and validation of novel animal model of chemotherapy-induced neuropathic pain

Authors: *A. .[1], V. TIWARI[2];
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Abstract: Development and Validation of Novel Animal Model of Chemotherapy-induced Neuropathic Pain. Akhilesh and Vinod Tiwari. Neuroscience and Pain Research Laboratory, Department of Pharmaceutical Engineering and Technology, Indian Institute of Technology (BHU), Varanasi, 221005, Uttar Pradesh, India **Abstract:** Chemotherapy-induced neuropathic pain (CINP) is among the most common side effects with the use of anti-cancer drugs but till date no effective treatment is available for the management of the same. One of the reasons for this could be the unavailability of preclinical animal models which can best represent the clinical situation. Chemotherapeutics agents are generally prescribed in the combination of two or three drugs and based on this observation, in this study we have used a cocktail of chemotherapy (paclitaxel, cisplatin, and vincristine) for the development of novel model of CIPN and compared with a conventional paclitaxel model using Sprague Dawley rats. Results revealed that rats administered with cocktail of chemotherapy have significantly increased the pain sensitivity which persisted up to the 8th week however, the paclitaxel-treated rats showed pain up to the 5th week. Next, we found an upregulation of mRNA expression of different receptors such as TRPA1, TRPV1, TRPM8, NR2B, and neuropeptides including substance P and CGRP in the DRG and spinal cord of the cocktail treated rats as compared to both PTX and vehicle-treated rats on day 35. The levels of neuroinflammatory markers such as TNF-α and IL-1β and nitrosative stress was also increased in cocktail-treated rats compared to both PTX and vehicle-treated group. Further, using the pharmacological tools we observed that on 14th day both cocktail and PTX treated rats showed significant pain sensitivity which was significantly attenuated by gabapentin 40 mg/kg *i.p.* The treatment with gabapentin also reversed the

molecular and biochemical alterations in the DRG, spinal cord, and sciatic nerve of both cocktail and PTX treated rats as compared to the vehicle administered group. The novel cocktail-treated animal model for CINP successfully demonstrated the face, predictive and constructive validity and therefore it could be utilized as an additional resource for unraveling the mechanism responsible for the development of pain and screening of pharmacological agents against CINP.
Keywords: Peripheral Neuropathy; Cocktail Chemotherapy; Chronic pain: Novel animal model; TRP Channels.

Disclosures: A. .: None. **V. Tiwari:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.06

Topic: D.02. Somatosensation – Pain

Title: Prazosin alleviates chemotherapy-induced neuropathic pain

Authors: *H. HU, X. HUO, A. YI, H. BIRLA, F. WANG, Y.-X. TAO;
NJMS, Newark, NJ

Abstract: Peripheral neuropathy is a common severe side effect of neurotoxic chemotherapy and can persist from months to years after chemotherapy completion, causing significant challenges for cancer survivors. Patients often can't tolerate chemotherapy-induced peripheral neuropathy (CIPN), leading to dose reductions or premature treatment discontinuation of chemotherapy. Painful CIPN is often poorly managed because it is frequently unresponsive to standard pain treatments, representing a pressing unmet medical need. Prazosin, an alpha-1 adrenergic receptor antagonist, is primarily used to treat hypertension, benign prostatic hyperplasia, and post-traumatic stress disorder (PTSD) in clinical practice, often used as a pharmacological tool in preclinical studies. Here, we investigated the potential analgesic effect of prazosin on paclitaxel-induced neuropathic pain. We found that oral administration of 5 or 10 mg/kg prazosin attenuated paclitaxel-induced mechanical allodynia and cold pain hypersensitivity in a dose-dependent manner. This analgesic effect was also observed when prazosin was administered intrathecally and intraplantarly. However, pretreatment with prazosin did not prevent the development of CIPN. It has been shown that TRPA1 and TRPV1 are involved in chemotherapy-induced pain hypersensitivity. Interestingly, prazosin also reduced TRPV1- and TRPA1-mediated nociception. Our *in vitro* study shows that prazosin blocked capsaicin (a TRPV1 agonist)- and AITC (a TRPA1 agonist)-induced Ca^{2+} responses, suggesting that its analgesic effect may be mediated via TRPV1 and TRPA1 channels. Our study provides the first evidence that prazosin has a strong analgesic effect on CIPN. Our findings may suggest that prazosin is an attractive alternative for the treatment of CIPN.

Disclosures: **H. Hu:** None. **X. Huo:** None. **A. Yi:** None. **H. Birla:** None. **F. Wang:** None. **Y. Tao:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

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Program #/Poster #: 291.07

Topic: D.02. Somatosensation – Pain

Support: ZIA NR000020-06

Title: Association between Brain-Derived Neurotropic Factor rs6265 polymorphism and neuropathic pain in breast cancer survivors

Authors: *T. GOTO[1], L. XIANG[1], L. N. SALIGAN[1], D. VON AH[2];
[1]Natl. Inst. of Nursing Res., Natl. Inst. of Nursing Res., Bethesda, MD; [2]The Ohio State Univ. Col. of Nursing, Columbus, OH

Abstract: Breast cancer is the second leading cause of cancer mortality among women in the United States. Survivors of breast cancer often report psychoneuropsychological (PNP) symptoms, to include fatigue, depression, anxiety, pain (bodily and neuropathic), and sleep disturbance. These distressing symptoms can be long-lasting and can negatively impact health outcomes and productivity, in general. We recently reported a link of a single-nucleotide polymorphism (SNP) of brain-derived neurotrophic factor (BDNF) rs6265 (Val66Met) with cancer-related fatigue (CRF). This current study attempted to validate the association of *BDNF* rs6265 polymorphism with CRF and explore the associations of *BDNF* rs6265 polymorphism with other PNP symptoms reported by 414 breast cancer survivors. Study participants were divided into three groups according to their *BDNF* rs6265 genotypes: C/C, C/T, and T/T. We observed that bodily pain (p = .03) and neuropathic pain (p = .005) were significantly different among the SNP groups. Interestingly, those with T/T genotype had the worst neuropathic pain but the mildest bodily pain. CRF only tended towards significance (p = .09) using the Kruskal-Wallis test. While we observed a significant difference in the proportion of their cancer stages among the SNP groups, which may also affect the symptom scores, there were not enough T/T subjects in cancer stages I and II. Therefore, we further analyzed only stage III subjects. Neuropathic pain remains significantly different among the SNP groups in stage III subjects (Kruskal-Wallis test, p = .007). No other PNP symptoms were significant in the SNP groups among stage III subjects. Post-hoc multiple comparisons adjusted by the Holm method showed that T/T subjects reported significantly higher neuropathic pain scores than those with C/C (p = .007) and C/T (p = .01) genotypes. BDNF has important roles in neurodegeneration. These results suggest that stage III breast cancer survivors with T/T *BDNF* polymorphism are more likely to experience severe neuropathic pain during and after treatment, more than any other genotype. Further validation of this finding is warranted.

Disclosures: **T. Goto:** None. **L. Xiang:** None. **L.N. Saligan:** None. **D. Von Ah:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

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Program #/Poster #: 291.08

Topic: D.02. Somatosensation – Pain

Support: GACR 20-19136S

Title: Paclitaxel induced neuropathic pain is prevented by dual PI3Kδ/γ inhibitor Duvelisib

Authors: P. ADAMEK, M. HELES, A. BHATTACHARYYA, M. PONTERASO, J. SLEPICKA, ***J. PALECEK**;
Inst. of Physiology, Czech Acad. of Sci., Inst. of Physiology, Czech Acad. of Sci., Praha, Czech Republic

Abstract: Development of painful paclitaxel-induced peripheral neuropathy (PIPN) represents a major dose-limiting side effect of paclitaxel chemotherapy treatment. Our experiments show a promising effect of Duvelisib (Copiktra™), a novel FDA approved PI3Kδ/γ isoform-specific inhibitor, in preventing increased mechanical sensitivity and pronociceptive signalling in dorsal root ganglia (DRG) and spinal cord dorsal horn (SCDH) in rat and mouse model of PIPN. Duvelisib blocked the development of mechanical hyperalgesia in both males and females. Duvelisib prevented paclitaxel-induced sensitization of TRPV1 receptors, increased PI3K/Akt-signalling in small-diameter DRG neurons and an increase of $CD68^+$ cells within DRGs. Specific optogenetic stimulation of inhibitory neurons combined with patch-clamp recording revealed that Duvelisib prevented paclitaxel-induced reduction of inhibitory currents in SCDH excitatory neurons. Enhanced excitatory and reduced inhibitory neurotransmission in the SCDH following PIPN was also alleviated by Duvelisib application. In summary, Duvelisib showed a promising ability to prevent neuropathic pain in PIPN. The potential use of our findings in human medicine may be augmented by the fact that Duvelisib is an FDA approved drug with known side effects. This work was supported by the Grant Agency of the Czech Republic GACR 20-19136S.

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Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

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Program #/Poster #: 291.09

Topic: D.02. Somatosensation – Pain

Support: NIH Contract No. 75N95019D00026.

Title: Evaluation of behavioral pain phenotype and optimization of the rat paclitaxel and oxaliplatin models of chemotherapy-induced peripheral neuropathy

Authors: *T. HANANIA[1], E. DUGAN[1], K. BUBAN[1], J. HAGEDORN[1], S. A. WOLLER[2], S. IYENGAR[3], M. O. URBAN[1];
[1]PsychoGenics, PsychoGenics, Inc., Paramus, NJ; [2]DTR, NIH/NINDS, Rockville, MD; [3]NINDS/NIH, Rockville, MD

Abstract: In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), the paclitaxel and oxaliplatin models of chemotherapy-induced peripheral neuropathy in the rat were optimized and validated. Adult male and female Sprague Dawley rats (n=10, each sex) were used in these studies. For the paclitaxel model studies, paclitaxel was injected at several doses (2 mg/kg, i.p.; 4 mg/kg, i.p.; 2 mg/kg, i.v.) on alternate days (Day 0, 2, 4, 6) to determine the optimal dose and route of administration. For the oxaliplatin model studies, oxaliplatin (3 mg/kg, i.v.) was injected 2 days per week for a period of 4 weeks. Hind paw tactile sensitivity was determined with von Frey filaments using the "up-down" testing method, and hind paw cold sensitivity was determined using the acetone test. In the paclitaxel model studies, 4 injections of paclitaxel on alternate days (Day 0, 2, 4, 6) produced bilateral hind paw tactile and cold hypersensitivity which was maximal by Week 5 and persisted through Week 6. The paclitaxel dose of 4 mg/kg, i.p. was found to be optimal for this model, and results from rat pharmacokinetic studies demonstrated that Cmax and AUC values associated with this dose were consistent with values associated with efficacy in the clinic. Interestingly, hind paw priming with acetone during Week 2 enhanced acetone cold hypersensitivity in this model during Weeks 3-6, while mechanical priming with von Frey filament stimulation did not affect the development of tactile hypersensitivity. In the oxaliplatin model studies, oxaliplatin injection (3 mg/kg, i.v.) 2 days per week for a period of 4 weeks produced bilateral hind paw tactile and cold hypersensitivity which was maximal by Week 6 and persisted through Week 8. The magnitude of tactile and cold hypersensitivity was similar in the paclitaxel and oxaliplatin models. Initial pharmacological characterization was performed in these models by examining the effects of morphine sulfate (0.3-3 mg/kg, s.c.) on bilateral hind paw hypersensitivity, and morphine sulfate (3 mg/kg, s.c.) was found to significantly inhibit bilateral tactile and cold hypersensitivity in the paclitaxel and oxaliplatin models at Week 6 and Week 7, respectively. The validation of the paclitaxel and oxaliplatin models of chemotherapy-induced peripheral neuropathy further highlights efforts within the NIH HEAL Initiative's PSPP program to validate clinically relevant endpoints and models to be incorporated into evaluating novel assets towards accelerating the development of novel non-opioid, non-addictive therapeutics.

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even if those funds come to an institution.; CRO. **K. Buban:** A. Employment/Salary (full or part-time):; Full time employee, PsychoGenics. 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.; CRO. **J. Hagedorn:** A. Employment/Salary (full or part-time):; Full time employee, PsychoGenics. 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.; CRO. **S.A. Woller:** None. **S. Iyengar:** None. **M.O. Urban:** A. Employment/Salary (full or part-time):; Full time employee, PsychoGenics. 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.; CRO.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.10

Topic: D.02. Somatosensation – Pain

Support: Rita Allen Foundation

Title: Sensory Neuron Dysfunction in Orthotopic Mouse Models of Colon Cancer

Authors: *C. GAFFNEY[1], M. BALOGH[1,2], J. ZHANG[1], N. KALAKUNTLA[1], N. T. NGUYEN[1,3], R. T. TRINH[1], C. AGUILAR[1,4], H. V. PHAM[1], B. MILUTINOVIC[1], J. M. NICHOLS[1], R. MAHALINGAM[1], A. J. SHEPHERD[1];
[1]MD Anderson, Houston, TX; [2]Pharmaceut. Analysis, Groningen Res. Inst. of Pharmacy, Univ. of Groningen, AD Groningen, Netherlands; [3]Grad. Sch. of Biomed. Sci., UT Southwestern Med. Ctr., Dallas, TX; [4]Neurosci. and Behavior Grad. Program, Univ. of Massachusetts Amherst, Amherst, MA

Abstract: Reports of neurological sequelae related to colon cancer are largely restricted to rare instances of paraneoplastic syndromes, due to autoimmune reactions. Systemic inflammation associated with tumor development influences sensory neuron function in other disease models, though the extent to which this occurs in colorectal cancer is unknown. We induced orthotopic colorectal cancer via orthotopic injection of two colorectal cancer cell lines (MC38 and CT26) in two different mouse strains (C57BL/6 and Balb/c, respectively). Behavioral tests of pain sensitivity and activity did not detect significant alterations in sensory sensitivity or diminished well-being throughout tumor development. However, immunohistochemistry revealed widespread reductions in intra-epidermal nerve fiber density in the skin of tumor-bearing mice. Though loss of nerve fiber density was not associated with increased expression of cell injury markers in dorsal root ganglia, lumbar dorsal root ganglia neurons of tumor-bearing animals showed deficits in mitochondrial function. These neurons also had reduced cytosolic calcium

levels in live-cell imaging and reduced spontaneous activity in multi-electrode array analysis. Bulk RNA sequencing of DRGs from tumor-bearing mice detected activation of gene expression pathways associated with elevated cytokine and chemokine signaling, including CXCL10. This is consistent with the detection of CXCL10 (and numerous other cytokines, chemokines and growth factors) in MC38 and CT26 cell-conditioned media, and the serum of tumor-bearing mice. Our study demonstrates in a pre-clinical setting that colon cancer is associated with latent sensory neuron dysfunction and implicates cytokine/chemokine signaling in this process. These findings may have implications for determining risk factors and treatment responsiveness related to neuropathy in colorectal cancer.

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Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.11

Topic: D.02. Somatosensation – Pain

Support: NIH Grant ROHDK117404

Title: Liver X Receptor activation modifies lipid composition and reduces Prostaglandin D2 levels in the dorsal root ganglia of western diet-fed mice

Authors: *N. ELSHAREIF, C. GAVINI, V. MANSUY-AUBERT;
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Abstract: Peripheral neuropathy is a common comorbidity associated with type 2 diabetes and obesity. As a result, chronic neuropathic pain is a disabling symptom in these populations. Effective interventions are available to treat diabetes and obesity, such as glucose management, diet, and exercise, however, these options fail to address neuropathic pain symptoms. Understanding the mechanisms behind the pathogenesis of neuropathic pain and neuronal dysfunction is crucial in order to identify effective therapeutic options that address its underlying causes. Recent studies focus on diet-induced lipid dysfunction associated with obesity as a potential target for neuropathic pain management. Here, we focus on the nuclear transcription factors Liver X Receptors (LXRs), which are known regulators of lipid trafficking, phospholipid remodeling, and inflammatory pathways. We have previously discovered that LXR activation using the synthetic agonist GW3965 protects obese mice from diet-induced mechanical allodynia. To further elucidate the role of LXRs in obesity-induced neuropathic pain, we used translating ribosome affinity purification (TRAP) to assess translatomic and lipidomic changes induced by LXR activation in sensory neurons of western diet (WD)-fed mice. Using the synthetic agonist GW3965, we observed that LXR activation regulates neuronal lipid

homeostasis in the dorsal root ganglia of WD-fed mice. Interestingly, treatment with GW3965 decreased prostaglandin D2 levels in sensory neurons, suggesting downstream mechanisms in which LXR activation may attenuate lipid-induced neuronal dysfunction and pain. We observed a decrease in sensory neuron free fatty acids, accompanied by an increase in various lipid species, including lysophosphatidylcholine, phosphatidylcholine, and cholesterol ester species, which may have interplaying mechanisms to address neuropathic pain dependent on LXR activation with GW3965.

Disclosures: **N. Elshareif:** None. **C. Gavini:** None. **V. Mansuy-Aubert:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.12

Topic: D.02. Somatosensation – Pain

Support: NIH/NINDS 1F31NS127357-01
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation

Title: The contribution of sciatic nerve inflammation to diabetic neuropathy

Authors: *S. HAKIM[1], C. J. WOOLF[2];
[1]Boston Children's Hosp. and Harvard Med. Sch., Harvard Univ., Boston, MA; [2]Children's Hosp. Boston, Children's Hosp. Boston, Boston, MA

Abstract: It is estimated that more than 200 million people worldwide will develop Diabetic Neuropathy (DN), a major complication of diabetes affecting the peripheral nervous system. DN is characterized by retraction of sensory axons in the periphery from their targets with a “glove and stocking” pattern and half of the patients with DN also develop neuropathic pain. Unlike type 1 diabetes, type 2 diabetes (T2DM) is accompanied by systemic metabolic dysfunction and many patients develop neuropathy while pre-diabetic, suggesting that features such as hyperlipidemia, hyperglycemia, and systemic inflammation may contribute to the risk of neuropathy. Macrophages are major drivers of the systemic inflammation accompanying the metabolic syndrome and are also key players in the Wallerian degeneration accompanying axon injury, but their role in DN remains elusive. Modeling DN in mice has been a challenge as streptozotocin injections, commonly used to model type 1 diabetes, do not faithfully represent the multifaceted characteristics of the metabolic disease that disproportionately afflicts most of today’s diabetes population. Because a typical American diet constitutes a high calorie content from both fat and sugar, standard high fat diets alone do not model the excess sugars and fats that the typical adult is exposed to.
We have examined a new diet-induced mouse model of T2DM that comprises high calorie content from both fat and sugar and found that not only do these mice develop obesity and T2DM, but they also develop DN symptoms. These include both mechanical allodynia as

determined by Von Frey indicating a feature of neuropathic pain, and heat hyposensitivity, measured using the Hargreaves and hot plate assays which reflect neuropathy. This model can be used therefore to study the effect of the Western diet on sensory neurons and explore whether inflammation plays a role in the neuropathy.
Using flow cytometry, we observed a significant increase in the number of Cd45+ immune cells in the sciatic nerves of mice with DN compared to age-matched controls and identified those to be monocyte-derived macrophages (Ly6C+Cd64+Cd11b+). This increased macrophage infiltration into the sciatic nerve in diabetic mice may contribute to the pathogenesis of DN by directly communicating with neurons via cytokine signaling or phagocytosis or indirectly by modulating the function of other cell types such as Schwann cells. In conclusion, we have set up a new mouse model to study painful diabetic neuropathy and find that sciatic nerves in diabetic mice are a site of macrophage infiltration, providing a means to explore if these immune cells contribute to the pathogenesis of diabetic neuropathy.

Disclosures: S. Hakim: None. **C.J. Woolf:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.13

Topic: D.02. Somatosensation – Pain

Support: Camden Health Research Initiative (CHRI)

Title: Diabetes increases the expression of Piezo2, Nav1.7 and TRPV1 in the small-diameter sensory neurons of rats

Authors: C. HO, ***M. O'LEARY**;
Biomed. Sci., Cooper Med. Sch. of Rowan Univ., Camden, NJ

Abstract: Painful diabetic neuropathy is a frequent complication of diabetes mellitus that has been linked to the dysfunction of peripheral sensory nerve fibers. Metabolic disorders and vascular dysfunction have been implicated in the pathophysiology of diabetic neuropathy but the molecular mechanisms that produce diabetic pain have not been clearly established. This study employed the streptozotocin (STZ) rodent model to investigate the effects of diabetes on the electrophysiology and gene expression of dorsal root ganglion (DRG) sensory neurons. STZ-treated diabetic rats displayed elevated serum glucose levels and increased sensitivity to heat and mechanical stimulation consistent with the development of painful diabetic neuropathy. The small-diameter (<30 μm) DRG neurons of these diabetic rats displayed increases in tetrodotoxin-sensitive (TTX-S) sodium current, reduced action potential thresholds and increased firing frequency by comparison to non-diabetic controls. The increases in TTX-S sodium current and neuronal excitability were found to coincide with a selective increase in the expression of Nav1.7 sodium channels. The neurons of diabetic rats also displayed increased sensitivity to capsaicin

and mechanical stimulation that paralleled increases in the transcripts encoding for heat sensitive TRPV1 and mechanosensitive Piezo2 channels. These findings suggest that the upregulation of Nav1.7, TRPV1 and Piezo2 expression act synergistically to promote neuronal hyperexcitability and to sensitize sensory neurons to exogenous heat and mechanical stimulation. The upregulation of these ion channels in small unmyelinated sensory neurons may contribute to the mechanical and thermal hyperalgesia observed in diabetic rats.

Disclosures: C. Ho: None. **M. O'Leary:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.14

Topic: D.02. Somatosensation – Pain

Support: DoD Grant W81XWH-20-1-0277

Title: Age related immunological changes in the plantar skin of diabetic mice

Authors: *J. M. NICHOLS, R. MAHALINGAM, H. PHAM, A. SHEPHERD;
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Abstract: With the incidence of diabetes and diabetic peripheral neuropathy (DPN) on the rise, it is important to understand the pathological mechanisms which contribute to DPN. In recent years, the immune system has been highlighted as a major driver of DPN pathology, with cytokines like TNFα, which is expressed by several immune cell types including macrophages, playing a key role. Since DPN often presents as a distal systemic polyneuropathy and incidence of DPN increases with age, we sought to characterize age related changes in the plantar skin immune cell populations of diabetic $Lepr^{db/db}$ mice as compared to WT littermates using single cell RNA sequencing (scRNAseq). Immune cells were isolated from the plantar skin of $Lepr^{db/db}$ and WT mice at 12 weeks (young) and 21 weeks (old) of age by digesting the footpads of each mouse in RPMI/1% Pen/Strep/20 mM HEPES containing 5mg/ml of Collagenase IV for 1.5hr at 37°C. The tissues were then gently dissociated on a 70µm filter to obtain a single cell suspension, which were stained with anti-CD45 for cell sorting with a FACS Aria flow cytometer. These purified $CD45^{+}$ cell samples were then processed using a NovaSeq6000 S2 Genomic Sequencer. ScRNAseq results showed several cell populations which varied in number based on age and disease; however, the most striking differences were seen in the 21 week old $Lepr^{db/db}$ mice. Cells which showed at least a 2-fold difference in this group compared to the other groups included: Conventional type 2 dendritic cells (cDC2), mast cells and basophils (M/B), dermal gamma delta T cells (dgdt), type 2 innate lymphoid cells (ILC2), conventional type 1 dendritic cells (cDC1), monocyte derived macrophages (MdMacro), and neutrophils (Neut). Additional analysis of pathway specific gene markers revealed that several immune functions were affected in 21 week old $Lepr^{db/db}$ mice. These included down regulation of innate

inflammatory responses, down regulation of IFNγ responses, and negative regulation of apoptosis, which would suggest a general down regulation of the immune responses of the hind foot without program cell death. Based on these results we conclude that dysregulation of the immune system occurs in the plantar skin of the hind foot. These immune cells are part of a microenvironment surrounding the nerves of the hypodermis that project into the epidermis. Thus we believe that it is reasonable to assume that dysregulation of this microenvironment in older mice could reduce the number of intraepidermal nerve fibers present within the epidermis of the hind foot. Future studies from our group will focus on immunofluorescent staining to confirm the results of the present study.

Disclosures: J.M. Nichols: None. **R. Mahalingam:** None. **H. Pham:** None. **A. Shepherd:** None.

Poster

291. Cancer Pain, Chemotherapy, Neuropathy, and Diabetic Neuropathy Pain

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 291.15

Topic: D.02. Somatosensation – Pain

Title: Small molecule hepatocyte growth factor (HGF)/MET positive modulators effectively reduce pain-related behaviors in a rat model of diabetic neuropathy

Authors: *A.-A. BERTHIAUME, K. KLEIST, R. TAYLOR, J. JOHNSTON, K. CHURCH; Athira Pharma, Bothell, WA

Abstract: INTRODUCTION. Hepatocyte growth factor (HGF) signaling through the MET receptor promotes neuroprotective, neurotrophic, and anti-inflammatory cascades. As neuropathic pain disorders, including diabetic neuropathy, have components of oxidative stress, nerve damage, and inflammation, positive modulation of the HGF/MET pathway may provide therapeutic benefit in these disease areas. We have developed a series of small molecule positive modulators (PMs) of HGF/MET to harness this system's therapeutic capacity. Here, we investigate the in vivo efficacy of several HGF/MET PMs for treating neuropathic pain in a rat model of streptozotocin (STZ)-induced diabetic neuropathy.
METHODS. In independent studies, diabetes was precipitated in rats with a single dose of STZ (55 mg/kg; IV). Neuropathic pain-related behaviors were established by day 14 post-STZ. HGF/MET PMs were administered once daily from day 15 to 28. Mechanical allodynia was assessed using von Frey filaments (1.4, 4, 10, 15, 26, 48, 60 g) one-hour post-dose on study days 21 and 28. In addition, on day 25, mechanical allodynia was evaluated prior to that day's dose, at which point the experimental compounds had been fully cleared from circulation.
RESULTS. The significant increase in mechanical allodynia observed in STZ-treated animals was effectively reversed by HGF/MET PMs from the first post-treatment timepoint evaluated (after 7 days of treatment; day 21 of the study), and this reversal persisted through the last timepoint evaluated (after 14 days of treatment; day 28 of the study). These behavioral readouts

were obtained while test compounds were in circulation and could represent acute and/or chronic effects of the HGF/MET PMs. Pain-related behaviors were also examined one-hour pre-dose on study day 25 (after 9 days of treatment). Because the half-life of these HGF/MET PMs is less than two hours, the compounds are not in circulation 23-hours post-dose. Interrogation of pain-related behaviors at this pre-dosing timepoint revealed a persistent reduction in pain by the test compounds, even when they are fully cleared from the plasma.
CONCLUSIONS. Overall, the HGF/MET PMs were independently found to significantly reduce pain-related behaviors at multiple doses in a rat model of STZ-induced diabetic neuropathy. These small molecule experimental compounds may represent viable therapeutic options for the treatment of neuropathic pain disorders.

Disclosures: **A. Berthiaume:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **K. Kleist:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **R. Taylor:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **J. Johnston:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma. **K. Church:** A. Employment/Salary (full or part-time):; Athira Pharma. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Athira Pharma.

Poster

292. Somatosensation in the Barrel Cortex

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 292.01

Topic: D.03. Somatosensation – Touch

Support: Deutsche Forschungsgemeinschaft (GZ: WA 3862/1-1)
NIH Brain R01NS104928
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Title: Emerging experience-dependent dynamics in primary somatosensory cortex reflect behavioral adaptation

Authors: *C. WAIBLINGER[1], E. DIMWAMWA[1], P. Y. BORDEN[2], M. E. MCDONNELL[3], G. B. STANLEY[1], A. R. REEDY[4];
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Pittsburgh, PA; [4]Integrated Cell. Imaging Core, Emory Univ. Sch. of Medicine, Emory Univ., Atlanta, GA

Abstract: The primary sensory somatosensory cortex (S1) is classically described as the origin of neuronal signals in response to tactile stimuli. In a recent study we have investigated the perceptual capabilities of S1 during learning and behavioral adaptation, using the mouse vibrissa system. We performed long-term chronic wide-field imaging of superficial layers (L2/3) in S1 with a genetically encoded voltage indicator (GEVI) in mice exhibiting context-dependent changes in detection behavior.We observed that the S1 region not only relays ascending sensory signals but also plays a secondary role in mediating long-term adaptive context-dependent behaviors. Chronic inactivation (ibotenic acid lesions) of the superficial somatosensory cortex layers abolished long-term context-dependent behavior suggesting this brain region is necessary for this adaptive process.Surprisingly, neuronal activity in S1 shifted from simply representing stimulus properties to transducing signals necessary for long-term adaptive behavior in an experience dependent manner. This finding suggests a signal transfer of these context-dependent changes between different brain structures along the sensory-motor arc, where S1 is transducing signals necessary for adaptive strategies in a dynamically changing environment. Moreover, we propose that this shift in cognitive signaling could even affect subcortical structures such as the primary thalamus, which we have recently shown to exhibit choice related signatures in highly trained animals. To understand the mechanism of this signal transfer, we are currently exploring the manipulation of corticothalamic neurons in mice trained on a tactile detection task. Specifically, we are optogenetically activating neurons in layer 6 of S1, which are cortical gain modulators that project both intracortically across layers as well as to the thalamus (L6ct). Optogenetic manipulations of these cells have been shown to change the signal-to-noise relationship suggesting enhanced detectability; however, the effect on behavior has yet to be explored. We hypothesize that in a dynamically challenging sensory environment this highly conserved circuit could be the node through which signals from several brain areas interact making it critical for an animals' survival strategies.

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Poster

292. Somatosensation in the Barrel Cortex

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 292.02

Topic: D.03. Somatosensation – Touch

Support: R01 NS117597
R01 HD054453

Title: The remarkably dynamic identity of adapting and facilitating neurons in mouse somatosensory cortex

Authors: *Z. DOBLER[1,2], S. MULA[2], C. PORTERA-CAILLIAU[2,3];
[1]Univ. of California, Los Angeles Interdepartmental Ph.D. Program in Neurosci., Los Angeles, CA; [2]Dept. of Neurol., [3]Dept. of Neurobio., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: To construct a stable and coherent experience of the external world, sensory circuits must adapt their activity to the statistics of the surrounding environment and filter out irrelevant stimuli. This is achieved in part via stimulus-evoked sensory adaption (SA), characterized by a progressive decrease in neuronal activity in response to repetitive sensory stimulation. While SA has been extensively studied at the level of individual neurons on timescales of tens of milliseconds to a few seconds, little is known about SA over longer timescales or at the population level. Here, we investigate population-level SA in the barrel field of the mouse somatosensory cortex (S1BF), which processes whisker inputs, using in vivo 2-photon calcium imaging (GCaMP6s) of layer 2/3 excitatory neurons in awake adult Slc17-Cre x Ai162 mice. The activity profiles of stimulus-responsive (SR) neurons varied widely across a population: in addition to previously described adapting neurons that decreased their firing with repetitive stimulation, we also found facilitating neurons which increased their activity, and still others that were neither adapting nor facilitating. Within each of these populations, individual responses to different whisker deflections were strikingly heterogeneous and stochastic. We also discovered that adaptation to one stimulus does not always generalize to different stimuli. Indeed, when we exposed mice to 10 whisker stimuli at one frequency followed by a second bout at an alternate frequency, we found that adapting neurons (but not facilitating neurons) exhibited significantly increased response peak amplitudes after switching to a higher frequency. Finally, we investigated the stability of population SA dynamics by recording the same neurons during bouts of 20 repetitive whisker stimulations across 8-9 days. Remarkably, most SR neurons did not maintain the same dynamics across days. Not only were the proportions of adapting or facilitating neurons dynamic over days, but the activity profile of individual neurons (adapting vs. facilitating) could change drastically from one day to the next. These results indicate that 1) Population-level SA is encoded heterogeneously in S1BF and does not universally generalize; 2) Adapting neurons are most sensitive to shifts in stimulus parameters; and 3) Exposure to the same repetitive stimulus over days shifts the balance between adaptation and facilitation at the population level.

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Poster

292. Somatosensation in the Barrel Cortex

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 292.03

Topic: D.03. Somatosensation – Touch

Support: NIH Grant R37 NS092367
NIH Grant F32 NS114327

Title: Neural correlates of history-based selective attention in mouse primary somatosensory cortex

Authors: *D. L. RAMAMURTHY, A. CHEN, D. E. FELDMAN;
Helen Wills Neurosci. Inst. and MCB Dept., UC Berkeley, Berkeley, CA

Abstract: Attentional selection prioritizes processing of behaviorally relevant stimuli to guide ongoing behavior, but the neural mechanisms of attention are incompletely understood. Mice provide powerful tools for discovery of the neural circuit mechanisms for attention. We recently developed an efficient behavioral paradigm that revealed spatially and temporally specific shifts of attention between specific whiskers on the mouse's whisker pad. Mice were trained to detect random single-whisker deflections (Go trials) interspersed with no-deflection (NoGo) trials. Mice used the recent trial history of stimulus-reward association guide selective tactile attention to recently rewarded whiskers, improving detection (d-prime) of those whisker stimuli on upcoming trials. This effect was spatially specific and occurred on a rapid trial-by-trial timescale, consistent with hallmark effects of attentional selection (in 10/11 mice tested). History-based effects lasted for ~10 s, and flexibly shifted from one whisker to another based on recent trial history. These effects exhibited a distinct somatotopic gradient, suggesting a substrate of modulation in primary somatosensory cortex (S1) or other early sensory areas. Here, we tested for neural correlates of attention in L2/3 PYR cells in S1, using 2-photon calcium imaging in Drd3-Cre; Ai162D (TIGRE 2.0-GCaMP6s) mice during the attention behavior (3 mice, 36 fields, 4374 PYR cells). We observed robust history-based cueing of PYR cell activity consistent with whisker-specific selective attention. Prior hits to one whisker boosted PYR cell dF/F responses to subsequent deflection of that same whisker, but not to other whiskers. This boosting only occurred after hit trials, not miss trials to the same whisker (i.e. it required prior conjunction of sensory stimuli plus reward), which was also true for the behavioral attentional effect. When prior hits to a whisker were followed by a NoGo trial, PYR cells showed history-dependent ramping up of baseline activity during the NoGo trial, likely representing an anticipatory signal to boost representation of the attended whisker. These neural correlates of attention were somatotopically structured, being greatest for the prior Go whisker and immediately adjacent whiskers in the same row, and weaker for more distant whiskers. Thus, history-based cueing boosts L2/3 PYR cell activity in a somatotopically precise manner in S1. These behavioral and imaging results demonstrate neural correlates of spatially selective tactile attention in mouse S1, and provide a new experimental paradigm to interrogate cell type-specific mechanisms underlying attentional control.

Disclosures: D.L. Ramamurthy: None. **A. Chen:** None. **D.E. Feldman:** None.

Poster

292. Somatosensation in the Barrel Cortex

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 292.04

Topic: D.03. Somatosensation – Touch

Support: DFG Grant
ERC
HBP

Title: What moves when mice move a single whisker to touch?Individuality and stereotypy in behavior

Authors: M. STAAB[1], K. SEHARA[1], N. LAURINE BAHR[2], S. DOMINIAK[4], M. E. LARKUM[5], *R. N. SACHDEV[3];
[1]Humboldt, Berlin, Germany; [3]Humboldt Univ., [2]Humboldt Univ., Berlin, Germany; [4]Humboldt-Universität Zu Berlin - AG Larkum, Humboldt-Universität Zu Berlin - AG Larkum, Berlin, Germany; [5]Humboldt Univ. of Berlin, Berlin, Germany

Abstract: A key function of the brain is to move the body through a rich, complex environment. In course of navigating the world, multiple sensory-motor components are linked into a coherent whole. When rodents engage their environment, they move their whiskers, as they extract tactile information. Even though the study of whisking has a long history, the details of what mice move, when they move and whether individual mouse behavior is stereotyped has still not been fully examined. Here we trained head fixed mice in a simple go-cue task to move a whisker -- the C2 whisker one side of the face to touch a sensor -- and tracked movement of whiskers and nose, and forces on the head post. Our analysis revealed that mice move adjacent whiskers in a coordinated fashion and that as mice move their whiskers, they move their nose and apply forces on the head post in a manner that reflects the behavioral epoch -- pretrial, cue onset, tactile trigger, licking and post-lick. The movement of whiskers explained variance in both nose movement and head post forces. Importantly, mice control the setpoint, amplitude and frequency of movement of individual whiskers bilaterally, independently. Even though mice achieved the goal of the task -to touch the sensor within 2 seconds -- how they did so, how they coordinated movement of the nose and forces on head post with movement of individual whiskers was specific for each mouse. The strategy they used was related to the distance they needed to move a whisker to contact the sensor. This work shows that mice can control details of individual whisker movement, while coordinating movement of their whiskers, face and head in a goal directed manner.

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Poster

292. Somatosensation in the Barrel Cortex

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 292.05

Topic: D.03. Somatosensation – Touch

Support: NIH/NNDS R01 Grant NS107599
Whitehall Foundation Grant 2017-05-71

Title: Sensory and motor representations and functions through learning in a selective detection task in mice

Authors: *K. ARULJOTHI[1], E. ZAGHA[1], E. KAUR[2];
[1]Psychology, Univ. of California Riverside, Riverside, CA; [2]UC Riverside, Riverside, CA

Abstract: An essential feature of goal-directed behavior is the ability to selectively attend to various stimuli during decision-making. Previous studies have observed an established attenuation of distractor sensory to motor signal propagation in distractor aligned cortices for a selective detection task in mice. Expert behavior exhibits many components for ignoring distractors, but the emergence of an attenuating filter through learning remains unknown. This study focuses on investigating the process from naïve through expert stages of impulse control for mice learning a go/no-go whisker-based selective detection task utilizing a transgenic pan-neuronal GCaMP mouse line for widefield Ca2+ imaging. One hypothesis is that in naïve mice, both target and distractor sensory signals propagate to the frontal cortex effectively and through learning, the distractor sensory to motor signal propagation is suppressed while the target signal propagation is unaffected. An alternate hypothesis would be that in naïve mice, neither target nor distractor sensory signals propagate to the frontal cortex, but through learning, target sensory signal propagates effectively to the frontal cortex while distractor sensory signal fails to propagate. This study also explores how task-relevant and task-irrelevant neural representations of movement in the cortex change through learning and what role whisker motor cortex (wMC) plays in sensory-motor processes through learning. By investigating the learning effects involved in the selection process, impairments in learning trajectories, such as in attention deficit disorders, can be further explored and better understood.

Disclosures: K. Aruljothi: None. **E. Zagha:** None. **E. Kaur:** None.

Poster

292. Somatosensation in the Barrel Cortex

Location: SDCC Halls B-H

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Program #/Poster #: 292.06

Topic: D.03. Somatosensation – Touch

Support: NIH Grant R01 NS117536
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Title: Representational drift impedes learning of a sensory cortical microstimulation task

Authors: *R. PANCHOLI, L. RYAN, S. PERON;
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Abstract: Neural activity is highly dynamic. Across cortex and hippocampus, the population of neurons representing a particular feature changes over time in a process called representational drift. Despite its ubiquity, it remains unclear whether representational drift has an impact on perception or behavior. Optical microstimulation of cortex is an effective means of studying drift as it enables precise genetic and spatial control of the stimulated population, circumvents neural processing prior to cortex, and forces animals to use the evoked activity to perform a behavioral task. We used volumetric two-photon calcium imaging to track neural activity as mice learned an optical microstimulation task. Mice were required to discriminate between a high and low number of optogenetic pulses delivered to several thousand opsin-expressing pyramidal neurons in layer 2/3 of primary vibrissal somatosensory cortex. Neural activity influenced animal choice on a trial-by-trial basis: for a given number of optogenetic pulses, trials evoking larger responses drove mice to report stronger stimulation. Mice learned the task at varying rates, though levels of evoked activity did not differ between animals that learned the task and those that did not. The photoresponsive population exhibited representational drift both within and across behavioral sessions. Both forms of drift were elevated among animals that failed to learn the task compared to those that did. Moreover, stimulus decoding from neural activity degraded more rapidly in mice that failed to learn the task. Our results show that the rate at which sensory cortical representations change constrains learning and impacts behavior.

Disclosures: R. Pancholi: None. **L. Ryan:** None. **S. Peron:** None.

Poster

292. Somatosensation in the Barrel Cortex

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Title: Gabrb3 is required for the functional integration of pyramidal neuron subtypes in the somatosensory cortex

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Abstract: Dysfunction of gamma-aminobutyric acid (GABA)-mediated inhibitory circuits is strongly associated with neurodevelopmental disorders. However, it is unclear how genetic predispositions impact circuit assembly. Using both pan-cortical and layer-specific ablation strategies, we show that *Gabrb3*, a gene strongly associated with Autism spectrum disorder (ASD) and Angelman syndrome (AS), is required for inhibitory synaptic function in contralaterally projecting pyramidal neurons of the somatosensory cortex. Utilizing in vivo two-photon and widefield calcium imaging in developing mice, we demonstrate that ablation of this gene leads to a developmental increase in local network synchrony and a long-lasting enhancement in anatomical as well as functional connectivity with contralateral—but not ipsilateral—targets. We propose that this pathway-specific effect is mediated by a selective requirement of *Gabrb3* for inhibitory synaptic function in contralateral-projecting pyramidal neurons. Our studies reveal a circuit-specific requirement for *Gabrb3* during the emergence of interhemispheric connectivity.

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Poster

292. Somatosensation in the Barrel Cortex

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 292.08

Topic: D.03. Somatosensation – Touch

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Title: Altered sensory representation in whisker primary somatosensory cortex of Cntnap2-null mice

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Abstract: Atypical sensory experience is a common feature of autism spectrum disorders (ASDs) and was recently recognized as a core diagnostic criterion. Prior studies have suggested that aberrant sensory processing may arise in the primary sensory cortex. In this study, we tested for abnormal tactile receptive fields and topographic map structure in whisker somatosensory cortex (wS1) of one mouse model of autism, the Cntnap2-null mouse. Cntnap2-null mice and wild-type littermates (n=4 each) were trained on a head-fixed whisker discrimination task in

which calibrated deflections were independently applied to 9 whiskers in a 3 x 3 array. Mice were trained to lick to all-whisker stimuli but to suppress licking to single-whisker stimuli, presented with or without background whisker deflection noise. Cntnap2-null and wildtype mice exhibited comparable behavior performance on the task. GCaMP8m was virally expressed in L2/3 pyramidal (PYR) neurons, and 2-photon Ca^{2+} imaging was used to measure whisker-evoked responses and receptive fields in wS1 from single-whisker stimuli during task performance. Under the no-noise condition, PYR cells in Cntnap2-null mice showed weaker whisker responses but normally sharp somatotopic tuning compared to controls. Background noise led to a reduction of response magnitude and broadening of tuning in controls, but not in Cntnap2-null mice. Sensory responsiveness was not elevated in the Cntnap2-null mice. Both Cntnap2-null mice and controls showed salt-and-pepper whisker map topography in L2/3, in which PYR neurons tuned for different whiskers were spatially intermixed, overlaid on an average somatotopic map aligned to L4 barrels. Whisker maps in Cntnap2-null mice showed more scatter than controls, with a lower proportion of PYR neurons tuned for the anatomical columnar whisker. Our data support the hypothesis that sensory representation in the primary sensory cortex is atypical and may contribute to sensory phenotypes in autism.

Disclosures: H. Wang: None. **D.E. Feldman:** None.

Poster

292. Somatosensation in the Barrel Cortex

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Title: Endocannabinoid-dependent formation of columnar axonal projection in the mouse cerebral cortex

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Abstract: Columnar structure is one of the most fundamental morphological features of the cerebral cortex and is thought to be the basis of information processing in higher animals. Yet, how such a topographically precise structure is formed is largely unknown. Formation of columnar projection of layer 4 (L4) axons is preceded by thalamocortical formation, in which type 1 cannabinoid receptors (CB1R) play an important role in shaping barrel-specific targeted projection by operating spike timing-dependent plasticity (STDP) during development (Itami, et al, 2016, Kimura et al. 2019). Right after the formation of thalamocortical projections, CB1Rs start to function at L4 axon terminals (Itami et al., 2012), which coincides with the timing of columnar shaping of L4 axons. Here we show that the endocannabinoid 2-arachidonoylglycerol (2-AG) plays a crucial role in columnar shaping. We found that L4 axon projections were less organized until P12, and then became columnar after CB1Rs became functional. By contrast, the columnar organization of L4 axons was collapsed in mice genetically lacking diacylglycerol lipase α (DGLα), the major enzyme for 2-AG synthesis. Intraperitoneally administered CB1R agonists shortened axon length, whereas knockout of CB1R in L4 neurons impaired columnar projection of their axons. Our results suggest that endocannabinoid signaling is crucial for shaping columnar axonal projection in the cerebral cortex.

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Poster

292. Somatosensation in the Barrel Cortex

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Title: Mapping the cellular and sub-cellular circuit motifs underlying sensory-driven traveling waves from the cortical surface

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Abstract: Traveling waves in mammalian cortex mediate vital aspects of animal cognition, such as stimuli perception and working memory. Theoretical results suggest that these low-frequency oscillations preserve timing across long-range neural circuits; thus, revealing the circuit mechanisms for traveling waves is critical to our understanding for how distributed computations maintain millisecond precision. However, key aspects of the microcircuit dynamics underlying and supporting traveling waves remain unknown. Macroscopic ECoG and Utah Arrays are conventional tools for mapping the propagation of cortical local field potentials (LFP); however, these technologies limit concurrent recordings with powerful modalities such as two-photon imaging and silicon probes. In this work, we developed a customizable, microfabricated, thin-film surface array for multi-modal interrogation of the circuits driving traveling waves near the cortical surface. Specifically, with high-density mapping in single cortical barrels in vS1 we show variety of gamma-, beta-, and theta-band traveling wave activity in mouse barrel cortex following passive whisker stimulation in awake animals. Gamma and beta-band waves dominate the first 50 ms activity following whisker touch, followed by a delayed beta-theta coupled wave approximately 100 ms after sensory stimulation. Using our surface arrays in conjunction with simultaneous silicon-probe electrophysiology, pharmacology, and two-photon imaging, we show that this delayed traveling wave originates from sub-cellular signaling, with a prominent sink-source in L2/3 to L4. Silencing vM1 feedback and local application of baclofen in vS1 abolished only the delayed travelling wave. Together, we surmise that the delayed travelling wave reflects calcium spikes from Layer 5 (L5) apical dendrites. These results not only demonstrate a translaminar contribution to travelling wave initiation, but the possibility to leverage our highly scalable microfabricated arrays to map dendritic dynamics from the cortical surface. Importantly, we also discuss the behavioral implications of this delayed dendritic wave, specifically in an active-touch paradigm and during reinforcement learning.

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Poster

292. Somatosensation in the Barrel Cortex

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Program #/Poster #: 292.11

Topic: D.03. Somatosensation – Touch

Support: NIH NS117536

Title: Columnar-scale barrel cortex lesions degrade tactile discrimination but not detection

Authors: *L. RYAN[1], M. LAUGHTON[1], A. SUN-YAN[1], R. PANCHOLI[1], S. PERON[2]; [1]New York Univ., New York, NY; [2]New York Univ. Ctr. For Neural Sci., New York, NY

Abstract: Primary sensory cortices typically display functional topography, suggesting that even small cortical volumes may underpin the perception of specific stimuli. However, because traditional loss-of-function approaches have poor spatial resolution (&gt 1 mm radius of effect),

the behavioral necessity of smaller cortical volumes remains unclear. Rodent primary vibrissal somatosensory cortex (vS1) is topographically organized into patches of cortex known as 'barrels' that each predominantly receive input from a specific whisker. Perception during single whisker behavior may therefore depend on individual barrels. Here, we train animals implanted with a cranial window over vS1 to perform several single-whisker perceptual tasks. We then use high-power laser exposure centered on the barrel representing the spared whisker to produce lesions with an average volume of ~2 barrels (0.17 ± 0.09 mm^3, mean ± SEM, n = 7 mice; volume of a single barrel: 0.09 mm^3) and assess the impact on behavior for several days following the lesion. Due to previously implanted cranial windows, lesions can be performed in awake animals immediately prior to behavior. Columnar-scale lesions impair performance in a go/no-go discrimination task where mice must determine if an object is close to or far from its face. Lesioned animals with degraded discrimination performance can immediately perform a go/no-go detection task, in which they must simply report the presence or absence of touch, with high accuracy. Sham lesions of visual cortex produced no behavioral effect. Animals trained *de novo* on the go/no-go detection task as well as on a more complex two-lickport detection task with delayed response show no behavioral deficit following columnar-scale lesions. Thus, vS1 barrels are necessary for performing object location discrimination but not simple or complex object detection behaviors. Moreover, small (~10,000) populations of neurons in primary sensory cortex can contribute to the perception of specific stimuli.

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Poster

292. Somatosensation in the Barrel Cortex

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Topic: D.03. Somatosensation – Touch

Support: NIH-UL1TR001998
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Title: Effects of isoflurane on vascular dynamic during whisker stimulation

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Abstract: Isoflurane is a widely used anesthesia during functional studies in central nervous system. Different experimental conditions differentially affect neuronal and vascular function and results in inconsistent findings from lab to lab. Here, we characterized the effect of isoflurane on vascular response in time-dependent series of different consciousness stages. Cranial window surgery was prepared in both 3-4 months old male and female mice. The

animals were housed in a reversed light cycle and our study was conducted during the active phase. To investigate neurovascular function, the animals were anesthetized with isoflurane and mounted under multiphoton microscopy. We performed simultaneous air-puff stimulation of contralateral whiskers and recording of vascular dynamic response at pre-, during, and post-stimulation in the barrel cortex at each consciousness stage starting from fully unconscious condition where isoflurane levels were maintained at 1.5%. Then, the same measurements were done when levels of isoflurane were at 0.3% for partial unconscious and 0% for fully awake condition. Increased basal penetrating arteriole diameter, 1.55 (± 0.2) and 1.47 (± 0.17) folds were observed at both at 1.5% and 0.3% condition respectively, compared to fully awake condition. During whisker stimulation, the magnitude of vascular diameter was not changed in the unconscious condition. Interestingly, whisker stimulation induced vasoconstriction in partial unconscious stage where vascular diameter was reduced 11.2 (± 2.9) percent compared to its baseline. On the other hand, the magnitude of vasodilation was increased 23.4 (± 8.9) percent in fully awake condition during whisker stimulation-induced neurovascular response. Our results indicate that isoflurane plays roles in neurovascular coupling and suggest that fully awake active phase is the optimal condition for neurovascular experimental studies.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.01

Topic: D.04. The Chemical Senses

Support: Lundbeck Foundation (DNKS Scholarship 2020))
Helga og Peter Kornings Fond

Title: Neuroanatomy of the olfactory bulb and rhinencephalon in the Göttingen minipig

Authors: *B. SØGAARD[1], J. B. STEINMÜLLER[1,2], D. ORLOWSKI[3,1], T. OVESEN[4], J. H. SORENSEN[1], A. N. GLUD[1];
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Abstract: Olfaction is affected in diseases such as Alzheimer's disease, depression, and Parkinson's disease. It is thus relevant to assess the olfactory system in translational animal models of these diseases. The Göttingen minipig is an established non-primate, large animal model that is increasingly used in neuroscience. Despite olfaction being the dominant sensory modality of the minipig, the neuroanatomy of its olfactory system is currently not well described. Therefore, we aimed to investigate the olfactory bulb (OB), the olfactory cortex (OC) and their

connectivity.We used 5 female minipigs (age 11-15 months, mean weight 30.68 kg), which were injected in the OBs unilaterally (n=2) or bilaterally (n=3) with the fluorescent retrograde tracer, hydroxystilbamidine “FluoroGold” (FG), and the anterograde tracer biotinylated dextran-amine (BDA) (10kDa). After 4 weeks the minipigs were euthanized, and the brains were removed, slabbed, and cryosectioned into 40 µm thick coronal sections. After section mounting on slides, BDA was visualized using avidin-biotin-peroxidase complex solution followed by immersion in DAB-solution. We counterstained with toluidine blue. The FG-sections were mounted directly and counterstained them with DAPI to provide orientation during fluorescence microscopy.The Nissl-stained sections revealed a highly organized laminar structure in the OB. The outer-most layer consisted of the olfactory nerve fibers. Four major layers of the main OB then followed: Superficially the characteristic glomerular layer was found, followed by the external plexiform layer (EPL). Also visible was the mitral cell layer with large multipolar neurons, superficial to the underlying granular cell layer (GCL) which is the largest layer. In the center of the bulb, a large olfactory ventricle (OV) was found patent throughout the bulb and was lined with ependyma. A large white matter tract surrounded the OV. In the dorso-medial part of the caudal main OB, the dorsal mitral cell layer and nerve fiber layer split and were interposed by the accessory bulb with features similar to the main OB. Retrograde tracing revealed FG-labelled neurons and axons in most of the OB. We found that FG tracing was especially present in the rostral OB in all layers, however it decreased throughout the OB to only surround the OV.In conclusion, we found that the minipig OB and rhinencephalon resemble similar macrosmatic species, however, projections beyond the primary OC remain unknown and necessitates further research.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

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Kilian J. and Caroline F. Schmitt Foundation

Title: Cortical feedback regulation of adult-neurogenesis in olfactory bulb could contribute to representational flexibility of odors in olfactory piriform cortex

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Abstract: The main olfactory bulb (MOB) in rodents is one of two regions in the brain where substantial numbers of cells are continuously born and integrated throughout life (Lledo, Alonso, and Grubb, 2006). New cells are first generated in the subventricular zone, migrate through the rostral migratory stream and over 95% of them differentiate into granule cells (GCs) in the bulb. Nearly every stage of the process of adult-neurogenesis, including the rate of cell birth, the migration, the integration, and the survival of newborn cells is regulated by neural activity. One major source of this regulation is top-down feedback from piriform cortex (PCx), which contributes to the integration of adult-born GCs at the level of spine density (Wu et al., 2020). It is however unknown what role this top-down feedback regulation of adult-neurogenesis plays on the computations performed by MOB and PCx. We hypothesize that a critical role for top-down feedback is to stabilize recent experiences by altering the integration of adult-born GCs. To test this hypothesis, we built a realistic spiking neural network model which recapitulated the circuit architecture within and between MOB and PCx. We modeled adult neurogenesis by replacing a certain number of GCs and associated network connectivity on each model day and simulated the responses of piriform cell populations to an array of model odors. We found that while the same odors activated the same subset of glomeruli, the responses of piriform cells progressively changed across model days. To quantify this progressive change, we defined the cortical representation of an odor as the response magnitude of all piriform cells in the network to a given odor, and studied how these odor representations varied over time. We found odor representations varied as more GCs were being replaced in the network. Recent experimental study (Schoonover et al., 2021) has found this ongoing change in PCx and described it as representational drift. Our results point to a mechanism, adult-neurogenesis, that may underlie this representational flexibility in PCx. Furthermore, we suggest that a critical role for top-down feedback, is not only to alter odor responses on short time scales associated with odor discrimination (Chen and Padmanabhan, 2022), but also to codify recent olfactory experiences on longer time scales via adult-neurogenesis. This form of plasticity would serve to encode the saliency or valence of an odor in PCx by controlling adult-neurogenesis of GCs.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: NIH-NIDCD Grant DC016307

Title: Consequences of chronic olfactory inflammation on olfactory bulb projection neurons

Authors: *B. LAFEVER[1], F. IMAMURA[2];
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Abstract: Chronic olfactory inflammation in conditions such as chronic rhinosinusitis significantly impairs the functional and anatomical components of the olfactory system. Although the clinical findings of olfactory sensory neuron degeneration and olfactory bulb (OB) shrinkage are observed in many chronic rhinosinusitis patients, the consequences of olfactory inflammation on OB neurons are largely unknown. Chronic olfactory inflammation induced by intranasal administration of lipopolysaccharide (LPS) in rodents results in atrophy, gliosis, and pro-inflammatory cytokine production in the OB. In this study, we investigated the pathological effects of chronic olfactory inflammation on the OB projection neurons, mitral and tufted cells. To induce chronic olfactory inflammation, we performed unilateral intranasal administrations of LPS to mice for up to 10 weeks. Effects of chronic olfactory inflammation on the OB were examined using RNA-sequencing approaches and immunohistochemical analyses. We discovered that repeated LPS administration upregulated immune-related biological pathways in the OB after 4 weeks. We also determined that the length of tufted cell lateral dendrites in the OB was significantly reduced after 10 weeks of chronic olfactory inflammation. Similarly, the axon initial segments of tufted cells decreased in number and in length after 10 weeks of chronic olfactory inflammation. The lateral dendrites and axon initial segments of mitral cells, however, were largely unaffected. In addition, dendritic arborization and axon initial segment reconstruction took place among tufted cells following a 10-week recovery period. Our findings suggest that chronic olfactory inflammation specifically impacts tufted cells and their integrated circuitry, whereas mitral cells may be protected from this condition as they do not appear to be impaired. This data reveals a dichotomy in the vulnerability to inflammation for superficial versus deep OB neural circuits and further demonstrates unique characteristics in the ability of the OB to undergo neuroplastic changes in response to inflammatory stress.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: NIH/NIDCD Grant 3R01DC016222-05S1

Title: A novel class of olfactory projection neurons promotes social learning

Authors: *S. SHARP[1], T. TAN[3], E. M. ROBINSON[2], K. DRUMMEY[4], S. R. DATTA[1]; [1]Harvard Univ., [2]Harvard Med. Sch., Boston, MA; [3]Harvard Med. Sch., Harvard Grad. Program In Neurosci., Boston, MA; [4]Univ. of Washington, Univ. of Washington, Seattle, WA

Abstract: In order to minimize exploration of potentially dangerous environments, mice engage in an indirect form of learning known as the Social Transmission of Food Preferences (STFP). STFP allows mice to quickly form preferences for novel food odors upon detecting them on the

breath of conspecifics. The acquisition of STFP is mediated by a subset of olfactory sensory neurons that express guanylate cyclase D (GCD) and project to a “necklace” of interconnected glomeruli in the posterior olfactory bulb. Retention and expression of STFP are thought to involve the hippocampus, prefrontal and piriform cortices, and nucleus accumbens; however, it is not clear how initial glomerular representations of social odors are able to effect change in these higher-order centers of learning and memory. Here, we describe a novel class of projection neurons (GCD-PNs) that interface with social odor representations in the olfactory bulb. GCD-PNs are morphologically and molecularly distinct; we use dye electroporation and viral tracing to characterize unique inputs and outputs associated with these neurons. The discovery and characterization of GCD-PNs bridges a conceptual gap between primary olfaction and long-term memory formation, and suggests that certain olfaction systems are granted privileged access to downstream cognitive structures.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: NIH/NIDCD Grant 3R01DC016222-05S1

Title: Ms4a olfactory receptors mediate social learning and influence glomerular morphology

Authors: *E. M. ROBINSON, S. SHARP, S. R. DATTA;
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Abstract: The common mouse, *Mus musculus*, is not born knowing which food odors are safe, and instead must learn through experience. The Social Transmission of Food Preferences (STFP) is a form of single-shot learning that enables mice to trust novel food odors after detecting them on the breath of conspecifics. This form of learning is mediated by a subset of olfactory sensory neurons (OSNs) located deep within the folds of the nasal epithelium; these OSNs express guanylate cyclase D (GCD) and project to a series of “necklace” glomeruli in the posterior olfactory bulb. Our lab recently discovered that a novel class of olfactory receptors, the four-pass transmembrane MS4A proteins, are expressed in GCD OSNs. These MS4As were found to respond to a variety of biologically relevant molecules; however, it is not yet known whether these molecules are capable of driving STFP when paired with neutral food odors. Furthermore, it is not understood whether MS4A receptors contribute to the morphology of necklace glomeruli. In this project we aimed to determine whether MS4A ligands are capable of promoting STFP and whether the absence of MS4A receptors alters the morphology of necklace glomeruli. We found that several MS4A ligands induced a preference for novel food odors

compared to water and control odors. MS4A mutants were found to have altered glomerular morphologies compared to control animals.

Disclosures: E.M. Robinson: None. **S. Sharp:** None. **S.R. Datta:** None.

Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: BRAIN 1R01NS111673
TR01 5R01DC017876

Title: High-throughput sequencing of olfactory single neuron projections revealsnon-random spatial organization

Authors: *Y. CHEN[1], X. CHEN[2], B. BASERDEM[3], H. ZHAN[1], Y. LI[4], M. DAVIS[1], J. M. KEBSCHULL[5], C. SOITU[1], A. M. ZADOR[1], A. A. KOULAKOV[1], D. F. ALBEANU[1]; [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Stony Brook Univ., Stony Brook, NY; [4]Baylor Col. of Med., Houston, TX; [5]BME, Johns Hopkins Univ., Baltimore, MD

Abstract: In most sensory modalities, neuronal connectivity reflects behaviorally relevant stimulus features, such as spatial location, orientation, and sound frequency. In contrast, the prevailing view of the olfactory system, based on reconstructions of dozens of neurons, is that it lacks structured connectivity, as suggested by reports of broad and distributed connections both from the olfactory bulb (OB) to the piriform cortex (PC) and within the cortex. These studies have inspired computational models of circuit function that rely on random connectivity. It remains, nonetheless, unclear whether the olfactory connectivity contains spatial structure. Here, we use high throughput methods (MAPseq and BARseq) to analyze the projections of 5,309 bulb (8 mice) and 30,433 piriform cortex (5 mice) output neurons in the mouse (C57BL6J, male, 8-10-week old) at single-cell resolution. Surprisingly, statistical analysis of this much larger data set revealed that olfactory connectivity is not random. We identified previously unrecognized spatial organization in connectivity along the anterior-posterior (A-P) axis of piriform cortex at 200 μm resolution. We found that both the bulb projections to the cortex and the piriform cortex outputs form orderly gradients along the A-P axis of piriform cortex. For example, a neuron in the olfactory bulb that targets the anterior portion of the piriform cortex likely also projects to the anterior olfactory nucleus (AON); and neurons in targeted (anterior) piriform locus complete the triad by also projecting to the AON. Moreover, the same triadic organization is replicated at different positions within the piriform cortex, along its anterior-posterior axis, for other functionally distinct targets, such as the cortical amygdala and lateral entorhinal cortex. The matched connectivity, reminiscent of residual neural networks (ResNet) architecture, enables

parallel computations and further cross-referencing, since olfactory information reaches a given target region via both direct and indirect processing pathways. Our data supports the view that olfactory information leaving the olfactory bulb is segregated into parallel streams that may support different computations related to perception (OB-APC-AON), valence (OB-PPC-CoA) and action (OB-PPC-lENT), structured according to the triadic connectivity motifs. In ongoing experiments, using BARseq we are investigating the logic of projections of sister mitral and tufted cells grouped by glomeruli by relating the glomerular RNA barcodes from the dendrites of individual cells to their axonal projections across the brain.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 293.07

Topic: D.04. The Chemical Senses

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Title: Basal forebrain GABAergic projections to the olfactory bulb are rapidly recruited by odor encounters in a stimulus specific manner

Authors: *P. S. VILLAR[1], R. C. ARANEDA[1], D. F. ALBEANU[2];
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Abstract: Early sensory processing is flexibly adjusted by descending feedback projections from multiple brain regions. Among these areas, the basal forebrain (BF) contains varied populations of cells including GABAergic and cholinergic neurons that innervate several cortical regions and regulate their neural output. While the role of the BF in state dependent regulation of cortical activity, learning and attention is well established, less is known about the role of this brain region in early sensory processing. Here, we studied the modulation, by odor stimuli, of the activity of BF GABAergic inputs to the olfactory bulb (OB), the first processing stage for odor information, using multiphoton calcium imaging in awake mice.Odor presentation resulted in fast and sparse responses in BF GABAergic axons innervating the glomerular (GL) and granule cell layer (GCL) of the OB, in an odor and concentration specific manner. Across multiple odors, axonal boutons responded by either enhancement or suppression of baseline activity, while mixed-type responses were rarely observed. Odor presentations triggered significant responses to at least one out of 16 odors in ~33% of the imaged boutons in the GL (1273/3816, 5 mice) and in

~35% of the boutons in the GCL (1422/4029, 6 mice). From all responding boutons imaged in the GL, ~31% responded to odors exclusively with enhancement (393/1273 boutons), while ~65% did exclusively with suppression (827/1273 boutons). Similarly, in the GCL ~18% of the responding boutons showed only enhancement (259/1422 boutons), while ~81% showed only suppression (1157/1422 boutons). In contrast, only 4% of boutons in the GL and 0.4% in GCL exhibited both enhanced and suppressed responses, suggesting that MCPO GABAergic axons show a segregation in their response mode. Furthermore, using electrophysiology in acute brain slices, we demonstrate the existence of excitatory projections from the piriform cortex that target OB projecting GABAergic neurons of the BF. Our results indicate that upon activation of the olfactory cortex, the BF GABAergic projections to the bulb are rapidly recruited and exhibit diverse and stimulus specific responses. Interestingly, the activation dynamics of GABAergic boutons in response to odor stimuli are similar to the responses exhibited by boutons in glutamatergic feedback originating in the olfactory cortex. Thus, the OB integrates multiple fast excitatory and inhibitory descending signals that can rapidly modulate its output. These observations open new venues to investigate the functional roles of top-down GABAergic feedback in supporting olfactory perception during behavior.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Title: Projection neurone encoding of single sniff high frequency stimuli

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Abstract: Fast fluctuating odour stimuli are thought to contain information on the odour source landscape. Recent studies have suggested that this high frequency information may be accessible to the mammalian olfactory system. However, the structure and extent of this fast odour encoding is unclear. To explore this, we presented a series of 100ms temporally complex stimuli consisting of 5 x 20ms 'bins' of odour to anaesthetised mice (32 temporal patterns in total) whilst

recording from olfactory bulb (OB) projection neurons. Each stimulus was designed to fit within a single inhalation. Linear classifiers trained on cell responses were not only able to distinguish the patterns well above chance, but the confusion matrix showed a distinct structure. This structure was preserved over different odours, indicating that it arises from the temporal component of the stimuli and is not simply due to odour identity. Particularly, stimuli with the same total odour were readily distinguishable from one another, strongly suggesting that sub-sniff temporal information was robustly present in the output of the OB. To explain these results, we fit Linear-Nonlinear-Poisson models to the response of each recorded cell. We show that a weighted sum of the stimulus bins outperformed other alternative models, such as total odour or onset. The dominant weighting aligned with mouse inhalation, suggesting that differences in responses may be due to variations in airflow during the inhalation cycle. The Euclidean distances between predicted responses were found to strongly correlate with the structured pattern found in the classification analysis. Modelled cells using this dominant weighting with cell unique odour affinities and baseline firing values were found to closely replicate the confusion matrix patterns seen in the true neural data. These findings show that sub-sniff temporal information is robustly encoded into the early olfactory system, and that the phase of odour signal relative to inhalation is a driving factor in projection neuron activity and may possibly be a key mechanism in distinguishing sub-sniff temporal stimuli.

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Poster

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Program #/Poster #: 293.09

Topic: D.04. The Chemical Senses

Support: DC016133
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Title: Brief odor stimulation evokes short-term adaptation in mitral/tufted glomeruli in the mouse olfactory bulb

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Abstract: The ability to recognize and locate odors would be facilitated by neural circuits that adjust their responsiveness to changes in an organism’s sensory experience. Previous imaging studies demonstrated that repeated odor stimulation evoke a form of adaptation that is generated by processing within the mouse olfactory bulb. However, the recovery from this adaptation remains unclear. 2-photon calcium imaging was carried out in awake mice from the apical dendrites of mitral/tufted cells innervating the glomerular layer. A range of different odor-concentration pairings were used, and adaptation recovery was tested by varying the

interstimulus interval from seconds to 24 hours. Adaptation was modest in most glomeruli at lower concentrations at all interstimulus intervals. Higher odor concentration stimuli evoked heterogeneous responses across the glomerular population where some glomeruli adapted, while others exhibited stable responses. Most adapting glomeruli showed a significant, but incomplete recovery with a 30 second interstimulus interval between odor presentations, although extending the interstimulus interval beyond 3 minutes resulted in a complete recovery. Remarkably, odor-evoked response amplitudes and dynamics were also stable across imaging sessions separated by 24 hours. The results indicate that mitral/tufted glomeruli exhibit a relatively short-term form of adaptation, a function that could be useful for making rapid adjustments to the current sensory environment.

Disclosures: **N. Subramanian:** None. **D.A. Storace:** None.

Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.10

Topic: D.04. The Chemical Senses

Title: Adaptive top-down control of sensory gain by long-range cholinergic projections to the olfactory bulb

Authors: *B. YU[1,2], C. REN[3], Y. YUE[1], T. KOMIYAMA[1];
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Abstract: Long-range cholinergic projections from the basal forebrain are considered essential for modulation of gain and tuning in many sensory regions. However, the dynamics of the activity of these cholinergic projections in diverse behavioral contexts remain unclear. Here, we investigated how cholinergic projections in the olfactory bulb respond to behavioral contexts by imaging axonal activities of basal forebrain cholinergic neurons using two-photon calcium imaging. We found that cholinergic axons in the olfactory bulb show strong and phasic odor responses during learning of an olfactory discrimination task but not during passive exposure. These phasic odor responses increased during the initial days of learning and then decreased at the expert stage. The odor-evoked phasic responses were region-specific and not observed in the motor or somatosensory cortex. Furthermore, these cholinergic projections exhibited very strong odor responses during behavioral context shifts, such as the first trial of a behavioral session, the first trial after a break in a session, an introduction of novel odorant stimuli, and omission of odorant stimuli. These moments of exaggerated cholinergic activity coincided with strong odor responses by the principal mitral cells and granule cells. These findings suggest that long-range cholinergic projections coordinate the odor responses of the olfactory bulb dynamically to cope with the ongoing behavioral demand.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.11

Topic: D.04. The Chemical Senses

Support: Medical Research Council

Title: Modulation of olfactory bulb circuits by metabolic signals

Authors: ***M. ONCUL**, J. JOHNSTON;
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Abstract: Olfaction is important for the regulation of food intake and energy homeostasis and contributes to food choice and its consumption. Olfactory processing is influenced by the feeding status, satiety decreases and fasting increases olfactory acuity. Consistent with this, olfaction is modulated by changing levels of several metabolically regulated molecules such as insulin and glucose. Insulin is a key hormone for feeding, energy metabolism and cognition. The olfactory bulb has the highest density of insulin receptors in the brain. When insulin administrated exogenously, it causes physiological and molecular changes in the olfactory bulb and suppresses olfactory detection. However, it has not been fully characterized how insulin alters olfactory sensitivity. Here, we investigate how olfactory bulb circuits are regulated by metabolic signals and the role of these molecules on olfactory perception and feeding behaviour. Especially, we are investigating mechanisms underlying the role of insulin in the modulation of olfaction and identifying cellular targets of insulin in the mouse olfactory bulb. To explore these mechanisms, we are using variety of approaches including patch-clamp electrophysiology, 2-photon calcium imaging, immunohistochemistry and behavioural tests. We perform voltage clamp recordings to test the effect of insulin on the cells in the mouse olfactory bulb. We use genetically encoded Ca indicators and 2-photon imaging to test action of insulin at the circuit level and our preliminary results suggest that insulin modulates olfactory-nerve evoked responses. We perform immunostaining to show the distribution of insulin receptors in the olfactory bulb and behavioural analyses to test olfactory sensitivity depending on the feeding state. Overall, this study will help to gain insight into the role of insulin in olfactory information processing by nutritional state and how this regulates olfactory-driven behaviour in mice.

Disclosures: **M. Oncul:** None. **J. Johnston:** None.

Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

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5R01DC014487-03

Title: Axially independent optical control and readout of neuronal circuit activity using the olfactory bulb as a model

Authors: *D. ALBEANU, M. KOH, F. ANSELMI, S. SADHU, H. CHAE, P. GUPTA, D. HERNANDEZ-TREJO, P. VILLAR, A. BANERJEE;
Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: All-optical manipulating and measuring activity in the intact brain enables mapping the functional connectivity of layer-organized neural circuits at cellular resolution. To investigate the odor response properties of sister mitral and tufted cells, which share input from the same parent glomerulus, we coupled one-photon patterned optogenetic stimulation with quasi-simultaneous readout of activity using multiphoton microscopy. We used a digital micro-mirror device (DMD) to create patterned illumination profiles. Axial separation of stimulation and readout planes was implemented by conjugating a motorized translatable holographic diffuser with the desired photo-stimulation plane, thus achieving widefield axial optical sectioning (15 µm x-y, 30 µm z-resolution, across 1.5 x 1.5 mm, calibrated up to a 500 µm axial shift). We controlled the activity of single glomeruli by stimulating OSN terminals in OMP-Cre x ReaChR mice on the surface of the main olfactory bulb. In parallel, we monitored responses (GCaMP6) of the output neurons (mitral and tufted cells) across different optical planes. We sampled mitral and tufted cell responses either within the photo-stimulation plane (glomerular dendritic tufts) or at 150-250 µm (cell bodies) below the photo-stimulation plane across a range of photo-stimulation intensities. Glomerular stimulation of OSN terminals selectively evoked responses in the targeted glomerulus and in the cell bodies of sister mitral and tufted cells receiving input from the photo-stimulated glomerulus and, further, revealed lateral inhibitory interactions between glomeruli. In ongoing experiments, we analyze the response properties of the identified sister cells as a function of brain state and experience.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: NIH Grant UF1 NS111692
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Title: A distributed odor code in the olfactory bulb of awake, behaving mice

Authors: *E. HANSON MOSS[1], D. PRIHAYATI[2], A. PATEL[2], J. REIMER[1], B. R. ARENKIEL[1];
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Abstract: Spatially stereotyped olfactory bulb (OB) activity has been extensively demonstrated when odors are presented under controlled conditions. When odors are presented with controlled respiration, under anesthesia, or as simple, monomolecular, low concentration odors, odor response maps are reliable, sparse, and easily separable. However, OB responses to more complex or naturalistic odors, presented in the awake state, are denser, more variable from trial-to-trial, and more temporally complex. Thus, an important, outstanding question is how dense, largely overlapping odor representations in the OB are separated into distinct odor percepts. Towards understanding how odors are identified and discriminated from highly mixed OB activity patterns, we examine large-scale population activity in the OB in response to odors using two-photon mesoscopic imaging of bilateral OBs through cranial windows in awake mice. In Thy1-GCaMP6F mice, which express a fluorescent calcium indicator in mitral cells and their glomerular apical dendrites, we directly monitor odor responses from OB glomeruli. We then use machine learning to define linear classifiers that identify odor presentations from glomerular response patterns. A linear classifier trained on subsets of odor responses is reproducibly able to decode odor identity from withheld responses, indicating that odor responses are largely reliable and stable over days, in agreement with much previous work. However, an analysis of the value added to the classifier by individual glomeruli shows that a larger portion of glomeruli contribute to the odor representation than would be suggested by rigidly stereotyped OSN inputs alone. These results suggest that odor codes in the glomerular layer are more distributed than previously appreciated. Intriguingly, a more distributed odor code in the OB may help enable robust odor encoding in the face of trial-to-trial variability, behavioral state variability, awake-state feedback, and neuromodulation.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.14

Topic: D.04. The Chemical Senses

Title: Odour adaptation in the olfactory bulb

Authors: *M. CONWAY, J. JOHNSTON;
Sch. of Biomed. Sci., Univ. of Leeds, Leeds, United Kingdom

Abstract: Odour adaptation allows organisms to acclimate to their environment whilst remaining sensitive to changes in the odour landscape. The neural mechanisms underlying odour adaptation and whether this occurs in the olfactory bulb has not been thoroughly explored. Input to the olfactory bulb is provided by olfactory receptor neurons, which arrange themselves into functional units (glomeruli) based upon the receptor they express, and transmit sensory information to a pool of output neurons, the mitral/tufted cells. Olfactory receptor neurons receive feedback inhibition onto their axon terminals from periglomerular cells, while mitral/tufted cells are subject to feedback, feedforward, and lateral inhibition via the dendrodendritic connections they form with periglomerular cells and deeply situated granule cells. We used two-photon imaging to measure the glomerular activity of olfactory receptor neurons, mitral/tufted cells, and periglomerular cells in anaesthetised mice, in response to 3s and 60s odour stimuli across concentrations spanning five orders of magnitude. We find that both the extent and the rate of adaptation varies widely across dorsal glomeruli. Fast and slow adaptation was observed; slower adapting responses had time constants of seconds to over a minute, whereas fast adapting responses with time constants <2 s were present in a subset of glomeruli. Within the same glomerulus, the relative strength and identity of the odour influenced the rate of adaptation and the recovery from adaptation. Response kinetics in mitral/tufted cells were diverse and activity often persisted for >10 s after odour presentation. In contrast, olfactory receptor neuron terminals and periglomerular responses were more homogenous and responses generally terminated at odour offset. Topical application of the $GABA_B$-receptor antagonist CGP35348 variably increased the amplitude of odour responses in olfactory receptor neuron terminals. We can provide a comprehensive view of odour adaptation across the different circuit components of the olfactory bulb.

Disclosures: M. Conway: None. **J. Johnston:** None.

Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.15

Topic: D.04. The Chemical Senses

Title: Circuits for odor discrimination and odor generalization in the mouse piriform cortex

Authors: *F. SANTOS-VALENCIA, K. FRANKS;
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Abstract: Animals must learn to both discriminate between and generalize across different odors to survive. For example, a mouse foraging for food must learn to discriminate the odor of a

rotten fruit from a fresh fruit. At the same time, the mouse must be able to generalize across different types of fruit odors. Where and how these two processes are implemented remains unclear. The piriform cortex (PCx) is only two synapses away from the olfactory epithelium and is the first and largest region in the olfactory pathway. The PCx exhibits extensive recurrent connectivity and receives inputs from multiple olfactory and non-olfactory brain areas, making it a likely candidate to implement odor discrimination and generalization learning. We have obtained large-scale extracellular neural population recordings in head-fixed mice performing odor discrimination in a two alternative forced choice task, and we have found that layer-specific odor representations emerged in PCx after odor learning. Mice were initially trained to discriminate between two distinct odorants. After becoming proficient, mice were presented with the two odorants and mixtures of the two at varying ratios while spiking activity was recorded in PCx. We found that responses to both the pure odorants and each of the mixtures were strongly decorrelated in layer (L.) 2. In L. 3, pure odorant-evoked responses were also strongly decorrelated, however responses to the odorant mixtures clustered with the dominant mixture component, which matched the mouse's behavior. This clustering of mixture responses was preserved in passive trails (i.e., after the mice had stopped licking). Interestingly, we failed to see these stark laminar differences in L.2 and L.3 odor responses in naïve mice, suggesting both decorrelation and generalization are active processes that require learning. Our data indicate that odor discrimination and generalization are implemented in different layers in PCx, and suggest that distinct cortical subcircuits - with distinct downstream projections - differentially transform sensory input to ultimately drive appropriate behaviors. In general, our findings shed light on the role of segregated cortical circuits and provide insight into how their specific computations might be linked to behavior.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: R01DC018075

Title: Piriform cortex takes sides: nostril-dependent emergence of cortical odor identity representations

Authors: *G. DIKECLIGIL[1], A. YANG[2], N. SANGHANI[2], J. STEIN[1], S. DAS[1], I. CHEN[1], K. DAVIS[1], J. GOTTFRIED[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Univ. of Pennsylvania, philadelphia, PA

Abstract: Many sensory systems rely on bilateral sensory organs to extract crucial perceptual information such as relative depth of objects in case of vision or location of sounds in case of

audition. Similar to audition and vision, the olfactory system also has bilateral sensory inputs, arising from the two nostrils isolated from one another by the nasal septum. However, unlike visual and auditory systems where the similarities and differences in cortical representations of ipsilateral and contralateral sensory inputs are well-studied, the extent to which the primary olfactory cortex differentiates olfactory input from the two nostrils is not well understood. To answer this question we recorded intracranial EEG signals from piriform cortex (PC) in patients with medically resistant epilepsy. Subjects participated in an odor identification task while stimuli (one of 3 unique odors or odorless air) were delivered to the left, right or bilateral nostrils via a computer controlled olfactometer. After each trial, subjects were asked to identify the stimulus they received in a four-alternative forced-choice task (odor identity judgment). In a subset of trials, a follow-up question was asked on whether subjects received the stimulus from the left, right or bilateral nostrils (laterality judgment). Subjects could successfully detect and identify odors in all three nostril configurations but could not identify whether odors were delivered from the left or right nostril above chance level. Analysis of odor-evoked neural oscillations in the time-frequency domain revealed that odors evoke identity specific neural oscillations such that odor-identity can be successfully decoded from PC neural oscillations using a support vector machine classifier in 9/10 subjects. While there were no significant differences in the average decoding performance across odor laterality conditions, there were significant difference in the timing of odor-identity representations. Using time-resolved decoding, we show that odor-identity information in PC emerges first in response to bilaterally delivered odors followed by ipsilateral and lastly contralateral odors. Overall, these findings suggest that time course of odor-identity representations in PC are sensitive to which nostril the odor information arises from and proposes a potential mechanism through which the olfactory system can extract odor laterality information.

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Poster

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Title: Cross-frequency coupling in orbitofrontal cortex underlying olfactory predictive coding

Authors: *G. ZHOU[1], G. LANE[1], S. SCHUELE[1], J. ROSENOW[1], S. BANDT[1], T. KAHNT[2], C. ZELANO[1];
[1]Northwestern Univ., Chicago, IL; [2]Cell. and Neurocomputational Systems Br., Northwestern Univ., Baltimore, MD

Abstract: The orbitofrontal cortex (OFC) plays a major role in multisensory integration and predictive coding. However, it is unclear how the OFC integrates information from other brain regions to achieve its function, especially in olfaction. Low and high frequency oscillations have been suggested to support communication between brain regions and local computations, respectively. We hypothesized that the OFC achieves olfactory predictive coding through cross-frequency coupling between low and high frequency oscillations. We used intracranial EEG recordings from a cued odor-sampling task and computed the strength of low-frequency phase and high-frequency amplitude coupling in the OFC. We first examined odor-induced response in the OFC using time-frequency analysis. The analysis revealed a significant gamma response in the posterior part of the medial OFC. Then, we calculated the phase synchronization and phase-amplitude coupling between the piriform cortex and these responsive OFC electrodes. We found that the piriform cortex and OFC had a strong functional connectivity in the low frequency range, and the high-frequency amplitude in the piriform cortex is significantly modulated by the phase of low-frequency oscillations in the OFC. Future analyses will include determining the frequency and direction of oscillatory coherence between OFC and PC following cues, and decoding of cue identity across oscillatory frequencies, prior to presentation of odor. These findings suggest that the orbitofrontal cortex might encode the identity of predictive codes in the olfactory system.

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Poster

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Title: Identifying classes of potassium and calcium channels that shape the firing properties of an unusual type of interneuron in the piriform cortex

Authors: *K. P. AUNG, J. M. BEKKERS;
Australian Natl. Univ., John Curtin Sch. of Med. Res., Canberra, Australia

Abstract: Ion channels are fundamental for the functioning of neurons, and their properties and distribution have been intensively studied in many brain regions. Less is known, however, about ion channels in neurons of the piriform cortex, which is a 3-layered paleocortex responsible for processing olfactory information. Here, we focused on ion channels in an unusual type of GABAergic interneuron found in layer 1a of the piriform cortex, horizontal (HZ) cells. HZ cells

are spiny interneurons that provide feedforward inhibition to glutamatergic neurons located in deeper layers. HZ cells fire action potentials in a pattern that is shaped by the presence of an afterhyperpolarization (AHP) with two components, fast and slow. We aimed, firstly, to identify the calcium-activated potassium (K_{Ca}) channel subtypes involved in these fast and slow AHPs and, secondly, to determine the contributions of different types of voltage-gated calcium (Ca_V) channels to the firing properties of HZ cells. Whole-cell patch clamp recordings from identified HZ cells were made in acute slices prepared from the piriform cortex of P18-25 GAD67-GFP mice. Current clamp experiments (for measuring firing properties) used high-K internal solution and normal artificial cerebrospinal fluid (ACSF) in the bath, whereas voltage clamp experiments (for measuring Ca_V currents) used a Cs-based internal solution and ACSF containing TEA and 4-AP to block potassium channels and TTX to block sodium channels. Experiments were performed at 33 ± 1 ^oC and blockers were applied either by bath perfusion or puffer pipette. Application of apamin, which blocks SK_{Ca} channels, completely inhibited the slow AHP and increased the firing frequency of HZ cells ($p < 0.001$, $n = 8$ cells). In contrast, application of the BK_{Ca} channel blocker, iberiotoxin, blocked about 70% of the fast AHP and significantly increased the action potential halfwidth ($p < 0.001$, $n = 7$). In voltage clamp experiments, application of specific Ca_V channel blockers showed that four subtypes of Ca_V channels ($Ca_V2.1$ [P/Q-type], $Ca_V2.2$ [N-type], $Ca_V2.3$ [R-type] and Ca_V3 [T-type]) were present and each was responsible for a similar percentage of Ca^{2+} entry into the HZ cell. R-type channels were the sole Ca^{2+} source for BK_{Ca} channels, whereas SK_{Ca} channels were activated by a non-linear sum of Ca^{2+} influx through all four subtypes of Ca_V channels. Our results reveal the functional diversity of K_{Ca} and Ca_V channels in an important type of interneuron in the piriform cortex. These findings add to our understanding of the specific classes of ion channels that shape the electrical activity driving complex brain processing.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 293.19

Topic: D.04. The Chemical Senses

Support: R01DC014443
R01DA049545
R01DC016519
T32DC015994
F31DC020364

Title: Identification and investigation of the role of an amygdala to ventral striatum circuit in the context of odor memory formation

Authors: S. E. RYU[1], ***S. E. SNIFFEN**[2], G. SKATES[1], N. L. JOHNSON[1], E. JANKE[3], A. CHAVEZ[1], M. KOKOSKA[1], D. VALLE[1], A. M. DOSSAT[2], M. MA[3], D. W. WESSON[2]; [1]Pharmacol. and Therapeut., [2]Neuroscience, Pharmacol. & Therapeut., Univ. of Florida, Gainesville, FL; [3]Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: Odors are potent triggers of emotional responses. The amygdala is well known for its role in influencing olfactory behavior and for its necessity in establishing Pavlovian fear responses. However, the mechanisms whereby the amygdala influences odor-evoked affective responses remain elusive. Using a combination of anterograde and retrograde viral tracing strategies, we identified two distinct populations of basolateral amygdala (BLA) projection neurons, specifically in the lateral nucleus of the BLA, which innervate the tubular striatum (TuS; also known as the olfactory tubercle). These neurons express either the drd1 or drd2 genes, which encode for the dopamine D1 or D2 receptor, respectively. In D1- and D2-Cre mice, we found that both cell types synapse throughout the entire span of the TuS, but three times more drd1 neurons synapse in the TuS than drd2 neurons. Furthermore, while the BLA is comprised of both GABAergic and glutamatergic neurons that can project to regions outside of the BLA, we found through both RNAscope and immunohistochemistry that these TuS projecting BLA neurons appear to be solely glutamatergic. Since work by several groups, including ours, has established the TuS as a key region for linking odor information with learned responses, we next investigated the potential contributions of this neural circuit to olfactory memory formation. Using an odor-evoked Pavlovian fear-learning task, we monitored the respiratory and freezing behaviors of D1-Cre, D2-Cre, and nontransgenic mice within a whole-body plethysmograph. First, we used cell-type specific fiber photometry of TuS projecting BLA neurons expressing GCaMP8m and found that BLA->TuS neurons are activated during Pavlovian odor fear learning. Next, we used a chemogenetic approach to test the causal roles of BLA->TuS neurons in fear learning. We infused the DREADD ligand, J60, locally into the TuS of mice with DREADDs expressed on BLA->TuS neurons, thereby silencing their activity. Preliminary results indicate that DREADD-mediated inhibition of BLA->TuS neurons perturbs normal Pavlovian fear learning. Given the expression of D1 and D2 receptors on BLA->TuS neurons, ongoing work is also exploring the involvement of dopaminergic transmission on the function of this circuit. Overall, our preliminary results contribute to a model whereby the D1 and D2 neuron BLA->TuS circuit is fundamental for odor learning and contributes to the formation of odor valence.

Disclosures: S.E. Ryu: None. **S.E. Sniffen:** None. **G. Skates:** None. **N.L. Johnson:** None. **E. Janke:** None. **A. Chavez:** None. **M. Kokoska:** None. **D. Valle:** None. **A.M. Dossat:** None. **M. Ma:** None. **D.W. Wesson:** None.

Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

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Topic: D.04. The Chemical Senses

Support: NIH Grant F31NS122489

Title: Odor-driven neural responses recorded in the amygdala-piriform transition zone

Authors: *C. DIAZ[1], K. M. FRANKS[2];
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Abstract: Many species rely critically on information about the environment that is transmitted through the olfactory system. In the main olfactory system of mammals, odorant molecules are first detected in the olfactory epithelium by olfactory sensory neurons, each expressing a single olfactory receptor type. Olfactory sensory neurons that express the same olfactory receptor all project to a unique pair of glomeruli in the olfactory bulb (OB). Information from the OB is then projected to multiple, distinct higher-order olfactory regions, whose output drives appropriate olfactory behaviors. One such region is the amygdala-piriform transition area (AmPir). As AmPir projects directly to the central amygdala, it is anatomically situated to process emotionally valent odor information. AmPir has been shown to be activated by predator odor using immediate early gene expression; however, little else is known about how odor information is processed in AmPir. Here, we recorded odor-evoked spiking activity in populations of neurons in awake, head-fixed mice in the AmPir and, for reference, in the neighboring posterior piriform cortex (pPCx). AmPir neurons are relatively broadly tuned, and, in our preliminary data, we find no marked differences between predator odor responses and responses to other monomolecular odorants that have been shown to be aversive, appetitive, or neutral to mice. Finally, odor responses in AmPir do not appear systematically different from those in neighboring PCx. Ongoing studies will fully elucidate and characterize the odor-driven responses in AmPir.

Disclosures: C. Diaz: None. **K.M. Franks:** None.

Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

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Program #/Poster #: 293.21

Topic: D.04. The Chemical Senses

Support: NIH COBRE Grant P20GM113109
Human Frontiers Science Program Grant RGP0016/2019
Johnson Cancer Center undergraduate research award

Title: Analysis of otpa-protein versus lhx5-,isl1-, vglut2a, and lhx2a-driven GFP confirms prethalamic extended (olfactory) amygdaloid territories in zebrafish

Authors: S. WEBER[1], L. WANER[1], E. CRANWELL[1], *T. MUELLER[2];
[1]Biol., [2]Kansas State Univ., Manhattan, KS

Abstract: Zebrafish is an important genetic model system for neural mechanisms of olfaction and neurodevelopmental disorders like autism that affect the sense of smell and taste. However, the zebrafish's extended (olfactory) amygdala (EA) and olfactory pallium have remained anatomically ill-understood. To overcome this anatomical knowledge gap and define the zebrafish EA, we analyzed the distribution of otpa-protein, calretinin, and GABA in zebrafish transgenic lines *Tg(lhx5:GFP), Tg(isl1:GFP), Tg(vGlut2a:GFP),* and *Tg(lhx2a:GAP-YFP).* In the latter GFP olfactory bulb neurons axons form the lateral olfactory tract (lot) that projects into olfactory pallial territories. In zebrafish, the lot specifically innervates the posterior ("Dp") and ventral most lateral aspects of the dorsolateral zone ("Dlv"). Comparing the distribution of otpa protein with those of GFP in *Tg(lhx5:GFP), Tg(isl1:GFP)* and *Tg(vglut2a:GFP)* we redefine the medial amygdala proper and confirm the prethalamic origin of the territory mislabelled as the "intermediate subpallial nucleus" ("Vi"). Also, the analysis of *Tg(lhx2a:GFP)* let us redefine territories often labeled as "Dp" and "Dlv." The differential expression of *lhx5*-driven GFP confirms that these territories correspond to the nucleus of the lateral olfactory tract (nLOT), whereas one previously overlooked territory should be considered homologous to the lateral pallium (mammalian entorhinal cortex). Altogether, our results define the extended amygdala in zebrafish thereby drastically facilitating its usability for spectrum autism disorders known to affect the sense of smell and social behavior.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.22

Topic: D.04. The Chemical Senses

Support: Hellman Fellowship
NDSEG Fellowship
NIH Grant R00DC014516-05
NIH Grant R01DC018313-01A1

Title: A bivalent, topographically distributed, and molecularly defined cortical amygdala circuit induces divergent valence behaviors

Authors: *J. R. HOWE[1], C. L. CHAN[2], M. BLANQUART[2], D. JIMENEZ[2], S. PREISSL[3], M. MILLER[3], X. HOU[3], C. M. ROOT[2];
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Abstract: Innate behaviors are observed across the animal kingdom, wherein individuals display stereotyped responses to specific evolutionarily relevant stimuli in the absence of prior learning or experience, suggesting developmental or genetic origins. Our past research has demonstrated that the cortical amygdala (plCoA) is a key brain region mediating innate motivational valence to

olfactory stimuli. However, little is known about the pathways linking these stimuli to valence, nor the genetic basis for such circuits. Here, we identify and characterize interlinked molecular organization, projection motifs, and subregions underlying the cortical amygdala's control of innate olfactory aversion and attraction. First, we found evidence suggesting divergent respective contributions of the anterior (aplCoA) and posterior cortical amygdala (pplCoA) to valence behaviors, where optogenetic stimulation of the anterior and posterior subregions induce aversive and attractive behaviors. Next, we performed single-nucleus RNA sequencing (snRNA-seq) and single nucleus ATAC-seq (snATAC-seq) separately on aplCoA and pplCoA, revealing correlated multi-omic gradients in gene expression and chromatin accessibility along the anterior-posterior plCoA axis with distinct populations of cell types across the two sub-regions. These gradients contained ensembles of glutamatergic neurons expressing either Slc17a6 or Slc17a7, which were enriched in aplCoA or pplCoA, respectively. Optogenetic stimulation of these ensembles showed that they selectively induce innate valence phenotypes, whereby the anterior Slc16a6-expressing neurons induce behavioral aversion, and posterior Slc17a7-expression neurons induce behavioral attraction. Further, we used anterograde and retrograde tracing to identify the outputs and topographical map of the output organization. The plCoA primarily projects to subcortical regions with functions mostly related to olfaction and valence processing. Interestingly, we found differentially enriched outputs for the aplCoA and pplCoA, that are topographically organized: the aplCoA primarily projects to the medial amygdala (MeA) and pplCoA projects to the nucleus accumbens (NAc). When interrogating these circuits, optogenetic stimulation of the plCoA-MeA projection induced aversion, and stimulation of the CoA-NAc projection induces attraction. Together, these results define the plCoA circuit as a hub for innate olfactory valence and characterizes the region's previously unknown molecular, and anatomical mechanisms that could allow it to assign innate valence to odors.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 293.23

Topic: D.04. The Chemical Senses

Support: NIH Grant R01AG062006-04

Title: Medial Temporal and Precuneus Activation During Verbal Odor Recognition Memory is Associated with False Alarm Errors

Authors: *C. FRANK[1], A. ALBERTAZZI[2], C. MURPHY[2,3];
[1]SDSU/UC San Diego Joint Doctoral Program in Clin. Psychology, SAN DIEGO, CA; [2]San Diego State Univ., San Diego, CA; [3]UC San Diego, San Diego, CA

Abstract: Deficits in odor recognition memory are thought to be among the earliest observed in the preclinical stages of Alzheimer's Disease (AD; Murphy, 2019) and those at genetic risk of AD may commit more false alarm errors before the onset of clinical symptoms (Gilbert & Murphy, 2004). This study sought to understand the neural correlates of false alarm errors using fMRI of elderly participants during a verbal-odor recognition memory paradigm. 27 elderly subjects completed an odor recognition memory test and functional and structural MRI at 3T. Immediately prior to MRI scanning, subjects were presented with 9 common household odors (Frank & Murphy, 2020). During fMRI acquisition, participants were presented with verbal labels corresponding to the 9 odors presented prior to scanning in addition to 9 distractor odors. Subjects indicated using a button box whether they recognized the verbal label as corresponding to an odor presented immediately prior. BOLD signal during correct recognition of an odor, failure to recognize an odor, correct identification of an odor as not presented, and incorrect recognition of an odor (false alarms) was evaluated as a function of the number of false alarm errors made during the odor recognition memory assessment in the neuropsychological battery using linear regression. Less activation in the bilateral precuneus, right parahippocampal gyrus, right fusiform gyrus, right hippocampus, right middle temporal gyrus, and bilateral superior and middle frontal gyri during false alarms while being scanned was related to false alarm rates during memory assessment ($p < .005$). Greater activity in the bilateral inferior temporal gyrus, fusiform gyrus, medial temporal pole, and perirhinal cortex during correct recognition while being scanned was associated with higher false alarm rates during memory assessment ($p < .005$). Less activation in the right inferior parietal lobule and supramarginal gyrus during correct recognition while being scanned was related to higher false alarm rates during memory assessment ($p < .005$). Given the roles the MTL and precuneus play in recognition memory and odor recognition, these results may suggest that poorer olfactory memory is related to alterations in these systems and that these systems may underlie age and preclinical AD related odor memory deficits. Future research should probe functional connectivity correlates of false alarms and how this relationship is modified by AD risk. Supported by NIH grant # R01AG062006-04 from the NIA to CM. Special thanks to Aaron Jacobson, Alan De La Cruz, Elizabeth Robinson, Jenna Lewis, and Dalia Chavarin for data collection and preprocessing efforts.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.24

Title: WITHDRAWN

Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: D.04. The Chemical Senses

Support: Boehringer Ingelheim Fonds PhD Fellowship
DIBS Research Incubator Award
Sloan Research Fellowship

Title: Mapping brain-wide responses to enteric nutritional stimuli in larval zebrafish

Authors: *M. ARINEL[1], K. M. MATOS-FERNANDEZ[1], M. D. LORING[1], E. P. DRAGE[2], E. A. NAUMANN[1];
[1]Dept. of Neurobio., Duke Univ., Durham, NC; [2]Dept. of Genet. and Mol. Biol., Univ. of North Carolina, Chapel Hill, Chapel Hill, NC

Abstract: Signals from the gut have been shown to drive changes in mood, behavior, and metabolism. This gut-brain communication is established by specialized sensory cells in the gut epithelium termed enteroendocrine cells (EECs), which encode information about nutrition and microbiota. Recent studies have shown that a subset of EECs form synaptic connections with vagal sensory neurons, sending enteric information directly to the brain through the vagus nerve. Yet, we lack basic conceptual insights into the way gut-brain circuits encode enteric sensory information to impact neural activity across the brain. The translucent zebrafish is an ideal model due to its optical accessibility across the entire gut-brain circuitry at single-cell resolution. Here, we demonstrate a method to study the effects of enteric stimuli on vagal and brain-wide activity in larval zebrafish. To map functional responses across the brain of zebrafish, we engineered an automated, computer controlled microgavage system to inject nanoliter volumes of distinct stimuli directly into the intestinal lumen of larval zebrafish while performing calcium imaging via volumetric two-photon microscopy. Using precisely timed, volume calibrated injections of chemical stimuli directly into the intestinal bulb, we show differential activation of vagal, hypothalamic, and hindbrain neurons, suggesting specific enteric sensory encoding across these brain areas. Finally, recording from zebrafish lacking EECs allow us to directly implicate these cells in driving specific enteric brain-wide neural activity.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.26

Topic: D.04. The Chemical Senses

Support: R01DC015426
T32MH067564
T32NS047987

Title: High-precision Mapping Reveals the Structure of Odor Coding in the Human Brain

Authors: *V. SAGAR[1], L. K. SHANAHAN[1], C. ZELANO[1], J. A. GOTTFRIED[2], T. KAHNT[1,3];
[1]Northwestern Univ., Chicago, IL; [2]Dept. of Neurol., Univ. of Pennsylvania, Philadelphia, PA; [3]Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Odor perception is inherently subjective. Previous work has shown that odorous molecules evoke distributed activity patterns in olfactory cortices, but how these patterns map onto individualized odor percepts has remained an open question. Addressing this question requires mapping neural responses to a large number of odors. Here we examine high-resolution functional magnetic resonance imaging responses to 160 monomolecular odors from individual subjects (18 h of fMRI hours per subject, N=3) to reveal the neural coding scheme underlying odor perception. We first show that detailed odor percepts beyond odor category are represented in piriform cortex, amygdala, and orbitofrontal cortex (OFC). This detailed neural representation of odor percepts beyond a simple odor category representation is most prominent in the OFC. We then show that computational encoding models can predict odor-evoked fMRI responses based on multidimensional perceptual spaces and that the dimensionality of the encoded spaces increases from the primary olfactory cortex to OFC. Importantly, whereas encoding of lower dimensions generalizes across subjects, higher dimensions reflect the subjective nature of odor percepts. These results indicate that detailed and idiosyncratic olfactory experiences reside in OFC in form of multi-dimensional representations of our olfactory environment.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: Grant-in-Aid for Scientific Research on Innovative Areas 18H04998 and 21H05808
JST-Mirai program JPMJMI17DC and JPMJMI19D1

Title: Spatiotemporal dynamics of odor representations in the human brain revealed by EEG decoding

Authors: *M. KATO[1], T. OKUMURA[2], Y. TSUBO[3], J. HONDA[4], M. SUGIYAMA[5], K. TOUHARA[1], M. OKAMOTO[1];
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Abstract: Humans can perceive various odors, from pleasurable floral ones to sickening ones. While involvement of multiple brain regions in such perceptual representations has been suggested, their spatiotemporal dynamics are still poorly understood. Previous studies on olfaction examined scalp-recorded olfactory evoked potentials (OERPs) and found that the late components of OERPs were modulated by odor pleasantness. However, as most of those studies used a few odors, the neural bases of different aspects of perception, such as pleasantness and quality, have been difficult to dissociate. In addition, those studies used a univariate analysis focusing on classical OERP components. Considering that multivariate pattern analyses (MVPA) often shows higher sensitivity compared to univariate analysis, neural representations of odor perception might have been overlooked. Here we studied how olfactory perception unfolds over time by using MVPA and representational similarity analysis (RSA), which infer neural representations by examining the correlations between neural and perceptual similarities. The neural similarities were quantified based on a pairwise odor decoding analysis on OERPs to 10 odors with diverse perceptual qualities, and their perceptual similarities were quantified using ratings on unipolar pleasantness and unpleasantness, and 45 semantic descriptors (e.g. flowery). The pairwise decoding accuracy reached significance at 100 ms after odor onset and reached a maximum at 350 ms, which suggests rapid emergence of neural representations of odor information. RSA revealed that neural representation underlying unipolar unpleasantness started earlier (300 ms) than that underlying unipolar pleasantness and perceived quality (500 ms) and evolved into a structure closest to the perception around 600 ms after odor onset. A source estimation showed that brain regions that represent odor information changed during the course of olfactory processing, starting with localized areas in the primary olfactory area at 100 ms, and then, by the time the sensor-level decoding accuracies were maximally correlated with perception (≥600 ms), spreading to larger areas associated with emotional, semantic, and memory processing. These findings indicate that, at the early stage of olfactory processing (<350 ms), initial odor information is coded in and around the primary olfactory area, and then, it evolves into their perceptual realizations (300 to >600 ms) through dynamic computations in widely distributed cortical regions, with different perceptual aspects having different spatiotemporal dynamics.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 293.28

Topic: D.04. The Chemical Senses

Title: Investigating the diverse response of vGlut2^{BF} neurons to odors with different valences

Authors: *P.-S. CHIN, B. ARENKEIL;
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Abstract: Basal forebrain (BF) is involved in food intake, body weight regulation, and sensory processing. Recently, we found that glutamatergic neurons in basal forebrain (vGlut2^{BF} neurons) are activated when the mice smell food odor and interact with food, and they are only activated prior to eating behavior. It seems that those neurons are able to pass food odor signal to tell the mice to eat. Interestingly, when we over-activated those vGlut2^{BF} neurons, this caused hypohagia and the mice ended up starving themselves to death. Also, we found that vGlut2^{BF} neurons are capable of responding to food odor and aversive odors (2-Methybutylamine and butyric acid). However, whether the vGlut2^{BF} neurons are involved in differentiating different odor information remains unknown. In my study, I found thatvGlut2^{BF} neurons respond to odors with different natural valences, and shows different response level and dynamics. Also, vGlut2^{BF} neurons were activated during the odor discrimination phase in a go/no-go olfactory-cued discrimination task, suggesting it may participate in processing the valence of olfactory information. Interestingly, in the task, those neurons show reward-related suppression and the suppression is independent of learning. Because of the diverse response, I am going to determine whether there are different populations of glutamatergic neurons in charge of different odor. Together, it shows that basal forebrain is a region that integrates all different odors, and may be able to transfer the information to affect downstream feeding and motivated behaviors.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

Support: DARPA, D21AP10162-00
NIH, R21DC018912

Title: Shallow networks run deep: How peripheral preprocessing facilitates odor classification

Authors: *P. PURI[1], S.-T. WU[2], C.-Y. SU[2], J. ALJADEFF[2];
[1]Dept. of Physics, [2]Dept. of Neurobio., UCSD, La Jolla, CA

Abstract: Drosophila olfactory sensory hairs (‘sensilla’) typically house two olfactory receptor neurons (ORNs) which can laterally inhibit each other via electrical (‘ephaptic’) coupling (Su, CY. et al., 2012, Nature) . ORN pairing is highly stereotyped and genetically determined. Thus,

olfactory signals arriving in the Antennal Lobe (AL) have been preprocessed by a fixed and shallow network at the periphery. To uncover the functional significance of this organization, we developed a phenomenological model of coupled ORNs responding to odor mixture stimuli. Our model includes two forms of nonlinearity which are essential to capture the biophysics of ephaptic interactions. We derived an analytical solution for the transient dynamics of the model, which shows that networks of coupled ORNs can extract the hedonic value (positive or negative) of certain odor mixtures via transient valence amplification. For a given odor mixture, the concentration ratio for which valence is most strongly amplified is determined by the asymmetry in coupling strengths of grouped ORNs. This coupling asymmetry arises from hard-coded morphometric asymmetries between grouped ORNs, and can be determined by fitting our model to in vivo electrophysiological measurements. Our model predicts that for efficient read-out of the amplified valence signal, which can then trigger a behavioral response, there must exist specific patterns of downstream connectivity that reflect the organization at the periphery. By analyzing the fly connectome data (Scheffer et al., 2020, eLife) from the AL to Lateral Horn (LH), we found evidence directly supporting this prediction. Specifically, connectivity patterns are more asymmetric for pairs of ephaptically coupled ORNs than expected by chance, suggesting that AL to LH projections are faithful to the genetically conserved interaction asymmetries at the olfactory periphery. We further studied the effect of ephaptic coupling on olfactory processing in the AL to Mushroom Body (MB) pathway. By analyzing a population level model of ORN, AL, and MB responses, we showed that stereotyped ephaptic interactions between ORNs lead to a clustered odor representation of glomerular responses. Such clustering in the AL is an essential assumption of theoretical studies on odor recognition in the MB (Babadi, Sompolinsky, 2014, Neuron). Thus, preprocessing of olfactory stimuli by a fixed and shallow network increases sensitivity to specific odor mixtures, and aids in the learning of novel olfactory stimuli. This preprocessing is crucial for odor-guided behaviors initiated by both the LH and MB pathways.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Topic: D.04. The Chemical Senses

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Karen Toffler Charitable Trust

Title: Lateral hypothalamic projections to the olfactory bulb constitute an anatomically distinct subpopulation of orexin neurons

Authors: *M. QI[1,2], D. A. FADOOL[1,2,3], D. A. STORACE[1,2,3];
[1]Biol. Sci., [2]Program in Neurosci., [3]Inst. Of Mol. Biophysics, Florida State Univ., Tallahassee, FL

Abstract: Olfactory cues play a key role in food consumption. Indeed, altered metabolic state can drive changes in olfactory sensory processing, anatomy, and perception. The underlying mechanisms of how olfactory cues might regulate food consumption and metabolic state remain poorly understood. We hypothesize that such olfactory-driven regulation could occur via circuitry connections to brain areas known to be involved in homeostatic regulation. Herein, we injected a monosynaptic retrograde tracer conjugated to Alexa Fluor 555 (CTB555) into the olfactory bulb in adult mice, which resulted in retrogradely labeled neurons in the lateral hypothalamus. We next sought to determine the genetic identity of the hypothalamic neurons innervating the olfactory bulb by combining tract tracing with immunohistochemistry for Orexin A, a neuropeptide released by neurons in the lateral hypothalamus. The numbers and spatial positions of labeled neurons were quantified in sequential sections through the hypothalamus. Twenty-two percent (± 4; s.e.m.) of the hypothalamic neurons that project to the olfactory bulb expressed orexin-A, and orexin-A positive fibers were expressed throughout all layers of the olfactory bulb. Remarkably, only 7.3 ± 1.2 % of all the orexin-A neurons projected to the OB, suggesting that the orexin-A neurons that innervate the OB constitute an anatomically distinct subpopulation. Overall, these results support a model in which olfactory sensory processing can be influenced by orexinergic feedback at the first synapse in the olfactory processing pathway.

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Poster

294. Hearing Loss

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Topic: D.05. Auditory & Vestibular Systems

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High Risk, High Return Research Program (2020)) in the Incheon National University
Research Grants Council of the Hong Kong (21104716 and 11102618)

Title: Opening cortical surface plasticity alleviates hearing loss-induced tinnitus

Authors: *S. PAK[1], M. LEE[1], S. YANG[2], S. YANG[1];
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Abstract: Hearing loss (HL)-induced tinnitus is largely driven by irreversible damage of the peripheral auditory system which chronically leads to abnormal neural responses and disrupted

frequency maps of the central auditory system. Whether and how electrical rehabilitation in the auditory cortex alleviate this intractable tinnitus remains speculative. Here, we propose that cortical stimulation can alleviate tinnitus by enhancing neural responses and promoting cortical map reorganization. For this, a graphene-based multichannel neurostimulator was used to monitor and activate cortical maps. Our results demonstrated that cortical surface stimulation increases cortical activities and reshapes the sensory maps, thereby alleviating HL-induced tinnitus. Furthermore, cortical surface possesses long-term potentiation even after HL, being implied as a cellular mechanism underlying sensory map remodeling. This suggests that cortical surface map remodeling can serve as an effective approach to facilitate functional recovery from sensory deprivation, providing a working principle of various treatment methods using cortical stimulation.

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Poster

293. Olfactory Central Mechanisms: Vertebrates

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Program #/Poster #: 293.31

Topic: D.04. The Chemical Senses

Title: Wide range of behavioral state tunings and the functions in sensory cortex

Authors: *Y. TANISUMI[1,2,3], K. SHIOTANI[4], J. HIROKAWA[5], Y. SAKURAI[5], H. MANABE[5];
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Abstract: How the sensory cortex shares and functions with top-down signals from higher-order regions is a significant question. We previously provided the first recordings of neuronal activity in the ventral tenia tecta (vTT) in the olfactory cortex, which receives top-down projections from the medial prefrontal cortex (mPFC) and projects to the broad olfactory areas. Here, we developed and combined electrophysiological recordings in the vTT with optogenetic mPFC-silencing while mice associated different four different odor cues with appetitive and aversive outcomes. First, we found individual vTT neurons encoded not only encoded odor-presentation associated with positive and negative value, but also the odor-evoked outcome waiting and positive/negative outcome phases. Second, we investigated whether the neural activity undergoes temporal scaling from the short to long intervals using the different-interval timing task. The neural tunings to the extension of short-delay to long-delay were expanded, suggesting the scalable vTT state encoding. Third, to reveal the source of the top-down signals, we expressed inhibitory opsin Arch in the mPFC and recorded from vTT neurons with and without the

optogenetic silencing of mPFC axons while mice conducted the task. Without mPFC-to-vTT inputs, the vTT-state representations were degraded and less integrated. Finally, we performed optogenetic silencing during the odor-outcome association task and the reversal learning to ask whether the mPFC-to-vTT inputs contribute to the learning of appetitive associations. Mice that experienced mPFC-to-vTT axons inhibition exhibited learning deficits. Taken together, our data suggest that the vTT acts as a hub that sends various context-dependent signals for learning from the mPFC to broad olfactory areas.

Disclosures: **Y. Tanisumi:** None. **K. Shiotani:** None. **J. Hirokawa:** None. **Y. Sakurai:** None. **H. Manabe:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.02

Topic: D.05. Auditory & Vestibular Systems

Support: Otolaryngology T32 DC012280
R01 DC011555

Title: Binaural Brainstem and Spatial Hearing Deficits in a Guinea Pig Model of Noise-Induced Cochlear Synaptopathy

Authors: ***M. BENSON**[1], J. PEACOCK[1], N. T. GREENE[2], D. TOLLIN[1];
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Abstract: Hearing loss is normally characterized by permanently raised auditory thresholds due to cochlear dysfunction. However, recent animal studies have revealed that brief, moderate-level noise exposure causes a permanent loss of ribbon synapses between inner hair cells and auditory nerve fibers, but only temporary threshold shifts that recover within a couple of weeks. Such noise-induced cochlear synaptopathy has therefore been called ‘hidden hearing loss’ because while there is a significant degeneration of ribbon synapses, the resulting hearing dysfunction is hidden from typical clinical assays. Here we used Guinea pigs to study the mechanisms leading to hearing deficits resulting from synaptopathy caused by a 2-hour 106 dB SPL 4-8 kHz octave band noise. We measured distortion product otoacoustic emissions (DPOAEs) and auditory brainstem responses (ABRs) to assay peripheral hearing. Next, we tested spatial hearing ability through the prepulse inhibition (PPI) of the acoustic startle reflex. Finally, we performed immunohistochemistry to confirm synaptopathy. ABR and DPOAE recordings were made prior to noise exposure, 24 hours post-exposure, and 1, 2, 4, and 8 weeks following exposure. Brainstem circuits subserving spatial hearing were assessed physiologically via the binaural interaction component (BIC) of the ABR and behaviorally through PPI (pre-noise and 8 weeks post-noise). PPI was used to measure hearing-in-noise ability, or spatial release from masking,

where the animal must detect a target (broadband chirp) at varying SPLs from one spatial location in the presence of background noise presented from various other spatial locations. This task approximates the 'cocktail party' effect, where the listener focuses on the speech of a specific talker while trying to ignore background noise. Results show that the noise exposure induces no permanent hearing loss, as measured by the DPOAEs and ABR thresholds, but results in a persistently decreased BIC amplitude relative to pre-noise exposure. Despite recovery of normal audibility, spatial hearing deficits persisted concomitant with the depleted BIC of the ABR. Cochlear synaptopathy was objectively confirmed by visualizing the loss of ribbon synapses in the cochlea. Labeling for the presynaptic ribbon (Ctbp2) and post synaptic ribbon (GluA2) allowed us to visualize any permanent changes to ribbon synapses. The results demonstrate that cochlear synaptopathy causes deficits in brainstem circuits known to be critical for binaural and spatial hearing and that these alterations lead to deficits in hearing abilities that require integration of inputs from both ears.

Disclosures: **M. Benson:** None. **J. Peacock:** None. **N.T. Greene:** None. **D. Tollin:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.03

Topic: D.05. Auditory & Vestibular Systems

Support: Hong Kong General Research Fund projects 11101020 & 11100219

Title: Sensitivity to Interaural Time Differences in Prosthetic Hearing: What Can Rats Teach Us?

Authors: ***J. W. H. SCHNUPP**[1], A. BUCK[1], S. BUCHHOLZ[2], T. A. PREYER[2], H. K. BUDIG[2], F. KLEINSCHROTH[2], N. ROSSKOTHEN-KUHL[2];
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Abstract: Early deaf human patients whose hearing is restored with bilateral cochlear implants (biCIs) are usually insensitive to interaural time differences (ITDs), an important binaural cue for binaural hearing. This insensitivity has usually been attributed to a lack of auditory input during a presumed sensitive period for the development of normal binaural hearing. However, our group was recently able to show that neonatally deafened (ND) rats who are fitted with biCIs in early adulthood and are given precisely synchronized binaural stimulation from the outset are able to lateralize ITDs with exquisite sensitivity, reaching thresholds of ~50 μs (Rosskothen-Kuhl et al., 2021, eLife doi: 10.7554/eLife.59300). Here we present results from several key follow-on psychoacoustic experiments with our ND biCI rats, which have yielded a number of new and important insights. First, by varying the pulse rate of the binaural stimuli delivered, we were able to show that ITD sensitivity remains surprisingly good for pulse rates of up to 900 pps, but drops

sharply at 1800 pps. Electric ITD sensitivity thus declines only at pulse rates higher than the upper limit for acoustic ITDs, and good ITD sensitivity with CIs is achievable at pulse rates used in clinical practice. Second, by independently varying envelope and pulse timing ITDs, we were able to show that ITD discrimination is dominated by the timing of the pulses, and envelope ITDs are essentially useless as a cue under CI stimulation. Third, by independently varying ITDs and ILDs, we were able to show that time-intensity trading ratios for electric hearing are as small as 20 μs/dB. Result 1 indicates that delivering good ITDs via CIs need not be incompatible with the high pulse rates needed for good speech encoding, but results 2 and 3 indicate that the essentially random pulse timing ITDs delivered by current, desynchronized clinical processors are a very significant problem. Pulse timing ITDs would normally be interpreted as powerful lateralization cues, which can confound even very large interaural level difference cues unless the animal becomes desensitized to ITDs.

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Poster

294. Hearing Loss

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Topic: D.05. Auditory & Vestibular Systems

Support: Scholarship of Göttingen Promotionskolleg für Medizinstudierende (Jacob-Henle-Programm/Else-Kröner-Fresenius-Stiftung, Promotionskolleg für Epigenomik und Genomdynamik, 2017_Promotionskolleg.04) - AM
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Priority Program 1926 “Next generation optogenetics” - AH, TM

Title: Optogenetic modulation of the auditory pathway for hearing restoration and neural network analysis

Authors: *A. MITTRING[1,2,3], T. MOSER[2,3,4,5], A. HUET[1,2,3,4];
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Synaptic Nanophysiology Group, Max-Planck-Institute for Multidisciplinary Sci., Göttingen, Germany

Abstract: To restore hearing to severely impaired patients, fast and spatially confined optogenetic stimulation of the spiral ganglion neurons (SGNs) represents a prospective alternative to electrical stimulation currently used in cochlear implants. Towards the development of the future optical cochlear implant (oCI), multiple channelrhodopsins (ChR) expressed in SGNs were tested for their capacity to activate the auditory pathway. Identifying the transfer function from light to SGN firing is critical to designing sound coding strategies of the oCI or employing optogenetic SGN stimulation to study auditory neural networks. Here, we investigated SGN light encoding with Chronos, the fastest naturally occurring ChR (Klapoetke *et al*, 2014; closing kinetics = 0.76 ms at 36°C, Keppeler *et al*, 2018). Mouse SGNs were optogenetically modified with trafficking-optimized Chronos (Chronos-ES/TS) using early postnatal viral gene transfer (AAV-PHP.B, human synapsin promoter, titer = 3.3-8.4 x 10e+12 GC/ml). Eight weeks later, *in vivo* stereotactic juxtacellular recordings from single putative SGNs and single putative anteroventral cochlear nucleus (AVCN) neurons were performed in response to patterned fiber-based optical stimulation. Three stimuli were measured to identify optimal optogenetic stimulation parameters: *i)* deterministic stimuli to probe the effect of different pulse durations, repetition rates and intensities; *ii)* forward masking protocols; *iii)* stimuli containing different repetition rates and pulse durations which are stochastically presented. By combining single neuron and population analyses, our data show: *i)* choice of pulse duration allows to modulate the spike rate of optically driven SGNs; *ii)* optogenetically modified SGNs require a few tens of ms to recover from a masking stimulus; *iii)* stochastic stimuli allow to capture within minutes the light encoding and recoding within the first stages of the auditory pathway.

Disclosures: **A. Mittring:** None. **T. Moser:** Other; co-founder and CEO of OptoGenTech GmbH. **A. Huet:** None.

Poster

294. Hearing Loss

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Program #/Poster #: 294.05

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01AG067791
UC Davis Departmental Funds

Title: Reorganization of auditory cortex in the aged rhesus macaque with high frequency hearing loss

Authors: *D. LU, B. BORMANN, K. STEWART, J. ROBERTS, J. JOHNSON, K. NEVERKOVEC, G. RECANZONE;
Univ. of California, Davis, DAVIS, CA

Abstract: Age-related hearing loss (ARHL) or presbycusis afflicts approximately 1/3 of the geriatric population and increases exponentially with age. While hearing aids can provide some benefit to a sub-population, there are also central changes, yet little is known about how natural aging influences auditory processing at the cortical level. To better understand the cortical changes in ARHL, we recorded single neuron activity in core auditory cortex in a passively listening male rhesus macaque monkey. Stimuli were 200ms duration tones with frequency ranging from 250Hz to 19kHz in 1/2 or 1/3 octave steps at levels of 30, 50 and 70 dB SPL, randomly interleaved with other acoustic stimuli. We compared the tonotopic gradient, spectral tuning, latency, spontaneous activity, and onset, sustained and offset driven activity. The animal was 26-27 years old at the time of testing, equivalent to 78-81 human years, and had bilateral high frequency hearing loss as measured by an ABR. Preliminary analysis of the best frequency (BF) of each recording location showed that the tonotopic representation in AI was not as systematically spatially organized as has been seen in younger monkeys. The rostral (low frequency) region showed a normal tonotopic gradient from low to high BF in the rostral to caudal direction, which we define as the non-reorganized zone. The caudal region that would normally represent the inaudible high frequencies showed a severely disrupted BF organization, which we define as the reorganized zone. Comparing neurons between the reorganized and non-reorganized regions showed that individual neurons simultaneously recorded from the electrode had more variance in their BF. The spontaneous, onset and offset, but not sustained, responses were greater in neurons in the reorganized zone compared to those in the non-reorganized cortex. Finally, the spectral bandwidth measured for neurons in the reorganized zone was greater at 30, 50 and 70 dB SPL compared to neurons in the non-reorganized zone. These data indicate that the responses of neurons in the reorganized zone are altered in both spectral and temporal domains across both the tonotopic gradient as well as between neighboring neurons. These differences may provide insights into the central mechanisms of ARHL.

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Poster

294. Hearing Loss

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Program #/Poster #: 294.06

Topic: D.05. Auditory & Vestibular Systems

Support: R01-DC017924

Title: Aging leads to impairment of spatial hearing abilities in the Mongolian Gerbil

Authors: *M. D. SERGISON[1], J. PEACOCK[1], M. A. BENSON[1], S. POLEG[1], N. T. GREENE[2], A. KLUG[1], D. J. TOLLIN[1,2];
[1]Physiol., [2]Otolaryngology, Univ. of Colorado Anschutz Med. Campus, Aurora, CO

Abstract: Aging is known to effect spatial hearing and speech in noise recognition, often even when hearing thresholds are normal. The Mongolian Gerbil (*Meriones unguiculatus*) is an animal with a hearing range similar to that of humans, providing a translational model to research how aging can lead to dysfunction of auditory pathways. However, whether aging gerbils exhibit spatial hearing deficits similar to what is seen in humans is not well known. Here, we examine if aging gerbils show a decrease in spatial hearing abilities compared to young gerbils. We ran a cohort of young (2-10 months) and aged (33-42 months) gerbils through a number of spatial hearing tasks that utilize the prepulse inhibition of the acoustic startle response (PPI). PPI requires no training and also offers a high-throughput measure such that a large number of animals can be tested in a relatively short timeframe. In our first set of experiments, we measured spatial acuity by presenting broadband noise that swapped speaker locations, acting as a prepulse, prior to presenting a startle stimulus. PPI of the startle response increased monotonically with wider angles of speaker swaps in young gerbils, but not in all aging gerbils. Next, the speaker swap was confined to a given hemifield, instead of swapping across the midline. While the results were not as robust as the midline swap, PPI increased with larger speaker swaps in young gerbils and did not in aging gerbils. Next, spatial acuity across the midline was assessed for low- (0.5 kHz) and high-pass (4 kHz) noise, in order to assess the abilities of young and old gerbils to localize sounds using interaural time differences (ITDs) and interaural level difference (ILDs), respectively. Similar to broadband noise, wider swap angles elicited higher PPI responses in young gerbils, but not in all aging gerbils. Lastly, to mimic detecting speech in noise, spatial release from masking ability was assessed. A broadband chirp was presented from the midline in the presence of broadband masking noise at different angles. PPI was largest when the maskers were at sources farther from the target and decreased as the masker intensity increased and were moved spatially closer to the target. We again saw this effect was strongest in young gerbils, with impaired detection abilities in the aging gerbils. Collectively, this data demonstrates that aging leads to impaired spatial hearing abilities in gerbils across a multitude of conditions, similar to what is known in aging humans. We hope to use PPI as a behavioral output measure to further investigate the mechanisms underlying age-related central auditory processing deficits.

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Poster

294. Hearing Loss

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Program #/Poster #: 294.07

Topic: D.05. Auditory & Vestibular Systems

Support: DFG Exc 2177/1

Title: Hearing loss in juvenile rats leads to hyperactivity and pronounced social play-fighting during development, together with cognitive disturbances in adulthood

Authors: *J. JELINEK[1], M. JOHNE[1], M. ALAM[1], J. K. KRAUSS[2], K. SCHWABE[1];
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Abstract: Background: In children, hearing loss has been associated with hyperactivity, disturbed social interaction, and cognitive deficits. Whether hearing loss is causal for these disturbances, or whether the dynamic interplay with deficient acquisition of spoken language and speech understanding plays a role, is unknown. To investigate the effect of hearing loss on behavioral deficits in a confined setting, especially in the absence of language which may act as confounding factor, we here tested the impact of hearing loss in juvenile rats on motor, social, and cognitive behavior.
Methods: In juvenile (postnatal day 14) male Sprague Dawley rats hearing loss was induced under general anaesthesia with intracochlear injection of neomycin (n=13). Sham-operated (n=8) and naive rats (n=8) served as controls. One week after surgery auditory brainstem response (ABR) -measurements verified hearing loss after intracochlear neomycin-injection, respectively intact hearing in sham-operated and naive controls. Thereafter, all rats were tested for locomotor activity (open field) and coordination (Rotarod), as well as for social interaction during development in weeks 1, 2, 4, and 8 after surgery, followed by training and testing for spatial learning and memory (4-arms baited 8-arms radial maze test).
Results: In the open field deaf rats moved faster and covered a greater distance than sham-operated and naïve controls (both $p<0.05$). Additionally the motor coordination on the Rotarod was disturbed in deaf rats ($p<0.05$). During social interaction, deaf rats showed significantly more play fighting during development ($p<0.05$), whereas other aspects of social interaction, such as following, were not affected. Learning the radial maze test was not disturbed in deaf rats, but retesting after eight weeks indicated a long-term memory deficit.
Conclusions: Hearing loss in juvenile rats leads to hyperactive behavior and pronounced play-fighting during development, together with deficits in long-term memory, suggesting a direct link between hearing loss and behavioral deficits. This model offers the opportunity for deeper investigation of developmental deficits resulting from hearing loss without language as potentially confounding factor.

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Poster

294. Hearing Loss

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant F30-DC017351
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NIH Grant R01-DC012557

Title: Locus coeruleus activity improves cochlear implant performance

Authors: *E. GLENNON[1], S. VALTCHEVA[4,1], A. ZHU[1], M. A. SVIRSKY[2,1,3,5], R. C. FROEMKE[2,1,3,5];
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Abstract: Rates of auditory perceptual learning and asymptotic speech perception performance with cochlear implants are highly variable across patients (Blamey et al 2013). Adaptation to cochlear implants is believed to require neuroplasticity within the central auditory system (Fallon et al 2009). However, mechanisms by which behavioral training enables plasticity and improves outcomes are poorly understood. Here we hypothesize that neural mechanisms that promote plasticity in the rodent auditory system are key to optimizing cochlear implant usage and might be especially helpful in cases of poor performance. We focus on noradrenergic modulation of rat auditory cortex by the locus coeruleus, which can enable robust and long lasting neural and behavioral changes (Martins & Froemke 2015; Sara 2015).We developed a new surgical approach for cochlear implantation in adult rats (King et al 2016). Our approach allows insertion of an 8-channel electrode array covering up to 360 degrees in the cochlea and allows rats to freely behave while using the implant to perform auditory tasks. Rats are trained on a go/no-go task and self-initiate trials to respond to a target tone. Previously, we showed in normal hearing animals that this task requires auditory cortex and is sensitive to cortical modulation and plasticity (Froemke et al 2013).Here we examined the effect of pairing locus coeruleus stimulation with an auditory stimulus on auditory learning when the animal has to relearn a tone identification task using a cochlear implant. Initial training was done using acoustic stimuli in normal hearing animals. Animals were then bilaterally deafened and unilaterally cochlear-implanted. Next, animals were retrained on the auditory task with the new target delivered by intracochlear electrical stimulation. Prior to daily behavioral training sessions for the new target, one group of rats underwent a 5-10 min pairing session. Pairing accelerated learning with cochlear implants ($N = 10$) compared to animals that did not receive it ($N = 16$, $p = 0.01$). We then conducted multi-unit and whole-cell recordings in the auditory cortex to assess activation of the cortex by the cochlear implant. Auditory cortical responses to cochlear implant stimulation reflected behavioral performance, with enhanced responses to rewarded stimuli and decreased distinction between unrewarded stimuli. Furthermore, fiber photometry from locus coeruleus predicted when implanted subjects ($N = 4$) began responding to sounds and longer-term perceptual accuracy. Adequate engagement of central neuromodulatory systems is thus a potential clinically relevant target for optimizing neuroprosthetic device use.

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Poster

294. Hearing Loss

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Program #/Poster #: 294.09

Topic: D.05. Auditory & Vestibular Systems

Support: CIHR Grant

Title: Differences in gray matter thickness covariances between hearing and deaf cats discovered using MRI

Authors: *S. G. GORDON[1], S. G. LOMBER[2];
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Abstract: Gray matter thickness derived from high-resolution magnetic resonance images is a useful non-invasive method of characterizing a property of the cerebral cortex. When combined with an atlas of the species in question, these measurements can be used to distinguish the thickness of anatomically relevant regions of interest (ROIs), as opposed to voxel-wise or whole-brain analyses. The purpose of this investigation was to examine if high-resolution MRI could be used to assess differences in cortical thickness covariance across ROIs between the brains of deaf cats and those of hearing controls. In this study, 29 adult hearing and 26 adult perinatally-deafened cats were scanned at 0.5mm isotropic resolution to look for structural covariance across ROIs from a cat brain atlas (Stolzberg et al., 2017). Deafness was induced during the first postnatal month by systemic administration of ototoxic drugs. Hearing status, or lack thereof, was confirmed by the recording of auditory brainstem responses, and all animals were scanned after reaching sexual maturity (>6 months of age). Thickness maps were obtained using the Advanced Normalization Tools (ANTs) DiReCT cortical thickness pipeline, and this data was then processed in MATLAB. Age and sex were regressed out of the raw thickness values independently for each group, and all subjects' values were normalized to the mean of that group. These corrected regional thickness values were then correlated against each other for each pair of ROIs within each group using Pearson's R, and corrected using the Benjamini-Hochberg method. Significant covariances were compared within and across the two groups. Plots of covariance vs. Euclidean distance between the ROI pairs were similar across hearing and deaf subjects, with each group showing increased significant covariances at very short distances (<5mm apart) and a more consistent distribution from 5mm out to the extremes (~35mm). The mean covariance for bilaterally homologous ROIs were found to be significantly higher than for non-homologous ROI pairs in both groups. Numbers of significant covariances within and across sensory modalities differed between groups. A notable result was the near-complete absence of somatomotor covariances in the deaf group as compared to the hearing group. Results from this work point to a change in the structural connectivity across the cat brain following deafness, further supporting the plastic nature of the brain as seen in past tracer and behavioral studies.

Disclosures: S.G. Gordon: None. **S.G. Lomber:** None.

Poster

294. Hearing Loss

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Program #/Poster #: 294.10

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant 5TL1TR001447-07

Title: Neuroplasticity in adult-deafened cochlear implant users: from synapse to speech perception

Authors: *A. E. HIGHT[1], E. GLENNON[2], Y.-S. CHENG[4], J. K. SCARPA[5], J. NEUKAM[2], N. CAPACH[3], M. INSANALLY[6], S. VALTCHEVA[7], M. A. SVIRSKY[3], R. C. FROEMKE[8]; [1]NYU Langone Med. Ctr., New York, NY; [3]Otolaryngology, [2]New York Univ. Grossman Sch. of Med., New York, NY; [4]Massachusetts Eye and Ear Infirmary, Boston, MA; [5]Weill Cornell New York-Presbyterian Hosp., New York, NY; [6]Otolaryngology, Univ. of Pittsburgh, Pittsburgh, PA; [7]Fac. of Medicine, Inst. for Vegetative Physiol., Univ. of Cologne, Cologne, Germany; [8]Otolaryngology, NYU Med., New York, NY

Abstract: Neuroplasticity is challenging to study due to scalability issues: neuroplasticity (and resulting timescales of learning and memories) range from milliseconds to years, from minuscule synapses to neuron conglomerates, and from sound detection to speech perception. We address this challenge by performing comparative studies in adult-deafened human and rat cochlear implant (CI) users. CIs are neuroprosthetic devices that restore hearing, but significant adaptation periods are required before human subjects attain maximum benefit from their devices. To connect human and rodent studies of CI use, we focused on types of adaptations that may generalize to speech perception (humans) and cortical encoding of CI stimulation (rodents).
(Human Studies) We longitudinally tracked adult deafened human CI users' (N=3) speech perception and auditory psychophysical abilities. Following initial activation, we sent subjects home with a tablet loaded with validated tests of spectral acuity (quick-spectral modulation detection, QSMD) and temporal acuity (modulation-detection test, MDT; gap-detection). Subjects completed these tasks every other day for the first ~30 days following activation. Speech perception was tested periodically in-lab using standard tests of word and sentence recognition. We found significant improvements in QSMD (frequency acuity) performance in 2 of 3 CI users and improvements in speech perception in all 3 CI users.
(Rodent Studies) Here, we trained rats on a 2-alternative forced choice (2AFC) task for sound frequency discrimination. Rats completed the 2AFC task with high discrimination (d'>1) after ~3 weeks of acoustic training (N=18) or ~1 week of CI training (N=5). Next, we measured excitatory and inhibitory postsynaptic currents (E/IPSCs) in rat A1 neurons, evoked by individual CI electrodes. Initial measurements revealed long-latency and irregular evoked synaptic responses. We found this initially poor excitatory-inhibitory correlation improved significantly after 2AFC training. Lastly, we performed micro-electrocorticography (μECoG) recordings of A1 in normal hearing and deafened rats with CIs. We found cochleotopic encoding of both tone-evoked and CI-evoked stimuli, but a supervised PCA/LDA decoder suggests encoding of CI stimuli may be initially degraded compared to acoustic stimuli.
We found CI-use induces an improvement in both speech perception and spectral discrimination

in humans and both spectral discrimination and cortical encoding (A1) in rats. Our next steps are to further elucidate the potential relationships between different types of adaptations ranging from speech perception to synaptic encoding.

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Poster

294. Hearing Loss

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Topic: D.05. Auditory & Vestibular Systems

Support: CIHR

Title: Connectivity alterations following perinatal deafness in the cat as assessed by probabilistic tractography

Authors: *A. SACCO, S. G. GORDON, S. G. LOMBER;
McGill Univ., Montreal, QC, Canada

Abstract: Following sensory deprivation such as deafness, areas and networks in the brain may adapt and rewire to compensate for loss of input, resulting in a potential myriad of shifts concerning local functionality, white matter integrity, network organization and more. These computational adaptations may engender regional disuse-driven atrophies and/or compensatory cross-modal plasticity (enhanced abilities in the remaining senses). To better elucidate the mechanism supporting compensatory plasticity, this study investigated structural connectivity differences between hearing (n=8) and perinatally-deafened (n=8) cats, a well-studied animal model for auditory deprivation. Using DTI, connectional changes were explored throughout the entirety of the brain in two analysis streams: region of interest (ROI) to ROI and modality to modality. For the latter, ROIs were combined according to primary function, resulting in 7 masks: auditory, visual, somatosensory, motor, frontal, non-cortical, and other. FSL's *probtrackx* was run from each gray matter ROI seed to the remaining 154 targets for ROI-ROI analysis, and from each seed modality to the remaining 6 targets for modality-modality analysis. For each case, relative streamline percentages were calculated between each seed to remaining target masks. Wilcoxon rank-sum tests were performed to compare percentages between groups and corrected for using the Benjamini-Hochberg procedure. Results suggest structural plasticity in various regions throughout the deaf brain, not limited to sensory cortices. This included a significant decrease in connectivity between dysgranular insular area and fourth somatosensory cortex of the left hemisphere for deaf compared to hearing ($p = 0.0463$). Near-significant differences ($p < 0.1$) included a decrease in connectional strength between visual area 18 and cingulate visual area, as well as increases between prepyriform cortex and area 21b, the dorsal

division of agranular insular area and the dorsolateral division of prefrontal cortex, and between posterior suprasylvian visual area and primary somatosensory cortex in deaf compared to hearing. For modality-modality analysis, results suggest reduced communication between motor cortex and non-cortical structures as indicated by a ~49% decrease in connectivity between the two modalities in deaf compared to hearing ($p = 0.0420$). Overall, this is the first study to examine tractography-based connectivity alterations following auditory deprivation in cats, and it suggests that deafness incites differential adaptations in areal communication both locally and globally. Funding was provided by CIHR.

Disclosures: A. Sacco: None. **S.G. Gordon:** None. **S.G. Lomber:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.12

Topic: D.05. Auditory & Vestibular Systems

Support: RGC/GRF 14605119

Title: Extent of Hearing Loss, Pre-operative Hearing Aid Use and Cortical Structural Alternation in Pediatric Cochlear Implant Candidates

Authors: *D. YUAN[1], I. H.-Y. NG[2], W. T. CHANG[2], W. C.-W. CHU[3], N. M. YOUNG[4], P. C. M. WONG[1];
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Abstract: Children with congenital hearing loss, compared to children with normal hearing, show reduced brain tissue volumes at auditory cortical regions due to deficient hearing input. However, few studies have examined the effects of hearing loss severity and amplification (e.g., hearing aid use) on structural changes to the auditory cortex. The current study investigated to what extent does residual hearing and use of hearing aids affect volume of auditory cortex in children with hearing loss. Twelve children with bilaterally congenital hearing loss who were candidates for cochlear implantation were recruited (eight females and four males, age = 25.25 ± 20.23 months). Voxel-based morphometry analysis was conducted to assess the gray matter volumes in the primary and secondary auditory cortex (Heschl's Gyrus and superior temporal gyrus). Hearing loss severity was quantified by residual hearing measured by pure-tone audiometry played at different frequency levels. Higher hearing threshold measured by pure tone audiometry represents worse residual hearing. The length of hearing aid use was also recorded. In general, the Wilcoxon signed-rank test showed that children in this cohort had significantly better residual hearing at low frequencies than at high frequencies ($Z = 2.371$, $p = 0.018$). The

multilevel regression revealed a significant main effect of high-frequency hearing threshold on gray matter volume in the left Heschl's gyrus (β = -0.564, p = 0.048) suggesting that children with better high-frequency residual hearing showed larger gray matter volume in the left primary auditory cortex. Moreover, there was a significant interaction effect between hearing aid use and hearing threshold on gray matter volume in the left superior temporal gyrus (β = 1.402, p = 0.029). Specifically, longer use of hearing aid modulated the loss of cortical volume in the left secondary auditory cortex related to hearing loss. This study provided preliminary evidence that the degree of hearing input is associated with neuroanatomical plasticity of the auditory cortex. Furthermore, the extent of this plasticity is modulated by hearing aid use before cochlear implantation.

Disclosures: D. Yuan: None. **I.H. Ng:** None. **W.T. Chang:** None. **W.C. Chu:** None. **N.M. Young:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The Chinese University of Hong Kong, Lurie Children's Hospital of Chicago. **P.C.M. Wong:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The Chinese University of Hong Kong, Lurie Children's Hospital of Chicago.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.13

Topic: D.05. Auditory & Vestibular Systems

Support: Czech Health Research Council NU20-08-00311

Title: Effects of tinnitus and presbycusis on functional involvement of the left and right auditory cortex.

Authors: *O. PROFANT[1,2], J. FUKSA[1,2], J. SYKA[1], J. TINTERA[3], A. SKOCH[3], V. SVOBODOVA[4], D. TOTHOVA[4];
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Abstract: Presbycusis and tinnitus are the two most common hearing related pathologies. Although both presumably originate in the inner ear, there are several reports concerning their central components. The aim of this study was to identify age, hearing loss, and tinnitus related functional changes, within the auditory system and its associated structures.Seventy-eight participants were selected for the study based on their age, hearing, and tinnitus, and they were divided into six groups: young controls, subjects with mild presbycusis or expressed presbycusis, young subjects with tinnitus, subjects with mild presbycusis and tinnitus, and subjects with expressed presbycusis and tinnitus. An MR functional study was performed with a 3T MR system, using an event related design (different types of acoustic and visual stimulations and

their combinations).The amount of activation of the auditory cortices (ACs) was dependent on the complexity of the stimuli; higher complexity resulted in a larger area of the activated cortex. Aging led to slightly increased activation especially of the right AC. Hearing loss decreased the amount of activated AC. The congruent audiovisual stimulation led to an increased activity within the default mode network, whereas discongruent stimulation led to increased activation of the visual cortex. The presence of tinnitus increased activation of the AC, specifically in the aged population, with a slight prevalence in the left AC. The occurrence of tinnitus was accompanied by increased activity within the insula and hippocampus bilaterally, where hearing loss produced minimal change. Overall, we can conclude that expressed presbycusis leads to a lower activation of the AC, compared to the elderly with normal hearing; aging itself leads to increased activity in the right AC. The complexity of acoustic stimuli plays a major role in the activation of the AC, its support by visual stimulation leads to minimal changes within the AC. Tinnitus causes changes in the activity of the limbic system, as well as in the auditory AC, where it is bound to the left hemisphere.

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Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.14

Topic: D.05. Auditory & Vestibular Systems

Support: Korea National Research Fund NRF-2020R1A2C1006254

Title: Gene expression changes after noise-induced hearing loss in auditory cortical area and hippocampus in C57BL/6 mice.

Authors: *M.-H. PARK[1,2], S.-Y. LEE[2], H. LEE[2];
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Abstract: Hypothesis and Background: Noise makes a direct damage to peripheral hearing organ. And peripheral hearing loss evokes changes in the central auditory system known as neural plasticity. Recently, several associations between hearing loss and cognitive function are emerged. Many studies are focusing to this associations using cellular and molecular techniques, biological imaging, and electrophysiological methods. In this study, we tried to identify some associations between central auditory and memory system after noise-induced hearing loss using RNA-sequencing analysis. Methods Twelve-week-old C57BL/6 mice were used for experiment. Hearing levels were measured using auditory brainstem response. Before the noise exposure, hearing thresholds were within normal ranges. Mice were exposed to white noise of 120 dB SPL

for two hours. After noise exposure hearing thresholds were increased to over 90dB SPL. Three months later, animals were euthanized, and bilateral auditory cortical area, hippocampus and cochleae were harvested. RNA-seq analysis was performed on each sample to identify which genes were expressed. This information was then used for comparative analysis of differential expressed genes (DEGs) compared to the samples from normal hearing control. The statistical significance of DEGs was determined by fold change (|FC|>2) and independent t-test (P<0.05). Results In the auditory cortical areas, RNA-seq analysis identified 89 DEGs, of which 62 were up-regulated and 27 were down regulated after noise exposure. In the cochleae, 102 DEGs were identified. 57 were up-regulated and 45 were down-regulated. In the hippocampus, 176 DEGs were identified. 141 were up regulated and 35 were down regulated. Gene ontology analysis and KEGG pathway analysis revealed that the signal pathways were involved after noise exposure. TNF signaling pathway and MAPK signaling pathway were down regulated in cochleae. cAMP signaling pathway was up regulated in auditory cortical area, and JAK-STAT signaling pathway and PI3K-Akt signaling pathway also up regulated in hippocampus. Conclusion: RNA-seq analysis revealed that several signaling pathways were involved in the auditory system and hippocampus after hearing loss. These pathways contribute to neural plasticity in the auditory cortical area. And in this study, hippocampus also showed change of gene expression after hearing loss. We need further evaluation for clarify the meaning of these changes in hippocampus.

Disclosures: **M. Park:** None. **S. Lee:** None. **H. Lee:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.15

Topic: D.05. Auditory & Vestibular Systems

Support: RNID - International Project Grant
BrainsCAN Accelerator Grant

Title: Differential plasticity in the auditory cortex, frontal cortex, and hippocampus following noise-induced hearing loss in a rat model

Authors: *S. V. PATEL, A. L. SCHORMANS, L. ZHAO, H. MANDER, S. SUNDARARAMAN, S. N. WHITEHEAD, B. L. ALLMAN, S. H. HAYES;
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Abstract: Hearing loss is known to affect brain regions both within and beyond the central auditory pathway, such as the hippocampus and frontal cortex. While it is well established that these brain regions normally respond to acoustic stimuli, it remains unclear how this sound processing is altered following hearing loss. To better understand the nature and extent of hearing loss-induced neuroplasticity in the frontal cortex and hippocampus, we characterized

changes in various types of sound processing (e.g., event-related potentials, sound-evoked gamma oscillations, sensory gating) in the weeks following loud noise exposure. The auditory brainstem response (ABR) was used to assess hearing sensitivity before and after rats were exposed to noise (115 dB broadband noise, 2 hours). Chronically implanted electrodes in the auditory cortex, frontal cortex, and hippocampus were used to investigate noise-induced changes in sound processing at 7, 14 and 21 days following noise exposure. The 40 Hz auditory steady state response (ASSR; 150 trials of a 0.5 second 40 Hz acoustic stimulus) was used to (1) determine the magnitude of sound-evoked responses (i.e., event-related potentials, ERPs) and (2) assess the ability of distinct brain regions to synchronize to the rapidly presented acoustic stimuli (i.e., inter-trial coherence, ITC). The ABR revealed increased hearing thresholds and impaired cochlear output. Despite this decrease in afferent input, the magnitude of the ERPs were maintained across all brain regions following noise exposure. In contrast, the ASSR showed a differential effect following noise exposure, characterized by no changes in the auditory cortex, but a persistent decrease in the frontal cortex and hippocampal ITC. To investigate whether region-specific molecular changes contribute to this differential effect in the ASSR, we used Western blotting to compare markers of synaptic connectivity (synaptophysin, PSD-95) and excitation/inhibition (NR2B, GAD65). Interestingly, in the frontal cortex, we saw decreased inhibition (GAD65) and excitability (NR2B), whereas only increased inhibition (GAD65) was observed in the hippocampus. In the auditory cortex, we observed increased synaptic markers (synaptophysin, PSD95) and increased excitation (NR2B). Ongoing analyses of additional acoustic paradigms will further investigate noise-induced changes in sound processing across these brain regions. Collectively, the present study provides evidence of region-specific alterations in sound processing following noise-induced hearing loss and highlights its complex interplay with accompanying molecular changes.

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Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.16

Topic: D.05. Auditory & Vestibular Systems

Support: funding by WSAudiology

Title: The Neurogram - a quantification of real-life hearing impairments based on electrophysiology

Authors: *F. SCHMIDT[1], L. REISINGER[1], P. NEFF[3], R. HANNEMANN[4], N. WEISZ[2]; [2]Ctr. for Cognitive Neurosci., [1]Univ. of Salzburg, Salzburg, Austria; [3]Swiss Federal Inst. of Technol. Geneva, Geneva, Switzerland; [4]WSAudiology, Erlangen, Germany

Abstract: The current gold-standard for the diagnosis of hearing loss is pure-tone audiometry. Yet, the artificial pure tones used to assess hearing thresholds in pure-tone audiometry do not resemble real-life listening situations. Therefore pure-tone audiometry only provides an incomplete picture of individual hearing impairment, as disorders such as supra-threshold hearing loss (i.e. hidden hearing loss) can not be captured. Additionally, pure-tone audiometry is vastly dependent on subjective feedback. This can be problematic as giving informed feedback is challenging for some patient groups (e.g. babies that are born deaf or elderly people with dementia). Here we propose the "Neurogram", a possible way to overcome the shortcomings of pure-tone audiometry by using a combination of system identification approaches, magnetoencephalography and a naturalistic listening situation (a radio play). By subsequently fitting linear encoding and decoding models we regress features of an acoustic signal (e.g. spectrograms) from related measured brain activity. We find that the decodability of acoustic information decreases with individual hearing capacity measured using pure-tone audiometry. Furthermore, we found a stronger relationship between subjective reports of speech perception (assessed using the Speech, Spatial and Qualities of Hearing Scale) and the here proposed "Neurogram" compared to pure-tone audiometry. In the future we aim to further develop this approach and work towards a diagnostic procedure that allows clinicians to fit hearing aids optimally based on a characterization of individual hearing impairment without solely relying on subjective feedback.

Disclosures: F. Schmidt: None. **L. Reisinger:** None. **P. Neff:** None. **R. Hannemann:** None. **N. Weisz:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.17

Topic: D.05. Auditory & Vestibular Systems

Support: William Demant Foundation Grant #20-1260
CIHR Doctoral Research Award

Title: Rapid adaptive plasticity associated with hearing restoration with over-the-counter hearing devices

Authors: *M. PERRON[1,2], B. LAU[1], C. ALAIN[1,2];
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Abstract: Age-related hearing loss is associated with communication difficulties, including difficulty in understanding speech-in-noise (SiN), in part due to structural and functional changes in the brain. Because there is evidence that the brain remains plastic during aging, hearing restoration may be effective in alleviating SiN difficulties in older adults with hearing loss. However, little is known about the short- and long-term neurobiological mechanisms associated

with hearing restoration. The purpose of this study is to examine the immediate behavioral and neurobiological effects of using bilateral personal sound amplification devices (PSAPs), which are inexpensive over-the-counter hearing devices, on SiN ability. Fifteen adults with normal to moderate hearing loss (ages 64-84, 10 females) [recruitment is ongoing] underwent a hearing assessment that included pure tone audiometry and the QuickSIN test. Participants also underwent a phonological discrimination task consisting of indicating whether pairs of syllables are the same or different under three noise levels. The task was performed twice in two counterbalanced sessions (with and without PSAPs), while brain activity was recorded by electroencephalography (EEG). Preliminary results revealed that individuals with poor QuickSIN scores benefited significantly from the devices in terms of reaction time (i.e., faster reaction time with the devices compared to without the devices). Cluster-based EEG permutation analyses revealed that PSAPs wearing is associated with increased transient alpha power over the right parietal cortex during first syllable processing (100-500 ms) and transient beta power over the left frontal cortex during second syllable processing (800-1200 ms). Interestingly, those who had a larger change in beta power were those who performed worse on the QuickSIN. Our results suggest that hearing restoration is associated with improved SiN abilities in older adults with SiN difficulties. Furthermore, hearing restoration only during a 3-hour session appears to be associated with neurofunctional changes in speech processing networks. In particular, alpha and beta powers may be neurobiological markers of the rapid adaptation to a new soundscape induced by hearing restoration, potentially reflecting modulation of attention or phonological discrimination ability due to a clearer soundscape.

Disclosures: **M. Perron:** None. **B. Lau:** None. **C. Alain:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.18

Topic: D.05. Auditory & Vestibular Systems

Support: Department of Defense W81XWH-17-1-0172 CDMRP
1I01 RX001986 RR&D Merit Award
1I01 RX001095 RR&D Merit Award

Title: Longitudinal analysis of noise exposure on neuronal activity and the role of non-dihydropyridine L-type calcium channel blocker Verapamil

Authors: *S. YALCINOGLU[1], S. KOTCHARIAN[2], R. BRAUN[2], A. HOLT[2];
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Abstract: People engaged in noisy activities are at greater risk for hearing loss, which affects ~48 million Americans. With no cure, current treatments can only manage hearing loss symptoms. Noise induced hearing loss (NIHL) can result in an increase in neuronal activity in

central pathways. Since voltage-gated calcium channels (CaVs) moderate neuronal activity, they serve as potential therapeutic targets for mitigating NIHL. Thus, we examined the role of verapamil, an L-type calcium channel blocker (CCBs), in preserving hearing function in rats after exposure to excessive noise. By using auditory brainstem responses (ABRs), gap inhibition of acoustic startle reflex (giASR), and manganese enhanced MRI (MEMRI) we address function and activity *in vivo* over time.Male Sprague-Dawley rats were divided into no noise (n=17), noise (n=18), no noise plus verapamil (n=5), and noise plus verapamil (n=7) groups with verapamil (30 mg/kg) or saline injected prior to noise exposure. The noise groups were unilaterally exposed to a 16 kHz, 106 dB SPL tone for 1 hour, while no noise groups were left in ambient noise. ABR hearing thresholds and amplitudes of waves I, II, and V were measured at 4, 12, and 20 kHz (1-90 days). 3 times per week for 12 weeks, giASR was measured at 6 frequencies (4, 8, 12, 16, 20, 24 kHz). Each group was subjected to a 20 ms startle noise to note maximum startle after a silent period, presence of a background tone, and background tone with a gap prior to startle. At baseline and 3 time points (0, 30, and 90 days), neuronal activity was visualized in the cochlear nucleus (CN), inferior colliculus (IC), medial geniculate body (MGB), and auditory cortex (AC) using MEMRI ($MnCl_2$ 66 mg/kg). There was no negative effect with verapamil use and when noise groups had a temporary threshold shift it decreased recovery time. Post noise exposure (day 0), wave I amplitude at 12 and 20 kHz decreased (68% $p<0.001$ and 67% $p<0.05$). 90 days post noise exposure wave II amplitude decreased (68% $p=0.01$) and wave V amplitude increased (200% $p=0.02$). An elevation of the wave V/I ratio was observed 1 day post noise exposure at 12 and 20 kHz. At 8, 12 and 20 kHz noise exposed animals showed enhanced inhibition of their startle. Post noise exposure there was a decrease in Mn^{2+} uptake in the CN (day 0) while it increased in the IC (day 30) and in all brain areas by 90 days ($p<0.05$).Our results demonstrate that CCBs can reduce the impact of noise induced hearing loss. Our observed changes in function and activity of central nuclei may provide compensation for NIHL. The relationship between neuronal activity and noise damage will be further addressed by investigating contributions of specific auditory related sub-nuclei and CaVs.

Disclosures: **S. Yalcinoglu:** None. **S. Kotcharian:** None. **R. Braun:** None. **A. Holt:** None.

Poster

294. Hearing Loss

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 294.19

Topic: D.05. Auditory & Vestibular Systems

Support: R01DC014101
R01NS116598

Title: Cre mouse lines without genetic, age-related hearing loss (AHL) optimized for auditory and multisensory neuroscience

Authors: *C. FOSS, T. OLSEN, J. BIGELOW, A. HASENSTAUB;
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Abstract: Modern molecular tools such as Cre-lox, Flp-FRT, etc. represent powerful tools for circuit dissection, but nearly all of the driver mouse lines for these systems are only commercially available on a C57 background. C57 mice have well characterized age-related hearing loss, caused by a single-nucleotide mutation in the cdh23 gene; they have been shown to have moderate high-frequency hearing loss (~20 dB, ≥32 kHz) by ~14-20 weeks of age, and more severe hearing loss is apparent across frequencies by 6 months of age (20-70 dB; Ison et al., 2007). Our preliminary results suggest this high-frequency hearing loss might occur even earlier, by around ~8-12 weeks of age. This is problematic for studies of hearing or multisensory phenomena and is especially an issue for behavioral studies in which mice must be trained for several months prior to recording.
CBA mice lack the mutant cdh23 gene, and CBA-C57 crosses have normal hearing (Frisina et al 2011; Burghard et al 2019). However, because the genomes of CBA and C57 mice vary in thousands of places (Lilue et al 2018), corresponding to both broad and deep phenotypic variation (Sultana et al 2019, Beckman et al 2020), subsequent generations will show unacceptable genetic and phenotypic variation depending on whether the CBA or C57 gene is passed along at each locus. Thus, in order to use such mice in multi-generation breeding schemes, it is necessary to backcross the Cre gene to a nearly pure CBA background.
Here, we backcross PV-Cre, SST-Cre, and VIP-Cre, the three Cre driver lines most essential for studies of cortical interneuron function, onto the CBA background for several generations. We assessed peripheral hearing sensitivity by recording auditory brainstem responses evoked by clicks and pure tones. We also crossed these CBA Cre lines to the Cre reporter line Ai14 (Madisen et al 2010), in order to confirm that Cre-driven recombination occurs in appropriate structures and neurons, consistent with the original Cre lines and with established aspects of PV, SST, and VIP expression. We will continue to backcross these mice onto the CBA background and monitor their hearing into old age. We expect these mice will be useful to other investigators performing behavioral studies of auditory or multisensory functions, and we will make them available on request.

Disclosures: C. Foss: None. **T. Olsen:** None. **J. Bigelow:** None. **A. Hasenstaub:** None.

Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.01

Topic: D.06. Vision

Support: Annexon Biosciences

Title: Classical complement initiator C1q mediates synapse and neuronal loss in a light damage model of photoreceptor degeneration

Authors: *A. TASSONI, J. VEREEN, E. CAHIR-MCFARLAND, L. MATTHEAKIS, T. YEDNOCK, Y. ANDREWS-ZWILLING;
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Abstract: Geographic atrophy (GA) is an advanced form of age-related macular degeneration leading to photoreceptor death and visual loss. Increased expression of complement components has been observed in retinal tissue from GA patients. The role of C1q and the classical complement pathway in driving disease progression is under investigation. We examined expression and tissue localization of complement proteins in the retina of mice exposed to light damage and determined the potential therapeutic benefit of classical complement inhibition in this model. BALB/c mice were exposed to white light to induce retinal damage and were assessed at Days 1, 3 and 7 post-light exposure. Classical complement component levels were measured in retinal lysates by ELISA, and C1q expression in tissue was assessed by immunohistochemistry. Synaptic externalization of phosphatidylserine (PS), a C1q ligand, was assessed by intravitreal (IVT) injection of the PS-binding probe PSVue. Microglial engulfment of synapses was assessed using IMARIS software. To assess the role of the classical complement pathway in photoreceptor cell damage, C1q activity was pharmacologically blocked by IVT injection of a C1q-inhibiting antibody. Additionally, retina specimens from GA patients were procured from the San Diego Eye Bank. Mice exposed to light damage exhibited progressive loss of photoreceptor synapses and cell bodies, as well as an increase in microglial cells across the outer plexiform (OPL, synapses) and outer nuclear layers (ONL, cell bodies). Following light damage, C1q expression was induced in microglia and was localized on photoreceptor synapses and cell bodies. There was a significant correlation between C1q levels in the OPL and ONL and photoreceptor synapse and cell body loss, suggesting a causal relationship. C1q-tagged synapses were engulfed by microglial cells upon damage, potentially through binding to synaptic PS externalization, which was observed following light damage. Treatment with a C1q-inhibiting antibody decreased complement component levels. Preliminary evidence showed reduced photoreceptor synapse and cell body loss and reduced inflammation. Furthermore, we confirmed C1q deposition on photoreceptor synapses in human GA retinal tissue, suggesting that this mechanism may be operative in humans. We provide the first evidence of C1q deposition on photoreceptor synapses in a light exposure model of retinal damage in mice and in human tissue from GA patients. Preliminary results suggest that inhibiting C1q protects against photoreceptor neuron damage. A Phase 2 study on a C1q-inhibiting antibody is ongoing in GA patients (ClinicalTrials.gov: NCT04656561).

Disclosures: **A. Tassoni:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **J. Vereen:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **E. Cahir-McFarland:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **L. Mattheakis:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Annexon Biosciences. **T. Yednock:** A. Employment/Salary (full or part-time):; Annexon Biosciences. E. Ownership Interest (stock,

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Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.02

Topic: D.06. Vision

Support: NINDS Intramural Program
EY14356 (Rachel O.L. Wong)

Title: Thyroid hormone receptor beta2 gain-of-function changes the spectral distribution of zebrafish cone signals

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Abstract: Thyroid hormone receptor β2 (trβ2), a splice variant of thyroxin receptor β, is a transcription factor/nuclear receptor selectively expressed in vertebrate long-wavelength-sensitive (LWS/red) retinal cones and is essential for LWS cone development. Using two gain-of-function zebrafish transgenics, we examine the physiological and morphological impact of excess trβ2 on zebrafish cone specification. The *crx:mYFP-2A-trβ2* transgenic expresses trβ2 early, in retinal progenitors, while *gnat2:mYFP-2A-trβ2;mpv17*$^{-/-}$ expresses trβ2 later, in differentiated cone cells. Here electroretinographic signals are isolated from cones by synaptic blockade with Na Aspartate and the cone spectral composition analyzed using an 8-opsin algorithm. In 5-day *crx:mYFP-2A-trβ2* embryos, UV- and blue-cone signals are attenuated while LWS2 (larval red-cone opsin) amplitudes increase. 5-day *gnat2:mYFP-2A-trβ2;mpv17*$^{-/-}$ spectra are indistinguishable from controls but LWS2 signals surpass controls during later embryonic and juvenile stages. By adulthood, both transgenics become entirely, or almost entirely, red cone dichromats, with LWS1 (adult red-cone opsin) amplitudes becoming super-normal, exceeding adult LWS2 signals, which are not affected. In adult controls, LWS1 and LWS2 signal amplitudes are about equal. There are mixed opsin cones in both transgenic larvae. In *gnat2:mYFP-2A-trβ2;mpv17*$^{-/-}$ mixed UV- red- opsin immunoreactive cones, a reporter for the UV opsin gene is suppressed, suggesting a UV-to-red cone transformation process. Larval cones

with gain-of-function transgenes are morphologically distinct from red, blue or UV cones, with wider inner segments and shorter axons than red cones, suggesting both cone spectral specification and shape are influenced by trβ2. The preprint is available at https://biorxiv.org/cgi/content/short/2022.06.02.494548v1.

Disclosures: **R.F. Nelson:** None. **A. Balraj:** None. **T. Suresh:** None. **L.J. Elias:** None. **T. Yoshimatsu:** None. **S.S. Patterson:** None.

Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.03

Topic: D.06. Vision

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Title: Ultrastructural comparison of photoreceptor synapses between macaque and marmoset retina

Authors: *J. ZUNIGA[1], K. MIAN[1], H. ZHAI[1], M. HOON[2,3], R. SINHA[1,2,3];
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Abstract: Rod and cone photoreceptors are unipolar neurons that transduce light into an electrical signal which is then relayed to the second-order neurons at specialized synapses.

Photoreceptor axon terminals comprise the specialized ‘triad’ synapse which consists of a plate-like ribbon structure that tethers vesicles and is opposed to three postsynaptic elements from two distinct second-order neuron types. The photoreceptor synaptic ribbons release glutamate onto the second-order neurons - bipolar and horizontal cells - in a sustained graded fashion. Traditionally old-world non-human primates, like macaques, have been used in vision research as their retinal structure is most similar to humans. Similar to macaques, marmosets, are cone-dominated primates that have a higher density of both rod and cone photoreceptors across all regions of the retina. While synaptic connectivity between photoreceptors and second-order neurons is well characterized in macaques, it is unclear how this synapse may differ in marmosets given the difference in rod and cone photoreceptor density. To gain insight into the ultrastructure of the first synapse of the marmoset retina, we performed serial block-face scanning electron microscopy (SBF-SEM) and reconstructed the photoreceptor terminals and their synaptic ribbons. We estimated the volume of the photoreceptor’s terminals, the number and size of ribbons per terminal, and the number of bipolar and horizontal cell post-synaptic processes (PSPs) in marmoset outer retina and compared that to observations from macaque outer retina. Our results from the peripheral marmoset retina indicate an increased number of synaptic ribbons and bipolar PSPs in the rod spherules of marmosets. This is especially interesting as marmoset rod spherules were found to be smaller than macaque despite having more ribbons and PSPs. These rod spherules also contain a novel synaptic structure near the main ribbon of the triad synapses consisting of a small cluster of ribbon segments with associated vesicles. Interestingly, marmoset cone terminals contain about a third of the number of ribbon synapses seen in macaque cone terminals. These differences in photoreceptor synaptic motifs and structures across primate models pique interest in their functional consequences on retinal circuits and vision.

Disclosures: **J. Zuniga:** None. **K. Mian:** None. **H. Zhai:** None. **M. Hoon:** None. **R. Sinha:** None.

Poster

295. Vision: Photoreceptors and Retina

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Title: Regional tuning of cone photoreceptor function in Marmoset retina

Authors: *A. SAHA[1,2,3], R. SINHA[1,2,3,4];
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Abstract: The fovea is a specialized region in the primate retina tightly packed with cone photoreceptors responsible for our high resolution central vision. Presence of the fovea gives rise to heterogeneity in the distribution and arrangement of photoreceptors in the primate retina. Cone density is maximum in the fovea and decreases gradually from the fovea to the peripheral retina. There are also morphological differences between the foveal and peripheral cones - the foveal cones have long, slender outer segments and axons, while the peripheral cones have shorter outer segments and axons. It is known that in primates, the peripheral vision has a higher temporal sensitivity than the central vision. This difference in temporal sensitivity originates in the cone photoreceptors. The foveal cones are two times slower compared to their peripheral counterparts in the macaque retina. However, it remains unknown if such functional differences in cones are consistent across primate species. Marmoset, a cone-dominant new world primate, serve as a unique model system to study the heterogeneity of cone signaling across the visual space. In this study, we use patch clamp electrophysiology to measure light evoked response properties from cones at increasing distances from the fovea in marmoset retina. We found that the cone kinetics vary across retinal space in a graded manner with the peripheral cones approximately 2-fold faster than the foveal cones. These kinetic differences across locations persist across a range of background light levels. Cones can efficiently signal over a broad range of mean luminance by decreasing their gain proportionately with background luminance. Additionally, cone responses also become faster with higher background luminance. These adaptive responses to mean background luminance are conserved in marmoset cones across regions and are comparable to the macaque cones. To identify the origin of the cone kinetic differences in marmoset retina, we measured the photocurrents in response to brief flashes of light as well as quantified the intrinsic biophysical properties of cones across locations. We found consistent differences in the kinetics of photocurrents across locations indicating that, the differences in response kinetics may originate in the phototransduction cascade. In summary, the differences of cone function across retinal locations is a conserved phenomenon across primate species.

Disclosures: **A. Saha:** None. **R. Sinha:** None.

Poster

295. Vision: Photoreceptors and Retina

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Regional Innovation Strategy (RIS)
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Title: Pathophysiological role of crystallin alpha B in neovascularization and fibrosis in mice age related macular degeneration model

Authors: *E. YU[1], W. GU[1], S. GU[1], E. HONG[1], A. KIM[1], H. KIM[1], J. MIN[2], J. YUN[1]; [1]Osong, Chungbuk Natl. Univ., Cheongju-si, Korea, Republic of; [2]Ulsan Univ. Hosp., Ulsan, Korea, Republic of

Abstract: Age-related macular degeneration (AMD) is the leading cause of blindness in Korea in people over 65 years of age. In general, it is classified into two types, dry (dAMD) and wet (nAMD). Macular fibrosis caused by choroidal neovascularization (CNV) in nAMD resulting in blindness with high probability. In our previous study, the concentration of crystallin alpha B (CRYAB), member of the small heat shock proteins, is associated with proliferative vitreoretinopathy (PVR) Grade. PVR is characterized by the growth of dense fibrotic contractile membranes. In this study, we investigated the role of CRYAB on CNV-induced fibrosis. In human aqueous humor, protein level of CRYAB was increased in patients of AMD and fibrosis, moreover, CRYAB was defined as having 0.87 area under the curve regarding fibrosis. Furthermore, proteomics studies identified Prss1, a protease as CRYAB binding protein that may produce cleaved form of CRYAB and augment activity to bind to fibrosis-related molecules. In laser-induced CNV mice model, CNV area was reduced in CRYAB knockout mice than WT mice in mice eyeballs, moreover, deficient of CRYAB decreased the expression of angiogenic factor and myofibroblasts marker. These data suggested that CRYAB may be involved on the development of CNV via proliferation of angiogenesis and fibrosis. We also found that CRYAB was present in a cleaved form with N-terminal cut off in eyeballs of laser-induced CNV mice. A CRYAB inhibitor, 3-methylglutamic acid ameliorates laser-induced pathological process, such as CNV development, expression of fibrosis markers and VEGF *in vivo* and *in vitro*. Interestingly, a protease inhibitor, AEBSF co-treatment with CRYAB inhibitor decreased expression of fibrosis markers and VEGF in *in vivo*. Taken together, our data suggested that CRYAB plays a key role in AMD as pathogenic factors via regulation of angiogenesis and fibrosis, and further research is needed on how the cleaved CRYAB form regulates angiogenesis and fibrosis.

Disclosures: E. Yu: None. **W. Gu:** None. **S. Gu:** None. **E. Hong:** None. **A. Kim:** None. **H. Kim:** None. **J. Min:** None. **J. Yun:** None.

Poster

295. Vision: Photoreceptors and Retina

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Program #/Poster #: 295.06

Topic: D.06. Vision

Support: RPB

Title: Endoplasmic reticulum and mitochondria defects in retinal organoids from vision loss patients

Authors: *M. DIAZ-AGUILAR[1,2,3,4], J. CHOI[2,3], H. MIN[2,3,4], D. DURAN[2,3], E. LEE[2,3], J. M. GRANDJEAN[5], R. L. WISEMAN[5], H. KROEGER[6], J. H. LIN[2,3,4];
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Abstract: Activating transcription factor 6 (ATF6) oversees the production of correctly folded functional proteins through induction/regulation of protein-folding enzymes and chaperones. Variants of ATF6 have been identified as causes of morphologic and molecular defects in cone photoreceptors that lead to achromatopsia (ACHM). ATF6 is expressed in all retinal cell types. But, apart from its described role in cone cells, it is unclear how ATF6 variants affect other retinal cells. To investigate ATF6 functions in other retinal cells, we analyzed the transcriptomes of seven major retinal cell types using retinal organoids derived from induced pluripotent stem cells (iPSC), from ACHM patients carrying biallelic ATF6[Y567N] disease variant, and control asymptomatic heterozygous family members. We performed bulk RNAseq on individual organoids (n=3 each for patients and controls) on day 290. Three RNAseq studies that analyzed non-diseased human retina provided the reference transcriptomic gene sets specifically associated with cones, rods, horizontal cells, bipolar cells, amacrine cells, retinal ganglion cells, and Müller cells (MC) (2019 Liang PMID: 31848347; 2019 Menon PMID: 31653841, 2020 Cowan PMID: 32946783). We then evaluated the expression of these retinal cell-specific gene-sets in ATF6 mutant ACHM retinal organoids (RO) based on the profiles obtained from normal human retinal cell type transcriptomes. Additionally, we investigated the ultrastructure of cone mitochondria and endoplasmic reticula (ER) in ACHM retinal organoids. We showed that cone photoreceptor-specific genes were significantly downregulated in ATF6-ACHM RO compared to controls (Wilcoxon, $P < 0.001$); MC gene expression was increased when comparing ATF6-RO versus controls (Wilcoxon, $P < 0.01$). Otherwise, there were no significant changes in gene expressions in all other retinal cell types in ATF6-ACHM RO. We found upregulation of ER-induced genes (Wilcoxon, $P = 0.002$) and dilated ER in the ATF6-RO. Finally, there were notable mitochondria damages, including disruption and disorganization in mitochondrial cristae in ATF6-RO. Overall, our data suggest three novel mechanisms that potentially contribute to ATF6-associated ACHM: Muller glial activation, abnormal activation of the ER-induced genes, and defect in mitochondria.

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Poster

295. Vision: Photoreceptors and Retina

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Topic: D.06. Vision

Support: NRF-2018R1D1A1B07051068
NRF-2021R1A2C1006087

Title: Retina Cytotoxicity of Silica and Titanium Dioxide Nanoparticles

Authors: *J. PARK, C. PARK;
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Abstract: The retina plays a key role in human vision. It is composed of cells that are essential for vision signal generation. Thus far, conventional medications have been ineffective for treating retinal diseases because of the intrinsic blood-retinal barrier. Nanoparticles are promising effective platforms for ocular drug delivery. However, nanotoxicity in the retinal tissue has not received much attention. This study used R28 cells (a retinal precursor cell line that originated from rats) to investigate the safety of two commonly used types of nanoparticles: silica nanoparticles (SiO_2NPs, 100 nm) and titanium dioxide nanoparticles (TiO_2NPs, 100 nm). Cellular viability and reactive oxygen species generation were measured after 24, 48, and 72 h of exposure to each nanoparticle. Cellular autophagy and the mTOR pathways were evaluated. The retinal toxicity of the nanoparticles was investigated *in vivo* in rat models. Both types of nanoparticles were found to induce significant dose-dependent toxicity on the R28 cells. A significant elevation of reactive oxygen species generation was also observed. Increased autophagy and decreased mTOR phosphorylation were observed after SiO_2NPs and TiO_2NPs exposure. The diffuse apoptosis of the retinal cellular layers was detected after intravitreal injection.

Disclosures: J. Park: None. **C. Park:** None.

Poster

295. Vision: Photoreceptors and Retina

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Program #/Poster #: 295.08

Topic: D.06. Vision

Support: NRF- 2021R1C1C1008042

Title: Effect of photobiomodulation in suppresion of oxidative stress on retinal pigment epithelium

Authors: *J. WON[1], **J. KIM**[2];
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Abstract: As the world undergoes aging, the number of age-related diseases has increased. One of them is disease related to retinal pigment epithelium (RPE) degeneration, such as age-related macular degeneration, causing vision loss without physical damage in the ocular system. It is the leading cause of blindness, with no cure. Although the exact pathogenesis is still unknown, the research shows that oxidative stress is one of the risk factors. Various molecules have been reported as anti-oxidative materials; however, the disease has not yet been conquered. Here, we would like to introduce photobiomodulation (PBM). PBM is a non-invasive treatment based on red and near-infrared light and has been used to cure various diseases by regulating cellular functions. Furthermore, recent studies showed its antioxidant effect, and due to this reason, PBM is arising as a new treatment for ocular disease. In this study, we confirm the antioxidant effect of PBM in retinal pigment epithelium via an RPE model with hypoxia. The function of RPE is protected by PBM against damage from hypoxia. Furthermore, we observed the protective mechanism of PBM by its suppression effect on reactive oxygen species generation. These results indicate that PBM shows great potential to cure RPE degeneration to help patients with blindness

Disclosures: J. Won: None. **J. Kim:** None.

Poster

295. Vision: Photoreceptors and Retina

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Support: NSERC Post Graduate Scholarship - Doctoral
Ontario Graduate Scholarship
Vision Science Research OSOTF Award
Medicine By Design
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Title: Investigating the mechanisms that regulate rod photoreceptor soma translocation in the developing murine retina

Authors: *A. GURDITA[1,2], V. Q. B. PHAM TRUONG[1,2], P. DOLATI[1,2], N. TACHIBANA[1], Z. C. LIU[1], A. ORTIN-MARTINEZ[1], M. IBRAHIMI[1,2], N. POKRAJAC[1,2], L. COMANITA[1], V. WALLACE[1,2];
[1]Donald K. Johnson Eye Institute, Krembil Res. Inst., Univ. Hlth. Network, Toronto, ON, Canada; [2]Lab. Med. and Pathobiology, Univ. of Toronto, Toronto, ON, Canada

Abstract: A current strategy for the late-stage treatment of some photoreceptor-related diseases is the regeneration or replacement of photoreceptor (PR) cells. However, the efficacy of this

approach may be limited by the inability of new PRs to correctly position and connect within the retina. We've developed an approach to track populations of rod PRs to determine the cell autonomous and non-autonomous mechanisms that regulate their positioning during development. Subretinal injections of lentivirus, encoding a fluorescent reporter and Cre recombinase, was used to label rod photoreceptors in post-natal $ROSA^{mTmG}$ pups. EdU was injected subcutaneously, immediately following subretinal injections, to label retinal progenitor cells (RPCs). Lenti-infected mouse retinas, beginning at post-natal day (P)0, were analyzed by immunohistochemistry (IHC) at several timepoints up to P14. P0 $Smo^{fl/fl}$ mice were simultaneously electroporated with empty-vector or Cre expressing plasmid and Lentivirus-RFP to target RPCs and post-mitotic rods, respectively. Rod soma positions were analyzed using sectioned and whole-mount retinas at P14. Rod soma position in P0-injected $Nrl^{-/-}$ and $Crx^{-/-}$ mice eyes were also analyzed at P14. Sparse labelling of temporally distinct post-mitotic rods demonstrates a stereotypic pattern of soma translocation and sublaminar positioning. P0-infected rod somas translocate basally from the outer limiting membrane toward the outer plexiform layer from P0 to P10. In contrast, EdU-labelled progenitor cell somas exhibit a rapid apical translocation followed by slow basal translocation. However, by P14, these EdU-labelled somas remain apically opposed to post-mitotic rod somas irrespective of the post-natal injection day suggesting that rod soma positioning is correlated with birth order. Conditional inactivation of smoothened in RPCs, consequently inhibiting Hedgehog induced mitogenic signalling, altered the final soma position of post-mitotic rods. This change in soma position suggests that rod soma translocation is regulated by non-cell autonomous mechanisms. Cell intrinsic regulators of photoreceptor maturation, such as Crx and Nrl were also assessed for impacts on rod soma translocation. In both $Crx^{-/-}$ and $Nrl^{-/-}$ retinas rod soma position within the ONL was disorganized. Both cell non-autonomous and cell autonomous factors mediate rod soma positioning. Understanding how photoreceptors migrate during development may reveal mechanisms implicated in disease and insights towards controlling these processes for the treatment of vision loss.

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Poster

295. Vision: Photoreceptors and Retina

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Topic: D.06. Vision

Support: RO1AR078663-01

Title: Sexual dimorphism of retinal thickness in development of the Ts65Dn mouse model of Down syndrome

Authors: *A. FOLZ[1], T. BELECKY-ADAMS[1], C. GOODLETT[2], R. ROPER[1];
[1]Biol., [2]Psychology, Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Individuals with Down syndrome (DS) present with a wide variety of phenotypes associated with the triplication of human chromosome 21 (Hsa21). Two of these phenotypes are a higher rate of refractive errors and increased retinal thickness when compared to the typically developing population. The cellular and molecular bases of altered retinal development in DS are not well understood, and identification of those mechanisms could provide new insights into mechanisms of neuronal development of individuals with DS.The Ts65Dn DS mouse model has a freely segregating segmental chromosome with approximately 50% of genes orthologous to Hsa21in three copies. Characterization of visual phenotypes in the Ts65Dn mouse model has been limited, but one constant is an increase in average retinal thickness, primarily evident in the inner retinal layers. Thus far, differences between male and female mice of trisomic Ts65Dn mice have not been studied. This study focused on determining whether there was a difference in retinal thickness between euploid and Ts65Dn trisomic mice, including sex as a variable.We hypothesized that retinal thickness at postnatal day (P)15 would be increased in the Ts65Dn mice compared to euploid mice and that sex differences may be present in the extent of the effect. To test this hypothesis, both male and female Ts65Dn and euploid eyes were dissected on P15, and retinas were sectioned at 14 microns and labeled with bipolar and retinal ganglion cell-specific antibodies. Using ImageJ, retinal thickness was measured from the outer edge of the photoreceptor outer segments to the inner edge of the ganglion cell layer, obtained at 200µm increments in both directions from the optic nerve. Stereology to determine retinal ganglion cell number is on-going. The retinal thickness was found to be significantly increased in the female trisomic mice as compared to female euploid mice at P15, but the male mice were not significantly different. There was also an increase in retinal thickness in the trisomic female mice when compared to the trisomic male mice, but there was no significant sex difference in the euploid mice.Although the retina and the brain are both composed of neurons derived from the embryonic neural tube, individuals with DS have hypocellularity in the brain and hypercellularity in the retina. This suggests that trisomy alters mechanisms involved in the proliferation, differentiation, and apoptosis of neurons in these different regions. By examining the mechanisms behind the increase in retinal thickness, we may find how trisomy alters the developmental regulation of the neurons of the retina and brain.

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Poster

295. Vision: Photoreceptors and Retina

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Program #/Poster #: 295.11

Topic: D.06. Vision

Title: Annual changes in dark adaptation over two-years for early/intermediate AMD and age-matched control patients.

Authors: *W. RIDDER, G. COMER, C. OQUINDO, P. YOSHINAGA, R. KHANKAN; Marshall B. Ketchum Univ., Marshall B. Ketchum Univ., Fullerton, CA

Abstract: Purpose: Previous reports have suggested that age-related macular degeneration (AMD) patients have abnormal dark adaptation. The purpose of this study was to investigate changes in dark adaptation over a two year follow up for early/intermediate AMD patients and age-matched normal patients**. Methods:** Thirty-five subjects initially took part in this study (20 AMD age 76.4 ± 9.60 and 15 normal controls age 73.4 ± 6.53; p = 0.28). Before the two-year visit, 3 normal and 7 AMD patients dropped out of the study. All subjects had a complete eye exam at each visit (VA, fields, OCT, fundus photos). The logMAR visual acuities for the test eye were not significantly different between the groups for the baseline visit (p = 0.57) or 1-year visit (p = 0.44) but were for the 2-year visit (p = 0.05). The AMD patients were grades 1 - 4 (simplified AREDS scale average baseline = 2.8 ± 1.02, year 1 = 3.0 ± 0.94, year 2 3.1 ± 0.93). The MacuLogix AdaptDx was used to measure dark adaptation. Visual acuity was determined with and without a 2-log unit neutral density filter. The subjects wore their optimal correction for all testing. **Results:** The rod intercept time from the dark adaptation test was significantly different at baseline (p < 0.001), year 1 (p < 0.001) and year 2 (p = 0.002) between the AMD and normal control subjects. The AMD and the normal control subjects rod intercept time did not change from baseline to year one or year 2 (all p values > 0.05). There was no significant difference between the AMD and control subjects for visual acuity or low luminance visual acuity until the 2-year visit (p = 0.05, p = 0.011, respectively). The visual fields were not different between the groups until the 2-year visit (mean deviation p = 0.001, pattern standard deviation p = 0.186, and foveal sensitivity p = 0.05). **Conclusions:** Dark adaptation was significantly different between the AMD and normal control subjects for each visit over the 2-years of this study. The visual acuity and low luminance visual acuity decreased for the AMD patients over the 2-years. Thus, as early/intermediate AMD progresses, peripheral functions are affected first followed by foveal functions.

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Poster

295. Vision: Photoreceptors and Retina

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UNADEV
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Variant

Title: Crx gene therapy for treating mouse models of dominant crx-associated retinopathies and beyond.

Authors: *J. E. ROGER[1], E.-K. GRELLIER[1], S. LOURDEL[1], C. LEGRAVEREND[1], M. PERRON[2];
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Abstract: Efficacy and safety of gene therapies for retinal diseases (RDs) have been proven with preclinical success translated into clinical effectiveness. However, this approach is rarely chosen for dominant forms. Based on our previous published work, we developed a mutation-independent AAV vector that could circumvent the clinical and genetic heterogeneity of *CRX* mutations in the transcription factor *CRX*. We also tested the neuroprotective potential for *CRX*-independent retinopathies. AAV-CRX is an AAV2/5 allowing the expression of human CRX specifically in photoreceptors, using Rhodopsin Kinase 1 promoter. The efficacy of AAV-CRX was assess by injecting at P30 either $Crx^{Rip/+}$ mice, a model of Leber Congenital Amaurosis, or $Tg(CRX^{R41W})$ mice, carrying the human CRX^{R41W} mutation causing cone dystrophy. The effects on CRX-independent RD was also tested in *rd10* mice by injecting at P14. The efficacy was assessed by immunohistochemistry, electroretinogram (ERG) and using a Dark/Light box test.AAV-CRX injection led to specific expression of CRX in photoreceptors with no toxicity. Three months after subretinal injection in $Crx^{Rip/+}$ mice, we observed: i) a rescue of rod and cone opsin expression, ii) a rescue of outer segment formation, iii) some degree of ERG response whereas it remained flat in controls iv) a fully restored behavioral response to light stress. $Tg(CRX^{R41W})$ characterization revealed a dose-dependent deleterious effect of CRX^{R41W} expression. Indeed, heterozygous $Tg(CRX^{R41W})$ carrying a single insertion displayed a functional retina while homozygous $Tg(CRX^{R41W})$ exhibited reduced retinal function after 3 months. These results support the relevance of increasing the amount of CRX^{WT} to counteract the dominant-negative effect of mutant *CRX*. The beneficial effects of AAV-CRX were observed in $Tg(CRX^{R41W})$ mutant mice with a cone only retina ($Nrl^{-/-}$ background). Finally, we showed that AAV-CRX has also a beneficial effect on *CRX*-independent RD by preserving rod photoreceptors in *rd10* mice. Overall, our gene therapy approach shows promising results for treating *CRX*-associated RDs, as well as *CRX*-independent retinopathies. It also highlights the potential interest of gene therapy to treat patients with RD carrying dominant-negative mutations.

Disclosures: **J.E. Roger:** A. Employment/Salary (full or part-time):; Retina france. 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.; Variant, ANR, UNADEV, Retina France. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/EP2020/056199, Variant. **E. Grellier:** A. Employment/Salary (full or part-time):; Retina france. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PCT/EP2020/056199. **S. Lourdel:** A. Employment/Salary (full or part-time):; Retina France. **C. Legraverend:** A. Employment/Salary (full or part-time):; CNRS. **M. Perron:** A. Employment/Salary (full or part-time):; CNRS.

Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.13

Topic: D.06. Vision

Support: UCLA BSCRC research award
Research to Prevent Blindness
Knights Templar Eye Foundation
Kingenstein Philanthropies
ARVO Foundation for Eye Research

Title: Molecular dissection of the foveal maturation in the marmoset retina

Authors: *L. ZHANG[1], M. CAVALLINI[1], R. XIN[1], Q. ZHANG[2], G. FENG[2], J. R. SANES[3], Y.-R. PENG[1];
[1]Dept. of Ophthalmology and Neurobiology, Univ. of California, Los Angeles, Stein Eye Inst., Los Angeles, CA; [2]Dept. of Brain and Cognitive Sci., McGovern Inst. for Brain Research, Massachusetts Inst. of Technol., Cambridge, MA; [3]Ctr. for Brain Sci. and Dept. of Mol. and Cell. Biology, Harvard Univ., Cambridge, MA

Abstract: High-acuity vision enables primates to perceive the visual world with high spatial and chromatic resolution. This function stems from the emergence of a specialized retinal structure, the “fovea”, present only in primates among mammals. Little is known about the molecular mechanism that governs formation and maturation of the fovea. In this study, we used single-cell RNA sequencing (scRNA-seq) and Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) to characterize postnatal maturation of the fovea in the common marmoset (*Callithrix jacchus*). We used scRNA-seq to analyze the marmoset foveal and peripheral retina at neonatal and adult stages. We profiled over 150k cells and identified over 60 cell types in each region at each stage. The foveal cell types are largely similar to those we previously identified in macaque and human retina, demonstrating a conservation in the cellular composition in the primate fovea. Comparison of cell types between neonates and adults showed that most cell types are fully differentiated at neonatal stage. We then asked which cell types account for the regional specificity. Interestingly, Müller glia and M/L-Cone showed the highest transcriptomic fovea-specificity at both neonatal and adult stages. This is consistent with the observation that the foveola center is formed only by cone and MG processes, both of which exhibited remarkable morphological and functional specializations. Thus, we derived region- and development- specific molecular signatures for these cell types. These signatures nominated molecular players and pathways underlying foveal cone postnatal elongation, outer segment development and packing. MG regional differences include genes implicated in regulation of growth, regulation of insulin-like growth factor and extracellular matrix organization. We then inferred the regulatory network underlying the regional differences in cones and validated most of the inferred transcription factors by ATAC-seq. To understand further how the interaction between MG and M/L-Cone might shape the fovea specification, we derived ligand-target gene signaling paths between them. We found fovea-specific genes of the MG, such as *CTGF* and

FGF9, might regulate M/L-Cone regional specificity via cell-cell communication. Altogether, our study provides molecular atlas of the marmoset retina and suggests potential molecular mechanism of postnatal fovea specification. Our study will serve as a framework for studying region specialization in complex neural structures.

Disclosures: **L. Zhang:** None. **M. Cavallini:** None. **R. Xin:** None. **Q. Zhang:** None. **G. Feng:** None. **J.R. Sanes:** None. **Y. Peng:** None.

Poster

295. Vision: Photoreceptors and Retina

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Program #/Poster #: 295.14

Topic: D.06. Vision

Support: NIHR
Moorfields eye charity
UCL
KCL

Title: Investigating a role for Wnts in promoting donor/host synaptic connectivity for transplantation of photoreceptors in advanced retinal degeneration.

Authors: *M. TARIQ, E. L. WEST, M. J. BRANCH, M. KHAZIM, M. BASCHE, C. PROCYK, A. J. SMITH, R. R. ALI, R. A. PEARSON;
Stem Cells & Regenerative Med., King's Col. London, London, United Kingdom

Abstract: Retinal degenerations lead to the irreversible loss of rod and cone photoreceptors, the cells responsible for detecting light. We have recently shown feasibility for rescue of visual function by transplantation of human pluripotent stem cell (hPSC) derived photoreceptors in murine models of advanced retinal degeneration. Nonetheless, the extent to which functional synapses are formed between the donor photoreceptors and host bipolar cells is relatively limited. To achieve comprehensive repair of the degenerated retinal circuitry, it is necessary to identify strategies to improve donor cell neurite outgrowth and synapse formation in transplantation.
In the developing retina, the role of Wnt proteins has been described in photoreceptor axonal outgrowth during the period of synapse formation in development. We seek to determine whether these developmental pathways can be used to promote donor/host synaptic connectivity following transplantation of photoreceptors into advanced models of retinal degeneration, by re-expressing candidate molecules into the adult host retinal environment.
A neurite growth assay for postmitotic murine rod photoreceptors showed upon bath-application of Wnt5a/b there was a significant increase in both the length of photoreceptor neurites (33.7μm±15.1) compared to controls (19.1μm±9.5; $P < 0.001$) and in the proportion of photoreceptors that formed neurites (86.4%±5.6) compared to control (34.4%±17.1; $P < 0.001$);

the latter finding has not been previously reported. A method to isolate and culture hPSC-derived cone photoreceptors was developed, and upon bath-application of Wnt9a there was a significant increase in the length of cone photoreceptor neurites (71.6μm±31.5) compared to control (34.6μm±14.5; $P < 0.001$).
To further explore the pathways regulating photoreceptor neurite outgrowth and photoreceptor-bipolar cell synapse formation, a microfluidic device has been designed to spatially separate the two cell types and provide a synaptic chamber, an arrangement analogous to the cellular and synaptic laminae of the retina. For the isolation and subsequent culture of bipolar cells, we have identified a novel method using CD15, a cell surface marker, resulting in a highly enriched bipolar cell population.
These results strongly indicate a role for Wnt signalling in the neurite extension and synaptic connectivity between photoreceptors and bipolar cells and the methodologies established here will allow for further investigation. To determine if this strategy can improve photoreceptor transplantation, we will establish the over-expression of Wnts in vivo followed by cell transplantation.

Disclosures: **M. Tariq:** None. **E.L. West:** None. **M.J. Branch:** None. **M. Khazim:** None. **M. Basche:** None. **C. Procyk:** None. **A.J. Smith:** None. **R.R. Ali:** None. **R.A. Pearson:** None.

Poster

295. Vision: Photoreceptors and Retina

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Topic: D.06. Vision

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BrightFocus Foundation
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Pilot Grant from Wisconsin National Primate Research Center
McPherson Eye Research Institute's David and Nancy Walsh Professorship in Vision Research

Title: Organization and connectivity of the rod photoreceptor synapse across different locations in the primate retina

Authors: *K. MIAN[1], J. ZUNIGA[2], A. SAHA[3,4,5], P. J. DERR[8], M. HOON[6,5], R. SINHA[3,5,7]; [2]Neurosci., [3]Dept. of Neurosci., [4]Cell. & Mol. Biol. Grad. Training Program, [5]McPherson Eye Res. Inst., [6]Ophthalmology and Visual Sci., [7]Dept. of Ophthalmology & Visual Sci., [1]Univ. of Wisconsin, Madison, Madison, WI; [8]Dept. of Neurobio., Harvard Med. Sch., Boston, MA

Abstract: Rod photoreceptors mediate dim light vision and convey their signals to second-order neurons, rod bipolar cells (RBCs), through the well-characterized triad synapse in the mammalian retina. This synapse is unique to the retina and is composed of two lateral processes from horizontal cells (HC) and a central invaginating process from an RBC. On the presynaptic side, the rod axon terminal consists of a specialized ribbon structure. Unlike conventional synapses that signal via action potentials, ribbon synapses convey information through graded release of glutamate. The synaptic "unit" of ribbons consists of the presynaptic ribbon at its active site with postsynaptic partners, HCs and RBCs, that receive the graded signal. Each ribbon can have one or more synaptic units. A hallmark of the primate retina is an anatomical specialization called the fovea, which is in the center of the retina and lacks rods. As the distance from the fovea increases, rod density first increases in the parafovea (~1-2 mm) and central retina (~3-4 mm) before decreasing again in the peripheral retina (>6 mm). The connectivity between rods and RBCs is understood, but how the number of rods synapsing onto a single bipolar cell (convergence), and how the number of bipolar cells synapsing with a single rod (divergence) changes across primate retinal regions remains unknown. Furthermore, variations in ribbon number, length, and synaptic unit have not yet been estimated in detail across retinal regions. This study aims to determine these differences in outer retinal connectivity to better understand signaling and sensitivity in the primate dim light pathway across retinal locations. To assess these differences, we performed serial block-face scanning electron microscopy and reconstructed neurons and their connectivity at the subcellular level. Our results indicate that rod terminal volume changes depending on retinal location. Additionally, the convergence ratio of rods to RBCs changes across the retina, with the highest degree of convergence in the peripheral retina and the lowest degree of convergence in the parafovea. Changes in the divergence of the dim light pathway were observed for peripheral rods that contacted a higher number of RBCs than rods in other regions. These differences in convergence and divergence across retinal locations are accompanied by significant changes in the number of rod photoreceptor ribbons and their synaptic units across regions. Altogether, these findings have implications for how the sensitivity of the primary dim light pathway may potentially differ across primate retinal locations due to changes in the synaptic connectivity at the first retinal synapse.

Disclosures: K. Mian: None. **J. Zuniga:** None. **A. Saha:** None. **P.J. Derr:** None. **M. Hoon:** None. **R. Sinha:** None.

Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.16

Topic: D.06. Vision

Title: A characterization of a mouse model of age-related macular degeneration (AMD) generated by laser-Induced choroidal neovascularization and pharmacological improvement by aflibercept

Authors: *K. PARK, H. PARK, Y. KIM, A. LEE, H. LEE, M. LEE, A. LEE, T. LEE, J. E. FRIEDMAN, P. J. SWEENEY, L. C. PARK;
Naason Sci., Cheongju-si, Korea, Republic of

Abstract: Age-related macular degeneration (AMD) is a medical condition in which damage in the macula of the retina results in blurred or loss of vision. Out of the two types of AMD, dry and wet, wet AMD is responsible for 90 % of AMD-associated vision loss. Wet AMD is caused by abnormal expression of vascular endothelial growth factor (VEGF) results in choroidal neovascularization. These abnormal blood vessels can rupture the Bruch membrane and disturb the retinal pigment epithelium (RPE) layer, damaging photoreceptors and leading to vision loss. The therapeutic effect of aflibercept (Eylea™, Bayer, Germany) on CNV progression was evaluated. CNV was induced in male C57BL/6 mice (n=8 per group) by photocoagulation with the 532 nm laser (200 mW, 100 ms duration, 50 μm spot size, Iridex Oculight Tx, Mountain View, USA) in one eye. Immediately after photocoagulation, the control group was injected with PBS, and the treatment group was injected with aflibercept (2 mg/ml), both via intravitreal injection using a 25 μl Hamilton syringe. 7 and 14 days after laser model generation, the CNVs were visualized using optical coherence tomography (OCT) and fluorescein angiography (FA) using the Spectralis HRA-OCT device (Heidelberg Engineering, Heidelberg, Germany). At the end of the study, the mice were euthanized, and the eyes were flat-mounted for immunohistochemical analyses. Treatment with aflibercept (n=8 per group) led to reduction of CNV volume by 60% compared to the group treated with PBS measured by OCT imaging. Aflibercept treatment also reduced the intensity of leaks observed by FA. At the end of the study, the CNV area measured by immunohistochemical staining with isolectin B4 was significantly reduced in the group treated with aflibercept compared to the group treated with PBS. The area of retinal fibrosis measured by collagen I staining was also reduced in the group treated with aflibercept by a lesser extent compared to isolectin B4. In addition, the longitudinal effects of wet AMD and the treatment with Aflibercept on a daily behavioral performance including circadian rhythm are evaluated in their home cage using the automated home cage analysis system. In conclusion, we showed that aflibercept was effective on the laser-induced CNV model for wet AMD using in vivo and ex vivo analyses. The laser induced CNV model provides a multifaceted preclinical model for wet AMD.

Disclosures: K. Park: None. **H. Park:** None. **Y. Kim:** None. **A. Lee:** None. **H. Lee:** None. **M. Lee:** None. **A. Lee:** None. **T. Lee:** None. **J.E. Friedman:** None. **P.J. Sweeney:** None. **L.C. Park:** None.

Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.17

Topic: D.06. Vision

Support: Department of Biotechnology India Grant

Title: Lipid Metabolilizing genes modulate Anti-VEGF response in Age-related macular degeneration.

Authors: *A. ANAND[1], K. SHARMA[1], R. MANKOO[2], S. SHARMA[2], N. SHARMA[3];
[1]Dept. of Neurology, P.G.I.M.E.R, P.G.I.M.E.R, Chandigarh, India; [2]PGIMER, Chandigarh, India; [3]noble life sciences, Maryland, MD

Abstract: Age related macular degeneration is a multifactorial disease. SNPs in certain genes have been associated with the disease. Wet form of the disease is treated by intra-vitreal administration of anti-VEGF injections . The response to the treatment varies among individuals depending upon unknown factors. In this study we have investigated the association between genetic variation and anti-VEGF response in AMD patients. For this study 78 AMD patients were recruited from Advanced Eye Centre, PGIMER Chandigarh. Details of Anti-VEGF treatment was noted for each patient. Blood sample was collected from the patients for isolation of serum & DNA. Retrospective record of administration of Anti-VEGF therapy was consulted for 11 patients. SNP genotyping (rs920915), LIPC (rs920915) and APOE (rs769449), pro-angiogenic genes including ADAMTS9 (rs6795735) and TIMP3 (rs5749482), regulatory genes e.g. B3GALTL (rs9542236), IER3 (rs3130783), HTRA1 (rs11200638) & SLC16A8 (rs8135665, monocarboxylic transporter protein) was done using Taqman assays and ELISA was used to estimate their proteins in Serum.We found that Alcohol intake and previous cataract surgery were modulators of Anti-VEGF response. B3GALTL and LIPC variants were associated with Anti-VEGF response in Indian AMD patients. Homozygous ‘CC’genotype of LIPC variant show enhanced expression of regulatory (HTRA1, B3GALTL and IER3), monocarboxylic transporter protein SLC16A8, & LIPC levels. We also found that APOE levels increased in patients with successive VEGF injections. The results show significance of lipid metabolizing molecules (including APOE & LIPC) in AMD which can influence the Anti-VEGF outcome in AMD patients.

Disclosures: A. Anand: None. **K. Sharma:** None. **R. Mankoo:** None. **S. Sharma:** None. **N. Sharma:** None.

Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.18

Topic: D.06. Vision

Support: 4VA-Foundation minigrant

Title: Characterizing the effects of light on the early onset photoreceptor function loss in Mucolipidosis type IV

Authors: R. CISTULLI, M. PAMONAG, ***M. WALKER**;
James Madison Univ., Harrisonburg, VA

Abstract: Purpose: In this study we are testing the changes in gene expression that underlie early photoreceptor function loss in the Mucolipidosis type IV (MLIV) retinal degeneration model. Retinal degenerative diseases (RDDs) are a diverse group of disorders that are responsible for loss of vision in 2.2 billion people worldwide. The genetic origins of RDDs are heterogenous, with mutations in more than 200 genes resulting in the loss of photoreceptor function which leads to photoreceptor cell death and retinal degeneration. We hypothesize that in the MLIV mice, the loss of function in the retina is driven by downregulation of key photoreceptor proteins and disruption to intracellular trafficking of phototransduction components. Our research aims to elucidate the pathophysiology that drives early onset changes in photoreceptor protein expression and localization in the MLIV retina. Methods: To measure loss of retinal function, we recorded the activity within the retina using electroretinograms. We also used western blots to measure protein expression and immunohistochemistry to visualize protein mislocalization. We are also using RNAseq to test differential expression within the MLIV retina at 6 weeks. Results: Our results show that photoreceptor function loss begins at 6 weeks and there is a decrease in expression of key phototransduction components such as rhodopsin, cyclic nucleotide gated (CNG) channels, and BBS4 protein in rod photoreceptors of MLIV mice. In addition to a decrease in expression, we also observed mislocalization of rhodopsin in the inner segment of photoreceptors. Conclusion: We are able to measure a functional change in photoreceptors that precedes cell loss. These changes result in decreased expression and mislocalization of phototransduction components in the MLIV retina. This study may lead to the identification of early photoreceptor disease markers for detecting retinal degeneration.

Disclosures: R. Cistulli: None. **M. Pamonag:** None. **M. Walker:** None.

Poster

295. Vision: Photoreceptors and Retina

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Program #/Poster #: 295.19

Topic: D.06. Vision

Support: CIRM
Research to prevent blindness
National Eye Institute
US Department of VA
VRSF
Bright Focus Foundation

Title: Network biology analysis of P23H rhodopsin interactome suggests novel protein degradation steps

Authors: *L. SAFARTA[1,2], K. KIM[1,2], J. CHIANG[3], J. COPPINGER[4], E.-J. LEE[1,2,5], J. LIN[1,2,6];
[1]Pathology, [2]Ophthalmology, Stanford Univ. Sch. of Med., Palo Alto, CA; [3]Neurobio., Okinawa

Inst. of Sci. and Technol. Grad. Univ., Okinawa, Japan; [4]Sch. of Pharm. and Biomolecular Sci., Royal Col. of Surgeons, Dublin, Ireland; [5]Ophthalmology, USC, Los Angeles, CA; [6]VA Palo Alto Hlth. Care Syst., Palo alto, CA

Abstract: Retinitis Pigmentosa (RP) is a blinding disease that involves degeneration of rod photoreceptor cells. A leading cause of autosomal dominant RP is the P23H rhodopsin (Rho) mutation. In this study, we use the P23H Rho knock-in mouse, which develops retinal degeneration that mirrors RP phenotype in patients carrying the orthologous variant. The P23H mutation in the rhodopsin gene causes a misfolded from of the rhodopsin protein. The mechanisms by which rods recognize and target misfolded rhodopsin for degradation are not fully understood. Here, we performed liquid chromatography with tandem mass spectrometry (LC-MS/MS) on P23H rhodopsin immunopurified from $Rho^{P23H/P23H}$ mice retinas to identify interacting proteins and compared the results with wild-type (WT) rhodopsin from $Rho^{+/+}$ mice. As a negative control, LC-MS/MS was performed on MEF cells. Spectral counts from WT, P23H, and MEF cells were recorded. We found 276 proteins associated to WT rhodopsin and 286 proteins associated to P23H rhodopsin. 113 proteins were common between both interactomes. We manually sorted and accessed publicly available databases to further understand our results. Resulting protein list from LC-MS/MS was input into the web-based application, gProfiler, executing enrichment analysis for Gene Ontology, Reactome, and the Kyoto Encyclopedia of Genes and Genomes. Further enrichment visualization was created through the Cytoscape plug-in, Enrichment Map. Our results suggest loss of essential rod functions, such as phototransduction, normal rhodopsin protein trafficking, and the retinal cycle, due to the P23H rhodopsin mutation. Using our list, we found evidence that protein quality control mechanisms such as ER associated degradation, ubiquitination, translation, and ER stress are significantly enriched in the P23H rhodopsin interactome. To identify important regulatory proteins within the P23H rhodopsin interactome, we generated protein-protein interaction networks of the proteins that interacted solely with P23H rhodopsin using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins). From this, we saw ribosomal, and ribosome associated quality control proteins with high connectivity, suggesting a novel step in P23H rhodopsin clearance. Lastly, we organized the two interactomes based on essential cellular compartments in the rod photoreceptor cell. P23H rhodopsin interactome shows enrichment of proteins from abnormal cellular compartments such as the synapse, axon, cytoskeleton, and ribosome.

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Poster

295. Vision: Photoreceptors and Retina

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Program #/Poster #: 295.20

Topic: D.06. Vision

Support: R01ActDC016295

Title: Dual AAV mediated delivery of PCDH15 in the neurosensory retinal cells rescues pathophysiology in an Usher syndrome animal model

Authors: *S. RIAZ[1], S. SETHNA[1], T. DUNCAN[2], T. M. REDMOND[2], S. RIAZUDDIN[1], L. CARVALHO[3], Z. M. AHMED[1];
[1]Otorhinolaryngology Head and Neck Surgery, Sch. of Medicine, Univ. of Maryland, Baltimore, MD; [2]Lab. of Retinal Cell and Mol. Biol., Natl. Eye Institute, Natl. Inst. of Hlth., Bethesda, MD; [3]Univ. of Western Australia, Lions Eye Institute, Univ. of Western Australia, Nedlands, Australia

Abstract: Abstract: Usher syndrome type 1 (USH1) is a neurosensory disorder characterized by hearing loss (HL), balance problems, and vision loss due to retinitis pigmentosa (RP). Amongst the known causative factors, variants in the PCDH15 gene are responsible for USH type 1F (USH1F). The p.R245X variant of PCDH15 (equivalent to p.R250X in mice), is the most common cause of USH1 in Ashkenazi Jews, with a carrier frequency of ~2%. The Pcdh15 knock-in mice ($Pcdh15^{KI}$) have profound HL by postnatal day 16 (P16), balance problems, and significantly attenuated visual function, assessed via electroretinography (ERG), with no apparent degeneration of retinal sensory cells. Herein, we investigated adeno-associated vector (AAV) based PCDH15 gene delivery as a potential therapeutic approach to improve the visual function in $Pcdh15^{KI}$ mice. Due to the size of PCDH15 and AAV's packaging capacity, we developed a dual AAV system where the human PCDH15 gene was split into two halves and packaged in separate AAV-Anc80L65 particles. We assessed the ability of the dual AAV PCDH15 system to restore expression of full length protocadherin-15, in $Pcdh15^{KI}$ mice-derived mesenchymal stem cells (MSCs), as well as in vivo subretinally injected retinae by immunofluorescence and immunoblotting. Retina function was assessed using ERG, while retinoid oximes were measured by HPLC. Our dual AAV based delivery of PCDH15 restored the expression of full length Protocadherin-15 in the KI MSCs, as well as in the neurosensory cells of the retina of the $Pcdh15^{KI}$ mice. Furthermore, subretinal delivery of PCDH15 in knock-in mice significantly improved ERG amplitudes at 1-, 2- and 3-months post injection, suggesting improvement in the functional activity of photoreceptors. At the molecular level, we found restoration of expression of RPE-specific visual retinoid cycle proteins, RPE65 and CRALBP, to WT levels. In parallel, quantification of retinoid oximes showed improved levels of chromophores in $Pcdh15^{KI}$ mice. Finally, we also observed restoration of light-mediated translocation of phototransduction cascade proteins, arrestin and transducin. In summary, our dual AAV-based PCDH15 delivery in the mutant mice retinae demonstrated functional and molecular restoration. Thus, dual AAV-PCDH15 vectors based gene replacement therapy may be a promising approach to treat blindness in USH1F patients in future.

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Poster

295. Vision: Photoreceptors and Retina

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 295.21

Topic: D.06. Vision

Title: Characterization of tecuzitécatl (tecu) mutants in behavioral paradigms.

Authors: *L. LUJANO PEREZ[1], J. RIESGO ESCOVAR[2];
[2]Lab. de Transducción de Señales, [1]Inst. De Neurobiología, UNAM Inst. De Neurobiologia, Queretaro, Mexico

Abstract: Characterization of tecuzitécatl (tecu) mutants in behavioral paradigms.**Authors**Laura Alejandra Lujano-Pérez[1], Juan Rafael Riesgo-Escovar[21,2] Instituto de Neurobiología, Universidad Nacional Autónoma de México campus Juriquilla, Querétaro, Mexico.**Disclosures**L.A. Lujano: None. J.R. Riesgo: None.
The brain of *Drosophila melanogaster* (*D. melanogaster*) processes information obtained from different sensory stimuli during its lifetime. Information from various sensory modalities, such as olfaction and vision, flow from the sensory organs to the central nervous system, and are at least partially processed in the brain Mushroom Bodies (MBs). For this study we isolated mutations in a gene we named *tecuzitécatl* (*tecu*), that codes for a secreted phospholipase A_2 (sPA_2) enzyme with a very reduced expression pattern. We show that mutations in this gene lead to faulty behavioral responses in visual paradigms in larvae and adults. We directly compared responses of control (*yw*) and two mutant *tecu* strains: $tecu^1$ and $tecu^2$, both of which have P element insertions within the locus. Both mutants share the same genetic background with the isogenized *yw* control strain. We performed larval phototaxis assays. Results show that mutant strains have significantly different responses (migrate more towards the light), whilst control larvae exhibit negative phototaxis. We then performed adult countercurrent assays, where mutant adult flies also have a defective response to light (mutant flies have significantly less positive phototaxis compared to the control). To elucidate where *tecu* function is needed, we performed electroretinograms on control and mutant flies. Preliminary results from both mutant alleles are consistent with a compromised synaptic communication between the photoreceptors and the laminar interneurons (L1 and L2) of the optic lobes of the brain. Taken together, these results show that *tecu* has an important role processing visual responses in *D. melanogaster*.

Disclosures: L. Lujano Perez: None. **J. Riesgo Escovar:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

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Program #/Poster #: 296.01

Title: WITHDRAWN

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.02

Topic: D.06. Vision

Title: Molecular characterization of retinal cell types in the lamprey retina

Authors: *Y.-R. PENG[1], L. ZHANG[1], A. MORSHEDIAN[1], S. GUCUM[1], A. P. SAMPATH[2], G. L. FAIN[3], A. PAHLEVAN[1];
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Abstract: Vision begins in theretina; vertebrates are known to share a common retinal plan composed of multipleclasses of neurons organized in layers to process visual information. It isgenerally understood that the vertebrate retina contains six main cell classes—rodand cone photoreceptors, horizontal cells, bipolar cells, amacrine cells,retinal ganglion cells, and muller glia. However, it remains unknown whetherall cell classes emerge from the conserved retinal program of ancestral vertebrates,or what cell types are contained in individual cell classes. In this study, weused single-cell RNA-sequencing (scRNA-seq) to profile the retinal cells of thesea lamprey (*Petromyzon marinus*), the oldest living vertebrate that is closestto an ancestral vertebrate. We sequenced over 22, 000 cells and detected allsix retinal classes. This suggests that the main cell classes have already beenspecified for vertebrates. Interestingly, transcription factors that are knownto determine the fate of some cell classes in mammals are found in the lamprey.Furthermore, we identified over 58 cell types from the six cell classes. On theone hand, we found strikingly conserved cell types between the lamprey and othervertebrates, such as mice and humans. On the other hand, we identified divergentgene programs in cell types that are unique to the lamprey. Therefore, thedataset provides a major resource to investigate the evolutionary basis of thecellular composition of the vertebrate retina.

Disclosures: Y. Peng: None. **L. Zhang:** None. **A. Morshedian:** None. **S. Gucum:** None. **A.P. Sampath:** None. **G.L. Fain:** None. **A. Pahlevan:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Title: Cell types and molecular architecture of the octopus visual system

Authors: *J. O. SONGCO-CASEY[1], G. C. COFFING[2], D. M. PISCOPO[1], J. R. PUNGOR[1], A. D. KERN[2], A. C. MILLER[1], C. M. NIELL[1];
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Abstract: Cephalopods have a remarkable visual system, with a camera-type eye, high acuity vision, and a wide range of sophisticated visual behaviors. However, the cephalopod brain is organized dramatically differently from that of vertebrates, as well as other invertebrates, and little is known regarding the cell types and molecular determinants of their visual system organization beyond neuroanatomical descriptions. Here we present a comprehensive single-cell molecular atlas of the octopus optic lobe, which is the primary visual processing structure in the cephalopod brain. We combined single-cell RNA sequencing with RNA fluorescence in situ hybridization to both identify putative molecular cell types and determine their anatomical and spatial organization within the optic lobe. Our results reveal six major neuronal cell classes identified by neurotransmitter/neuropeptide usage, in addition to non-neuronal and immature neuronal populations. Moreover, we find that additional markers divide these neuronal classes into subtypes with distinct anatomical localizations, revealing cell type diversity and a detailed laminar organization within the optic lobe. We also delineate the immature neurons within this continuously growing tissue into subtypes defined by evolutionarily conserved fate specification genes as well as novel cephalopod- and octopus- specific genes. Together, these findings outline the organizational logic of the octopus visual system, based on functional determinants, laminar identity, and developmental markers/pathways. The resulting atlas presented here delineates the "parts list" of the neural circuits used for vision in octopus, providing a platform for investigations into the development and function of the octopus visual system as well as the evolution of visual processing.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.04

Topic: D.06. Vision

Title: Alternative vision: Neuronal processing underlying polarization vision of cephalopod squid

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Abstract: Across the entire animal kingdom, animals from only three major groups evolved to have visions with high spatial resolution: cephalopods, vertebrates and arthropods. Different from arthropod eyes, only cephalopods and vertebrates adopted camera-type eye structures. Yet over 500 million years of independent evolution, the cephalopod visual system is everted and incorporates only one clear layer of nuclei of retinal cells that sense polarized light and generate action potentials with axons directly projecting to the optic lobe. Previous works focused on both the anatomy of the cephalopod retina and on the population of neuronal responses to the polarization direction. However, it remains unknown whether these retinal cells are independently sensing photons or acting coordinately with each other. Here, I performed multi-electrode array recordings from squid retinas by injecting light with constant and alternative polarization directions. My preliminary results show that squid retinal cells tend to respond stronger to flashes with changing polarization, suggesting a retinal process that involves a coordination of nearby cells. This work starts to uncover the neuronal codes of an independently evolved visual system that shares similarities with the camera-like eye structure, and potentially providing deeper insights into our understanding of the neuronal codes of retinal processes during visual perception.

Disclosures: J. Cai: None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

Support: Pfizer NCBiotech Postdoctoral Fellowship
Duke Institute for Brain Sciences Incubator Award

Title: Development of viral vectors for cross-species comparisons of collicular projecting retinal ganglion cells

Authors: *M. BOHLEN[1], A. RUDZITE[2], S. ROY[3], T. B. DAW[2], G. KUCZEWSKI[4], M. A. SOMMER[4], G. D. FIELD[3];
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Abstract: The superior colliculus (SC) is a major target of retinal ganglion cell (RGC) axons. However, our understanding of the morphological and functional diversity of retinotectal RGCs remains limited. Furthermore, we have limited understanding for how retinal input shapes signal processing in the SC. Specifically, what RGC types innervate the SC, what signals do they transmit about visual scenes, how conserved are these signals across species and how do these

signals influence behavior? Answering these questions requires tools for reliably labeling and manipulating neurons in species other than just mice. To overcome this limitation, we have been developing viral techniques in rats and non-human primates to label and ultimately manipulate signaling from retina to the SC. To tackle these fundamental questions, we have been testing viral vectors for their capacity to efficiently deliver and drive transgene expression in RGCs following superior collicular injections. Second, we seek to use these viral constructs to deliver fluorescent and neuronal actuator proteins to RGCs, and then use these transgenes to morphologically and physiologically define retinotectal RGC types in rats and primates. The outcome of these efforts has been viral constructs that are efficient at retrogradely delivering exogenous genes to RGCs in macaque and rat following injections into superficial SC. One capsid in particular, rAAV2-retro has been particularly effective at delivering transgenes to retinotectal RGCs. rAAV2-retro has produced a trove of morphological data on retinotectal RGC types in rat and primate. From the anatomical perspective, we have identified several previously described RGCs in both species that were not known to project to the SC. In general, when effective, these viruses provide approximately complete fills of transduced RGCs. The initial data produced from these injections suggested that a greater diversity of RGCs may project to the SC than previously appreciated. We also believe that it is likely that these viral constructs will be effective at labeling RGCs that project to the other brain regions and in other species. We will be examining these possibilities soon.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

Support: Duke Institute for Brain Sciences Incubator award

Title: Determining the visual signals sent by retinal ganglion cells to the superior colliculus

Authors: *A. RUDZITE[1], M. O. BOHLEN[6], S. ROY[2], M. NARAIN[3], T. B. DAW[4], G. KUCZEWSKI[3], G. D. FIELD[2], M. A. SOMMER[5];
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Abstract: The rodent retina sends visual information to the brain using ~40 distinct retinal ganglion cell (RGC) types. One major recipient of retinal input is a midbrain structure called the superior colliculus (SC). Classically, the SC is associated with orienting responses and sensory-motor integration, but recent work has also implicated it in attention and decision making. Despite the major projection from the retina to SC and its key role in various behaviors, our

understanding of the diversity and function of RGC input remains limited. This project aims to identify the morphological and functional diversity of retinotectal RGCs using techniques that can be utilized across a wide range of mammalian speciesWe made bilateral injections of rAAV2-retro into the SC of Long-Evans rats. The viral construct expressed mCitrine, a fluorescent reporter, and ReaChR, a red-shifted channelrhodopsin2 variant. We harvested retinas after a survival period and made ex-vivo measurements of RGC visual responses using a large-scale, multi-electrode array (MEA). Several visual stimuli were presented to functionally classify RGC responses, including checkerboard noise, drifting gratings, and full-field light steps. After measuring visual responses, L-AP4 (100 µM), DNQX (100 µM), and L-AP5 (100 µM) were bath applied to block synaptic transmission from photoreceptors to bipolar and horizontal cells. The success of pharmacological block was determined by eliminating visual responses to 100% contrast steps. Then bright, full-field flashes of 560nm light were presented using an LED to drive ReaChR responses in infected retinotectal RGCs. Following physiological measurements on the MEA, each retina was fixed using 4% PFA and then immunolabeled for confocal imaging. Morphological classification uses previous classification schemes (Sun et al., 2002b).We are in the process of identifying a diverse group of morphological and physiological retinotectal RGCs. For several center-surround receptive field RGC types, we have observed a nearly complete mosaic of receptive fields and dendritic fields, indicating an irreducible cell type. We have also identified direction-selective and orientation-selective retinotectal RGC types.We have developed a pipeline for identifying the function and morphology and RGCs that project to the SC. This pipeline does not rely on transgenic animals and thus holds the promise of being useful across a wide range of vertebrates. Indeed, initial testing of the viral construct in non-human primates indicates high transfection efficiency. Furthermore, this approach may prove useful in identifying RGC inputs to other brain areas in diverse species.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Title: The manifold organization of mouse retinal ganglion cell types

Authors: L. DYBALLA[1], A. RUDZITE[2], M. P. STRYKER[4], G. D. FIELD[3], *S. ZUCKER[1]; [1]Yale Univ., New Haven, CT; [2]Neurobio., [3]Dept. of Neurobio., Duke Univ., Durham, NC; [4]Ctr. for Integrative Neurosci, Dept Physiol, Univ. of California San Francisco, San Francisco, CA

Abstract: There are ~45 distinct types of retinal ganglion cells (RGCs) in mouse, which can be functionally classified by responses to visual stimuli (e.g. white noise and drifting gratings). Previous analyses of cell types used clustering algorithms that emphasize differences but frequently obscure similarities. We introduce a complementary approach using machine learning to infer a manifold, a kind of high-dimensional surface, of retinal ganglion cell responses. Instead of "pushing" distinct groups apart (e.g. tSNE), the manifold structure is defined by similarities. Each point in the manifold is an RGC, and cells that respond with similar dynamics to the stimulus ensemble are placed near one another. The advantage of this approach is that clustering is not artificially induced and that functionally related cell types which share e.g. common inputs can be identified.

We measured visual responses of (n = 1200) RGCs from ex vivo (N = 4) mouse retina using a large-scale multielectrode array. Stimuli included moving gratings and optic flows built from either dark or light dots or short line segments (Dyballa et al. PNAS 2018). Stimuli subtending ~60°×40° were presented for 1.25 sec separated by 0.75-sec mid-gray screens. Responses to each stimulus class were summarized by a histogram across time. Diffusion maps were used to construct a manifold that summarized how each RGC, within the context of other RGCs, responded to the stimulus ensemble.

The resulting manifolds have a distinctive structure. There are only a few disconnected components, e.g. ON, monophasic small RGCs, consistent with the clustering approach. But the bulk of cells define a highly articulated manifold (dimension ~5) consisting of a dense core from which a number of extended spike-shaped protrusions emerge. ON brisk transient and ON brisk sustained are organized along one protrusion, which attaches to a core consisting of most other RGC types.

We speculate that similarity in position on the manifold reflects similarity in circuitry or intrinsic properties or, alternatively, in how the stimuli drive responses across diverse RGC types. Our approach can incorporate additional stimuli, the responses to which may further separate the neurons in the dense core.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

Support: NIH grant EY031396

Title: Simple and complex orientation selective ganglion cells in the retina

Authors: *T. B. DAW, M. THAPA, K. RUDA, G. D. FIELD;
Dept. of Neurobio., Duke Univ., Durham, NC

Abstract: Extracting oriented edges in visual scenes is a fundamental and highly conserved computation in the visual system. Orientation selective (OS) ganglion cells (OS-RGCs) have been identified in the retina, but many features of their responses remain unclear. We sought to understand the receptive field (RF) structure of OS-RGCs, whether OS is maintained across rod and cone vision, and the biological and computational mechanisms contributing to OS in the retina. We measured RGC responses to visual stimuli from ex vivo rat retinas using a multi-electrode array. We used drifting gratings to identify OS-RGCs and determine spatial frequency tuning. We used checkerboard noise to estimate RF structure. Linear-nonlinear (LN) and nonlinear input models (NIM) were used to determine the extent to which OS-RGCs perform linear spatial integration. Consistent with previous work, we found OS-RGCs with peak tuning along either the horizontal or vertical meridians. However, among OS-RGCs, we identified at least two distinct types of RFs. Some OS-RGCs exhibited RFs reminiscent of cortical simple cells: their responses had narrow spatial frequency tuning and were highly phase-dependent. Importantly, they had an OFF-center RF flanked by ON-responsive zones, producing an even-symmetric RF that accurately predicted visual responses, indicating relatively linear spatial integration. Other OS-RGCs exhibited RFs similar to cortical complex cells: their responses had broad spatial frequency tuning that weakly depended on phase. Furthermore, like cortical complex cells, their linear RF had a speckled organization with small ON and OFF subzones that poorly predicted their visual responses, indicating nonlinear spatial processing. Both kinds of OS-RGCs maintained tuning down to rod-mediated light levels, and tuning was largely eliminated in both cell types by blocking GABAa receptors. Modeling results demonstrated that, compared to the LN model, an NIM that included one suppressive and three excitatory filters more accurately captured visual processing of OS-RGCs, particularly the nonlinear computations of complex cells. Overall, we demonstrate two distinct RF structures among OS-RGCs that parallel the division of simple and complex cells in primary visual cortex. These results suggest that simple and complex OS tuning emerges first in the retina, at least in rodents. Both forms of tuning span rod to cone vision. Finally, both forms of tuning depend on GABAa receptors, suggesting that they are mediated by feedforward inhibition onto RGCs. Future work aims to understand the projections of OS-RGCs and their responses to natural stimuli.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

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Title: Revealing nonlinear computations in the retina using random motion stimuli

Authors: *M. BÜTTNER[1,2], M. ZNIDARIC[1,3], R. DIGGELMANN[1,3], A. BUCCI[1,3], A. HIERLEMANN[3], F. FRANKE[1];
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Abstract: Visual processing starts in the retina where photons are encoded into spiking responses of Retinal Ganglion Cells (RGCs) before they are sent to brain areas further downstream. Traditionally the study of this process is conducted by analysing RGC responses to a multitude of light stimuli, such as full-field flashes, moving bars, and white noise. However, it has been shown that many RGCs of the mouse retina do not respond well to these stimuli. Furthermore, receptive field (RF) estimation using white noise analysis fails if a neuron primarily encodes a nonlinear function of the pixel intensities, e.g. the direction of movement. Here we present an analysis framework of RGC responses to a stimulus consisting of randomly moving objects (RMO), drawn from a white parameter space defining an object's location of appearance, size, color, speed, and direction of movement. Albeit the stimulus itself is high-dimensional and has strong spatio-temporal correlations, we were able to compute RFs of both linearly and nonlinearly (e.g. direction-selective (DS) cells) responding RGCs efficiently by reverse correlating against a sparse matrix representation of the stimulus, which describes the presence or absence of an object in space and time. We then estimated a neuron's tuning for object parameters by analysing its responses to objects moving over its RF. We extend this approach to analyse nonlinear response behaviours due to second-order interactions of temporally close objects. Using a data set of high-density multielectrode recordings of the ex-vivo mouse retina (n=5734 RGCs, 4 retinae), we identified nearly three times the number of RFs with the RMO compared to white noise using the same stimulus duration. Moreover, substantially more cells responded to the RMO (n=4148) than to either one of the classical stimuli, in this case, chirp sweep, moving bar, or white noise (n=3517). Notably, while white noise analysis does not allow characterising direction selectivity, with the RMO we could identify both RFs and direction tuning in DS cells. Finally, we observed suppression of the neural response in a population of ON-RGCs as temporally close objects pass over the cells' receptive field. We show that this effect persisted even after pharmacologically blocking inhibition. This nonlinear effect can likely be associated with a specific nonlinear computation upstream of the ganglion cells. With this work, we present a novel analysis framework of an easily parameterizable stimulus which can be used to reliably extract both the tuning towards object parameters (e.g. direction-selectivity) as well as RFs of visual neurons and investigate nonlinear response behaviours.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Title: Analysis of the receptive field substructure of retinal ganglion cells with artificial neural networks

Authors: *M. YOUNG[1,2,3], T. GOLLISCH[1,3];
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Abstract: A central question in sensory neuroscience asks how neural circuits perform complex computations across cascaded cell layers to faithfully encode complex stimuli. Specifically in the retina, retinal ganglion cells already extract salient features of the visual scene, such as texture and motion. This occurs in part through the nonlinear integration at the ganglion cell of inputs across an array of bipolar cells. The characteristics of such signal integration determine the ganglion cell's response to various visual stimuli and the cell's overall computational role in early visual processing.
Thus, developing computational analyses that can model retinal ganglion cell responses to various visual stimuli and emulate nonlinear spatial integration are highly desirable. Of particular interest have been models that structure the ganglion cell's receptive field into subunits, where each subunit is thought to reflect input from individual bipolar cells. These models have emerged to better reflect relevant aspects of retinal circuitry and capture stimulus encoding, especially compared to traditional approaches that use the receptive field to filter the stimulus. Attention has recently turned to artificial neural networks, which have already shown promise in predicting retinal responses to natural scenes and in inferring bipolar cell properties. However, none of these network models have explicitly sought to extract ganglion cell subunits from two-dimensional visual stimuli.
Here, we introduce two neural network models for detecting the layout of subunits within the receptive fields of retinal ganglion cells: a neural network that seeks to predict spikes given a visual stimulus and an autoencoder. When applied to previously recorded data from ganglion cells in the salamander retina stimulated with spatiotemporal white noise, both of our neural network models can retrieve subunit layouts within the ganglion cell's receptive field. Not only do the subunits fully tile the ganglion cell's receptive field, but specific subunits also arise from neural networks with different numbers of hidden units, emphasizing our model's biological feasibility. The identified subunit layouts allow improved predictions of ganglion cell responses to held-out white noise images compared to linear models. These models are also further compared with other methods of subunit identification.

Ultimately, these models can facilitate the development of detailed stimulus-response models for ganglion cells that are capable of mapping onto retinal circuits, thus helping elucidate how these complex computations arise from sensory circuits.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Title: Functional Classification of Foveal Ganglion Cells in the Living Primate Eye

Authors: *S. S. PATTERSON[1], T. GODAT[2], K. KOHOUT[1], Q. YANG[1], W. H. MERIGAN[1], D. R. WILLIAMS[1];
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Abstract: While the fovea is best known for its specialized role in high spatial acuity mediated by a high density of midget ganglion cells (RGCs), both anatomy and transcriptomics indicate that at least 15 rarer RGC types are also present. However, our understanding of the visual information these RGC types convey to the brain is limited because both the fovea and rarer RGC types are difficult to address with standard physiology approaches. Here, we address these gaps in knowledge by using adaptive optics and calcium imaging to explore the diversity of RGC foveal types in the living primate eye.
Experiments were conducted in two macaque monkeys expressing GCaMP6s in the foveal ganglion cell layer. We used a fluorescence adaptive optics scanning light ophthalmoscope with a 3.69 x 2.70 degree field of view to measure the calcium activity in response to visual stimuli presented directly to the photoreceptors. With this approach, we could simultaneously survey the response properties of hundreds of distinct cells. We classified cells based on their responses to a battery of visual stimuli tailored to *in vivo* calcium imaging. The stimuli assessed the strength of ON and OFF input, spectral tuning, receptive field size, temporal tuning, direction selectivity and adaptation to changes in mean light level.
21% of cells exhibited response properties inconsistent with midget RGCs and could be classified into at least 12 functional groups. These groups include both rarer RGC types and

displaced amacrine cells. We observed a range of response properties, including ON-OFF responses, non-canonical receptive field organization, direction selectivity and suppressed-by-contrast responses. This classification enables functional identification of the rarest foveal RGCs in the living eye, laying the foundation for future experiments directly targeting these elusive cell types to determine their roles in vision.
Our results reveal an unappreciated functional diversity in the primate fovea. While the dominance of midget RGCs observed is consistent with the fovea's specialization for color and spatial vision, our results indicate that this is an incomplete picture of visual processing in the fovea and reveal several unexpected forms of visual information present in the foveal output.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Program #/Poster #: 296.12

Topic: D.06. Vision

Title: The receptive field construction of midget ganglion cells in macaque retina

Authors: M. A. SOMARATNA, *A. W. FREEMAN;
Univ. of Sydney, Univ. of Sydney, Sydney, Australia

Abstract: Introduction. The anatomy, connectivity and physiology of the macaque retina are increasingly well understood but attempts to combine these three viewpoints into a comprehensive and quantitative model have been limited. We have constructed a signal processing model for retinal receptive fields based on known structure and function; the model focusses on pathways leading to midget ganglion cells. **Methods.** Each neuron was simulated with a linear differential equation, and model responses were calculated by simultaneously solving the equations for all neurons. The published data used for the model were available as a function of eccentricity, and we could therefore calculate responses at any given eccentricity. For postreceptoral neurons, anatomical eccentricity was converted to its functional equivalent by subtracting the lateral offset due to Henle fibres and other postreceptoral displacements. **Results.** Our main findings are as follows. 1. The radius of the ganglion cell centre mechanism depended mainly on optical spread of the stimulus at the fovea, and on ganglion cell dendritic radius at eccentricities greater than 1°. Centre radius calculated in this way matched well with empirical physiological measurements. 2. Surround responses are known to result from the feedback between cones and H1 horizontal cells. We used frequency domain analysis to compute surround responses independently from those for the centre. Contrary to the popular difference-of-Gaussians model, the relationship of surround to centre was best viewed as divisive rather than subtractive. 3. The horizontal cell's receptive field is determined by both the dendritic fields of, and electrical connections between, horizontal cells. Surround radius in the model was

determined by the passage of signals from cone to horizontal cells and in the reverse direction, and was therefore larger than the radius of the horizontal cell receptive field. 4. We calculated spatial frequency responses to a drifting grating for a variety of cone contrasts, motion directions and eccentricities. Responses were bandpass with a low-frequency cut that depended on the cone contrasts. The descending arm at high frequency was variable because of aliasing within the cone spatial array. **Conclusion.** Modelling of midget ganglion cells helps to link spatial aspects of physiological measurements with observed retinal structure.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Title: Rod-cone coupling and visual temporal sensitivity: an operant assay for measuring absolute visual thresholds in the mouse

Authors: *S. LAMAGNA, Y. UMINO, B. E. KNOX, E. SOLESSIO;
Ctr. for Vision Research, Dept. of Ophthalmology & Visual Sci., SUNY Upstate Med. Univ., Syracuse, NY

Abstract: The role of electrical coupling between rods and cones in vision remains unresolved. Interphotoreceptor coupling enhances the signal-to-noise ratio of rod cell responses in electrophysiological recordings. Thus, we hypothesize that rod-cone coupling contributes to absolute visual sensitivity. Connexin 36 (Cx36) mediates rod-cone coupling under dim, rod-driven (scotopic) conditions in mammals. Cone-specific Cx36 knock-out (XO) mice lack gap junctions between rods and cones (Jin et al 2020, *Science Advances*). In this report, we developed an operant behavior assay to measure the absolute visual thresholds in these mice. XO mice and WT littermates were trained to complete a forced choice test via operant conditioning. Mice were tasked with visiting one of two ports conditioned on a full-field flash stimulus ranging in duration from 60 -1000 ms. First, mice learned the task with a bright 2 s stimulus then were introduced to briefer stimuli and dimmer conditions. After achieving a stable response for a dim 60 ms stimulus, mice completed multi-intensity sessions. Sensitivity indices (*d'*) were calculated from the Hit and False Alarm rates, and fit with psychometric functions to obtain absolute visual thresholds (i.e., the intensity at $d' = 1$). At the shortest duration (60 ms), both XO (n=5) and WT (n=2) had similar thresholds ($p = 0.38$; Mann-Whitney test): 0.04 R*/rod/sec (SD = 0.07) and 0.03 R*/rod/sec (SD = 0.02), respectively. The XO group displayed an inverse linear relation between flash duration and threshold from durations of 110-1000 ms, where the average XO 1000 ms threshold was 0.2×10^{-3} R*/rod/sec (SD = 0.8×10^{-4}). By contrast, WT mice displayed

constant thresholds from 256-1000 ms, where the 1000 ms WT threshold was higher than that of the XO mice ($p = 0.048$; Mann-Whitney test), at $0.8x10^{-3}$ R*/rod/sec (SD = $0.7x10^{-4}$). This assay advances the tools for measuring murine visual sensitivity by quantifying how stimulus duration effects absolute thresholds. At the shortest flashes, we detected no difference between the thresholds of WT and XO mice. At the longest flashes, we found XO mice are capable of detecting dimmer stimuli than WT. Work is ongoing to increase the statistical power of these experiments. Although the number of animals tested so far is small, our results raise the possibility that coupling between rods and cones contributes to the temporal properties of absolute visual sensitivity.

Disclosures: **S. Lamagna:** None. **Y. Umino:** None. **B.E. Knox:** None. **E. Solessio:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.14

Topic: D.06. Vision

Support: Loveless funds (Aalto University internal grant)

Title: Single-photon responses drive the pupillary light response at visual threshold.

Authors: *G. PEINADO ALLINA[1], P. ALA-LAURILA[2];
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[2]Dept. of Neurosci. and Biomed. Engin., Univ. of Helsinki & Aalto Univ., Aalto, Finland

Abstract: The retina has two major functions: it generates a conscious image representation of the world, and it drives other body functions that observers cannot make conscious experience of, such as the circadian clock or the pupillary light reflex (PLR). Decades of studies have revealed the striking sensitivity of image forming vision in darkness as well as the neural mechanisms allowing such sensitivity. However, similar knowledge is by enlarge missing from non-image forming vision. Here, we combine pupillometry and psychophysics to directly compare the threshold sensitivity of image-forming and non-image-forming vision. To do so, dark-adapted human observers were presented with a two-alternative forced-choice task in which light stimuli were presented in one of two possible time intervals marked by an auditory cue. Simultaneously, videos of the pupil were digitized, and the obtained PLR traces were subjected to a two-interval forced-choice ideal observer analysis. Stimuli could be varied in wavelength, intensity, duration, and/or in their spatial extent as they could be delivered to the full visual field (Ganzfeld) or to a small spot in the retina. We report four primary findings: 1) Surprisingly, the PLR reaches identical sensitivity to single photons as human psychophysics for full-field visual stimuli. 2) The integration time of the PLR is indistinguishable from that of conscious vision. 3) The PLR displays a strong thresholding non-linearity supporting coincidence detection of spare photons. 4) Image forming vision is two orders of magnitude more sensitive than the PLR for

stimuli restricted to small spatial scales (~70 µm spot, 10° eccentricity). Overall, our experiments show that unconscious vision has access to an exquisitely sensitive neural pathway. Spatial pooling over large areas of the retina makes the PLR at least 4 orders of magnitude more sensitive than previously reported and constitutes a powerful window to study the processing of signals arising from the outer retina in dim light in live human subjects non-invasively.

Disclosures: G. Peinado Allina: None. **P. Ala-Laurila:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

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Program #/Poster #: 296.15

Topic: D.06. Vision

Support: University of Helsinki HiLIFE Grand Challenge
Aalto University Centre for Quantum Engineering, CQE
Aalto University Infrastructure Grant, Loveless

Title: Temporal resolution of human vision at visual threshold

Authors: *J. TIIHONEN[1], M. KILPELÄINEN[2], F. RIEKE[3], R. KIANI[4], P. ALA-LAURILA[5]; [1]Fac. of Biol. and Envrn. Sci., [2]Dept. of Psychology and Logopedics, Univ. of Helsinki, Helsinki, Finland; [3]Dept. of Physiol. & Biophysics, Univ. of Washington, Seattle, WA; [4]The Inst. for the Study of Decision Making, New York Univ., New York, NY; [5]Univ. of Helsinki & Aalto Univ., Espoo, Finland

Abstract: Human vision can reliably detect a handful of photons in darkness. Decades of work have revealed the key neural mechanisms for this striking sensitivity. However, the temporal resolution of vision at these extremely low light levels has remained underexplored. Classic perceptual studies such as flicker fusion suggest a ~200 ms integration time, which could limit the temporal resolution of dark-adapted rod vision. However, based on the dynamics of retinal cell responses, we hypothesize that this limit is task dependent and could be up to an order of magnitude finer.
We quantified the temporal resolution of human observers and primate ganglion cells (ON parasol ganglion cells) in matching conditions at the sensitivity limit of vision. We utilized twodistinct paradigms: 1) one in which we quantified the capability of the human observers and ganglion cells to estimate the duration of a flash stimulus, and 2) one in which the observers and ganglion cells had to define which of two flashes presented in different locations appeared first. We constructed a tightly constrained model for comparing the psychophysical and retinal performances at the lowest light levels.
We present three novel findings on the temporal aspects of vision in darkness. First, we find a 5 times finer temporal resolution (~40 ms) in the psychophysical two-flash temporal discrimination task than expected from classic measures of integration time. Second, by utilizing

a dim red light in darkness, we show that humans can further improve their response kinetics by utilizing cone-driven signaling even when fully dark adapted. Third, our modeling shows that retinal ganglion cells can reach the fundamental kinetics limit set by rod photoreceptors, whereas human perceptual performance falls short of this limit by ~3-fold.
Overall, our results establish that temporal resolution of vision at the limit of visibility varies with task, ranging from ~200 ms in tasks limited by the rod integration time (e.g., duration estimation) to only tens of milliseconds in tasks that require accurate representation of stimulus onset (e.g., onset discrimination). We provide a mechanistic explanation for the observed range of temporal resolutions, quantitatively linking the photoreceptor mosaic, ganglion cells and human time perception at visual threshold.

Disclosures: **J. Tiihonen:** None. **M. Kilpeläinen:** None. **F. Rieke:** None. **R. Kiani:** None. **P. Ala-Laurila:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: D.06. Vision

Support: Academy of Finland grant 296269
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Ella and Georg Ehrnrooth Foundation
Victoria Foundation
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University of Helsinki Research Foundation

Title: The origin of noise in the retinal ON pathway at low light levels

Authors: *L. SMEDS[1], S. DENY[2], P. ALA-LAURILA[2,1];
[1]Univ. of Helsinki, Helsinki, Finland; [2]Aalto Univ., Espoo, Finland

Abstract: The capability of humans, mice and many other species to detect just a handful of photons is one of the most remarkable examples of sensory signal processing. It has been hypothesized that the detection is limited by spontaneous isomerizations of rhodopsin in rods, called pigment noise. However, the precise contribution of pigment noise has never been rigorously quantified at the level of ganglion cell inputs. Here we quantified the contribution of pigment noise and other noise sources in the mouse retina in darkness and dim background light (up to 0.5 R*/rod/s) where signals traverse the mammalian retina via the most sensitive rod pathway (the rod bipolar pathway). We recorded the input currents of the most sensitive ON type retinal ganglion cells (ON sustained alpha cells), since our earlier work has shown that these ganglion cells drive behavioral detection of light increments. By utilizing pharmacological tools, we quantified the contributions of noise originating from the outer retina (photoreceptors and

their synapses) and the inner retina (bipolar cells and downstream) in ganglion cell synaptic inputs. We found that half of the noise reaching ganglion inputs originates in the inner retina and the other half in the outer retina. However, contrary to the classical hypothesis, only ~2-3% of the total noise reaching the most sensitive mouse ON ganglion cells at starlight originates from pigment noise.

Disclosures: **L. Smeds:** None. **S. Deny:** None. **P. Ala-Laurila:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

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Title: Retinal loss sets a fundamental limit to the variance of visual signals originating from single-photon stimulation

Authors: *K. DOVZHIK[1,2,3], J. TIIHONEN[4], A. TAVALA[2,3], P. ALA-LAURILA[1,4];
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Abstract: The uncertainty arising from Poisson statistics of light has until recently precluded the possibility to stimulate the visual system with a precise number of photons. Recent studies utilizing a single-photon gun (SPG) postulate that it is possible to produce sub-Poisson signal statistics in retinal rods [1] and in human psychophysics [2]. However, the key prerequisite for these results has remained untested, namely that the loss of single-photon signals in the retina strictly constrains the extent at which biological signal statistics can deviate from Poisson statistics. Now we test the impact of single-photon loss on the variability of visual signals from rods to human psychophysics at the sensitivity limit of vision. We stimulated toad (Bufo marinus) retinal rods axially using a state-of-the-art SPG and measured their light-sensitive current using suction pipette technique. We measured the minimum loss of single-photon signals in rods. Finally, we estimated the minimum loss of single-photon signals across the relevant pathway in the primate retina (rod bipolar pathway) to analyze the minimum number of trials

required for distinguishing the biological signal distributions originating from a Poisson source or a SPG. We estimate that the minimum cumulative single-photon loss in rods is 86% (source loss: ~75%, rod loss ~43%), requiring a minimum of ~3 000 trials in optimal conditions for a statistically significant difference between the response distributions arising from a Poisson source or a SPG. The cumulative single-photon loss in human psychophysics experiments even with a lossless SPG was estimated to be ~99.8%, requiring a minimum of more than 2.2 billion trials (210 years of experiment) and a perfect memory for distinguishability in response distributions between Poisson and SPG stimulation. We show that due to the loss of single-photon signals it is impossible to distinguish the response distributions originating from Poisson or SPG sources in retinal outputs or at the level of perception on a biologically relevant timescale. Our results require a fundamental re-evaluation of the previous results relying on SPG stimulation and set tight constraints to which extent visual signal statistics can deviate from Poisson statistics at the sensitivity limit.
[1] Phan et al. (2014). Phys. Rev. Lett. 112, 213601.[2] Tinsley et al. (2016). Nature Communications 7, 12172.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

Support: NIH F32 EY032401
NIH R01 29869
NIH R01 EY04067
Glaucoma Research Foundation
VA Senior Career Scientist Award

Title: Progress towards integrating theories of efficient coding and metabolically efficient coding as constraints on retinal ganglion cell biophysical properties.

Authors: *B. SMITH[1], C. F. MCHUGH[2], N. C. BRECHA[3], S. A. BARNES[4];
[1]Neurobio., UCLA, Los Angeles, CA; [2]Doheny Eye Inst., Los Angeles, CA; [3]Dept Neurobio., David Geffen UCLA Sch. of Med., Los Angeles, CA; [4]Ophthalmology, Doheney Eye Inst., Pasadena, CA

Abstract: A first principles approach to understanding the process of information encoding in the retina requires a quantitative evaluation of the underlying constraints on the system. Noise, the statistics of natural scenes, metabolic cost, and the limited dynamic range of retinal ganglion cells (RGCs), have all be proposed as constraints governing how information is encoded in the optic nerve. Because each RGC subtype responds to a unique feature in visual space it is possible

that the balance of constraints is unique to each subtype.
We asked how the biophysical properties of RGC subtypes balance metabolic constraints against the need to adapt to changes in contrast. We have developed voltage clamp protocols that allow voltage clamp of Na_v channels in identified RGC subtypes in the intact retina. We first present preliminary results supporting classical results in metabolic efficient coding, showing that metabolic efficiency is inversely correlated with maximal spike frequency and spike half width in alpha and M1 RGCs. ON αRGCs are more efficient compared to OFF αRGCs consistent with a predominance of negative contrast in natural images. Changes in variance of Gaussian white noise (GWN) current injection causes adaptation in spike rate and drives a physiological elevation in reactive oxygen species (ROS). We found that in sustained but not transient αRGC subtypes, strengthening the ability of the RGC to buffer ROS using antioxidants significantly reduces variance adaptation during 30 s of high (144 pA^2) variance GWN, principally by elevating spike rate following 20 s of high variance GWN. In contrast artificially elevating cellular ROS reduced adaptation by reducing spike rate during the first 10 seconds of high variance GWN. H_2O_2 drove a hyperpolarizing shift in inactivation and a significant reduction in peak Na_v current in sustained αRGCs as well as increasing the rate of entry into the slow inactivated state. We found that sustained αcells had larger reductions in peak current when $Na_v1.1/1.3$ channels were blocked. Blocking $Na_v1.1/1.3$ channels prevents further modulation by H_2O_2 both in voltage clamp recordings of Na_v current and in current clamp recordings of variance adaptation. We propose that the need for repetitive spiking in sustained RGCs necessitates an electrogenic phenotype that is resistant to slow inactivation of Na_v channels. We suggest that ROS elevation acts specifically on $Na_v1.1/1.3$ channels, slowly reducing availability and mimicking the effects of slow inactivation to facilitate adaptation to contrast. This represents a direct influence of metabolism on adaptation to input statistics in a subset of RGCs with high metabolic demand.

Disclosures: **B. Smith:** None. **C.F. McHugh:** None. **N.C. Brecha:** None. **S.A. Barnes:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.19

Topic: D.06. Vision

Title: Bidirectional modulation of AMPA and $GABA_A$ receptors in retinal ganglion cells using DART.2

Authors: *H. YAN[1,2], M. RUDZITE[1], M. THAPA[1], B. SHIELDS[2], P. P. VAGADIA[3], M. MCDONNELL[4], S. THANNA[4], A. B. REITZ[4], G. E. SCHILTZ[3], M. R. TADROSS[1,2], G. D. FIELD[1,2];
[1]Neurobio., Duke university Sch. of Med., Durham, NC; [2]Biomed. Engin., Duke Univ., Durham, NC; [3]Chem., Northwestern Univ., Evanston, IL; [4]Fox Chase Chem. Diversity Ctr., Doylestown, PA

Abstract: Glutamate and GABA are two principle excitatory and inhibitory neurotransmitters in the retina. Because these two neurotransmitters are released and sensed by diverse retinal cell types, it has been technically challenging to dissect their contributions to the stages of visual processing from photoreceptors to feature-selective retinal ganglion cells (RGCs). **DART** (Drugs Acutely Restricted by Tethering) is an emerging technology that can solve these challenges. The method achieves cell-specific delivery of pharmaceuticals by instructing cells to express the HaloTag Protein (HTP), which captures and locally accumulates a drug linked to the HaloTag Ligand (HTL), yielding a cell-specific pharmacological effect. In this study, we characterized and validate newly developed **DART.2** reagents on a genetically defined subset of RGCs. Intravitreal injection of AAV_{7m8}-DIO-$^{+}$**HTP**$_{GPI}$-dTomato in PV-Cre mice provided genetic access to a set of RGCs. Electrophysiological measurements from these $^{+}$**HTP** RGCs revealed that application of **YM90K.1**$^{\mathbf{DART.2}}$ blocks AMPAR-EPSCs within minutes, with no impact on $GABA_A$R-IPSCs. Conversely, **gabazine.1**$^{\mathbf{DART.2}}$ antagonizes $GABA_A$R-IPSCs with no impact on AMPAR-EPSCs. We also evaluated newly developed PAM reagents: **CMPDA.2**$^{\mathbf{DART.2}}$ potentiated AMPAR-EPSC amplitudes and integrated currents and extended the decay times of synaptic currents. Similarly, **diazepam.1**$^{\mathbf{DART.2}}$ enhanced $GABA_A$R-IPSC amplitudes and integrated currents, and prolonged decay time. We confirmed that all four reagents had no impact on neurons expressing a control "double dead" $^{\mathbf{dd}}$**HTP** virus, confirming the cell type-specificity of the method. To further understand the role of $GABA_A$ receptors in RGC visual processing, we evaluated spontaneous action potential firing via current-clamp or multi-electrode array (MEA) recordings. Thus far, we have found that **gabazine.1**$^{\mathbf{DART.2}}$ increases spontaneous firing in the $^{+}$**HTP** RGCs, with no effect on $^{\mathbf{dd}}$**HTP** expressing cells. In sum, **DART.2** enables bidirectional modulation of endogenous AMPAR and $GABA_A$R-mediated synaptic signals onto genetically defined RGCs. These reagents offer the unprecedented opportunity to interrogate synaptic computations in visual processing. In future experiments we aim to use these constructs to determine the contributions of specific neurotransmitters onto specific cell types that shape visual processing.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.20

Topic: D.06. Vision

Support: European Union's Horizon 2020 grant agreement No 861423

Title: Seamless high-density microelectrode array electrophysiological recordings of rod-degenerated retina enable comparison of different artificial vision restoration strategies

Authors: *A. E. COJOCARU[1], A. CORNA[1], M. REH[2], G. ZECK[1];
[1]Vienna Univ. of Technol., Vienna, Austria; [2]Univ. of Tübingen, Tübingen, Germany

Abstract: We convey most of the information about the world through the visual system, therefore the loss of sight counts as one of the most debilitating maladies that a person can suffer from. Up to the present day, according to world health organization (WHO), around 2 billion people have a near or distance vision impairment. Pathologies such as retinitis pigmentosa (RP) or age-related macular degeneration (AMD) are some of the most frequently encountered for vision impaired patients. Because these cases imply advanced retinal layer degeneration, the last cell layer, the retinal ganglion cells (RGCs), present as an attractive target to vision restoration. We conduct ex vivo experiments on rod-degenerated mice models (rd10), as well as transgenic mice expressing channelrhodopsin-2 (ChR2), aged between 112 and 281 days post-natal. For our extracellular recordings we make use of the high-density microelectrode arrays (MEA) based on complementary metal oxide semiconductor (CMOS) technology (Bertotti et al. 2014). We use electrical stimulation in an epiretinal configuration, as well as optogenetic stimulation delivered at 460 nm, both in patterned stimuli to assess the spatial resolution inferred from spiking RGCs. For these stimulation modalities, we use two MEA types, one containing a "native" oxide layer with an active area of 1 by 1 mm^2, shown to be able to simultaneously stimulate and record, and one conveying an area of 2.3 by 2.3 mm^2, offering the possibility for low noise electrophysiological recordings. We show that seamless optogenetic and electrical stimulation can be used with these types of chips and that the artefact typically induced by such a stimulation can be subtracted from the recorded data. We will compare the spatial resolution achieved by the two artificial stimulation strategies (optogenetic and electrical) based on the activity of the very same RGCs. The results may contribute towards the selection of an optimal artificial vision restoration strategy.

Disclosures: **A.E. Cojocaru:** Other; entrain Vision project, European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 861423. **A. Corna:** None. **M. Reh:** None. **G. Zeck:** Other; entrain Vision project, European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 861423.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.21

Topic: D.06. Vision

Support: NIH Grant EY029259

Title: Electrical receptive fields are narrowed in degenerated retina, whiles stimulus driven spontaneous activity spreads more widely.

Authors: *N. W. OESCH[1], **M. CARLETON**[2];
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Abstract: Vision restoration strategies aim to restore vision by replacing the function of lost photoreceptors with optoelectronic hardware, or through gene therapy. One complication to these approaches is that retinal circuitry undergoes remodeling after photoreceptor loss. Circuit remodeling following perturbation is ubiquitous in the nervous system and understanding these changes is crucial for treating neurodegeneration. In retinal degeneration, much work has focused on changes to the retinal circuitry that give rise to spontaneous oscillations, however, how these circuit changes influence the encoding of restored retinal activity is not well understood. Here we use subretinal electrical stimulation to reveal new insights into the circuit changes and retinal processing that occur in response to retinal degeneration. We examined how retinal degeneration mediated remodeling alters the spread of both evoked and spontaneous activity. We found that electrical stimulation synchronizes oscillatory activity, over space and through time. Intriguingly, we found that this synchronization of spontaneous activity cannot be explained by a general increase in receptive field size. In fact, the electrical receptive field was narrower for *rd10* mice than *wt,* a surprising finding given the increased gap junction coupling that has been observed in models of retinal degeneration. These findings indicate that different circuit mechanisms are involved in mediating feed forward excitation, and the lateral spread of spontaneous activity, and remain somewhat segregated during disease. Importantly, we demonstrate that even as spatial electric receptive fields are preserved in degenerated retina, electrical stimulation can synchronize spontaneous activity generating increased redundancy in the retinal code.

Disclosures: **N.W. Oesch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Nanovision Biosciences. **M. Carleton:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

Support: NIH Grant EY024567

Title: Inhibition differentially tunes responses of direction selective retinal ganglion cells near the threshold of vision.

Authors: *S. ROY, X. YAO, J. RATHINAVELU, G. D. FIELD;
Neurobio., Duke Univ., Durham, NC

Abstract: Detecting movement reliably under dim light conditions is critical for rodents to navigate their environment. Direction selective ganglion cells (DSGCs) in the retina encode

information about movements across a broad range of light levels and relay this information to the brain. Previous work has shown that adaptation to low, rod-activating light levels, differentially tunes responses of ON-OFF DSGCs (ooDSGCs), such that superior direction preferring ooDSGCs exhibit broad tuning while ooDSGCs preferring other directions exhibit narrow tuning. Here, we sought to identify other functional asymmetries between the populations of ooDSGCs. Using a multi-electrode array to measure the spiking activity of DSGCs from ex-vivo mouse retina, we found that the absolute sensitivity of superior ooDSGCs is ten-fold higher than other ooDSGC subtypes. This higher sensitivity could partly, but not fully, be explained by a larger receptive field size of superior ooDSGCs than other ooDSGCs. The remaining was explained by differences in the amount of GABA-ergic inhibition. Blocking GABA-ergic inhibition largely eliminated the difference in threshold responses across ooDSGC subtypes. This indicates that inhibition plays a major role in determining the absolute sensitivity of DSGCs. Using a conditional knockout mouse that eliminated connexin36 gap junctions between superior ooDSGCs, we found that gap junction coupling did not significantly contribute to the absolute sensitivity difference between the ooDSGC subtypes. We further show that GABA-mediated inhibition masks the OFF responses of ooDSGCs under scotopic conditions, making the responses more selective to light increments than to light decrements. Together, these results reveal that GABA-ergic inhibition plays a key role in differentially modulating absolute sensitivity and contrast preference of ON-OFF DSGCs under scotopic conditions.

Disclosures: **S. Roy:** None. **X. Yao:** None. **J. Rathinavelu:** None. **G.D. Field:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

Support: NIH grant EY05253 (JMA)
NIH grant EY007360 (SB)

Title: Spatiotemporal differences between ON and OFF retino-thalamic pathways

Authors: *A. J. TENGOLICS[1], J. JIN[1], R. MAZADE[2], S. BLOOMFIELD[1], J.-M. ALONSO[1]; [1]Dept. of Biol. Sci., SUNY Col. of Optometry, New York, NY; [2]Biomed. Engin., Georgia Inst. of Technol. and Emory Univ., Atlanta, GA

Abstract: Vertebrates use different ON and OFF pathways to process the properties of light and dark stimuli in visual scenes. In cat visual cortex, ON pathways show a preference for light stimuli with long duration and large size whereas OFF pathways show a preference for briefer and smaller dark stimuli (Mazade *et. al* 2019). We hypothesize that these ON/OFF cortical differences are inherited from the retina. To test this hypothesis, we recorded from mouse (*C57BL/6)* retinal ganglion cells (RGCs) using the loose patch clamp technique (resistance seal

<1GΩ) and measured RGC responses to stimuli with different size and duration. We targeted presumed alpha RGCs, which are a major retinal output to the visual thalamus. The stimuli were flashed squares with the maximum contrast of the visual display (dark on light background and light on dark background; luminance: 0 cd/m^2 for dark and 200 cd/m^2 for light) and all stimuli were projected on a screen size of 480x360µm. To measure RGC tuning to stimulus duration, the size of the stimulus was fixed at 180x180 µm and the stimulus durations varied between 16.66 and 1066.66 milliseconds. To measure RGC size tuning, the duration of the stimulus was fixed at 100 milliseconds and the size varied between 12 and 360 µm per square side. Consistent with our hypothesis, the average preferred stimulus duration was 1.4 times shorter for OFF than ON RGCs, although the difference did not reach significance (778±381 vs 571±399 milliseconds, n = 12/25, p = 0.171, Wilcoxon test). Against our hypothesis, the average preferred stimulus size was 1.2 times larger for OFF than ON RGCs, but the difference was not significant (252±153 vs 301±63 µm of square side, n = 2/11, p = 0.974, Wilcoxon test). Also consistent with our hypothesis, the responses from OFF cells were more strongly suppressed than the responses from ON cells by both stimulus duration (ON/OFF mean percentage = 6.6 / 9.8, n = 12/25, p = 0.453, Wilcoxon test) and size (ON/OFF mean percentage = 5.5 / 22.2, n = 2/11, p = 0.512, Wilcoxon test), but the comparisons did not reach significance. Finally, the stimulus duration generating the strongest rebound responses when turning off stimuli of non-preferred polarity (e.g. dark for ON cells) was significantly shorter in ON than OFF cells (ON/OFF means = 597±406 / 891±300ms, n = 11/25, p = 0.027, Wilcoxon test), as would be expected from shorter preferred stimulus durations for OFF- than ON-driven inhibition. These preliminary results are generally consistent with the notion that the spatiotemporal differences between ON and OFF pathways measured in cat visual cortex originate in the retina and are well preserved across species.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

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Program #/Poster #: 296.24

Topic: D.06. Vision

Title: CFTR and syntaxin-1a interact in chick retinal amacrine cells

Authors: *B. LEVISKAS, E. L. GLEASON;
Biol. Sci., Louisiana State Univ., Baton Rouge, LA

Abstract: Protein-protein interactions differ among cell types and the cellular localization of these interactions can further define protein function. Interactions between cystic fibrosis transmembrane conductance regulator (CFTR) and SNARE protein Syntaxin-1A (STX1A) have been confirmed in epithelia (Naren *et al*., 2000). Our lab has established a role for CFTR regulation of cytosolic Cl^- in neurons and previously demonstrated expression of CFTR

(Krishnan et al., 2017) and STX1A (McMains *et al*., 2011) in amacrine cells (ACs). Here we ask whether there is physical interaction between CFTR and STX1A in ACs. Protein homogenates were produced from embryonic equivalent day (EE) 18 chick retina (*Gallus gallus*) and adult chicken brain. Co-IP was achieved using CFTR antibodies in protein homogenates to form an immunocomplex with CFTR protein (the bait). Magnetic protein A/G beads then precipitated the target CFTR protein immunocomplex and other bound proteins. Once eluted, Co-IP products were blotted and probed for STX1A and SNARE protein SNAP-25, which give a prominent band at a size previously established to be the SNARE complex (Hayashi *et al*., 1994). To further test for *in vitro* interactions between CFTR and STX1A, a binding assay using recombinant phosphorylated whole CFTR protein as a supplement in protein homogenates was used in Co-IP. Immunoblots of the CFTR-supplemented sample shows a 2.3-fold increase in the STX1A probe signal, indicating that increasing CFTR concentration increases the level of CFTR-STX1A interactions. To rule out the possibility that CFTR and STX1A binding only occurs *in vitro*, colocalization of CFTR and STX1A was examined via immunocytochemistry in cultured ACs. Results show a Pearson's correlation coefficient of 0.69 ± 0.02 (mean ± SEM, n =7) in AC processes indicating substantial overlap in protein expression. Because STX1A commonly functions at synapses in neurons, we asked whether inhibition of CFTR effects synaptic function by making whole cell voltage clamp recordings of spontaneous postsynaptic quantal currents (SPCs) from EE18-21 ACs with ≥3 presynaptic ACs. CFTR inhibition ($CFTRinh_{172}$, 10 µM) increases the frequency of events/60s as compared to control [$CFTRinh_{172}$, 99.6 ± 25.9, (mean ± SEM, n =5, p =.003) control, 68.2 ± 20.6 (mean ± SEM, n =5) and wash 81 ± 24.1 mean ± SEM, n =5, p =.02)], suggesting that CFTR somehow limits the rate of spontaneous vesicle fusion. Together, these data demonstrate interactions between CFTR and STX1A in ACs. Furthermore, the elevation of SPCs frequency upon CFTR inhibition suggests a possible presynaptic role for CFTR.

Disclosures: B. Leviskas: None. **E.L. Gleason:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.25

Topic: D.06. Vision

Title: The role of intracellular Ca^{2+} stores in nitric oxide-dependent Ca^{2+} elevations in retinal amacrine cells

Authors: *L. ZHONG, E. L. GLEASON;
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Abstract: Our research has shown that NO can trigger Ca^{2+} elevations (Zhong and Gleason, 2021) involving Ca^{2+} store release (by an as yet unknown mechanism) and Cl^- store release by activating the cystic fibrosis transmembrane conductance regulator (CFTR) (Krishnan et al.,

2017). Our previous results also indicate that NO-dependent cytosolic Ca^{2+} elevations (NOdCE) are mandatory for releasing Cl^- via CFTR. Therefore, we investigated the mechanisms involved in the NOdCE in order to provide a better understanding of NO- and Ca^{2+}-mediated Cl^- regulation in neurons. TMEM16A, is a Ca^{2+}-activated Cl^- channel that is involved in the NO-dependent regulation of cytosolic Cl^- in chick amacrine cells (ACs) (Rodriguez et al., unpublished data). However, in epithelia TMEM16A has also been demonstrated to somehow enhance Ca^{2+} signaling via inositol 1,4,5-trisphosphate receptors (IP_3Rs) (Cabrita et al., 2017). Thus, we first evaluate the effect of a TMEM16A inhibitor T16Ainh-A01 on cytosolic Ca^{2+} by Ca^{2+} imaging. ACs were cultured from 8-day embryonic chick (*Gallus gallus*) retinas and experiments were performed after cells were in cultures for 6-10 days. To monitor cytosolic Ca^{2+} levels in cell bodies (CBs) and cell processes (CPs), cells were loaded with the fluorescence Ca^{2+} indicator Oregon Green™ 488 BAPTA-1 AM and tested in $0Ca^{2+}$ external solution to isolate the internal Ca^{2+} store. Under control conditions, NO increases cytosolic Ca^{2+} (mean peak amplitude fluorescence intensity values, 132.1 ± 10.6 A.U. (CBs, n=58) and 23.0 ± 3.0 A.U. (CPs, n=54)). Pre-application of T16Ainh-A01 (50 µM) decreases cytosolic Ca^{2+} responses (mean peak amplitude fluorescence intensity values, 21.7 ± 5.2 A.U. (CBs, n=39, $p<0.0001$, Welch's unpaired t-test) and 6.7 ± 0.9 A.U. (CPs, n=31, $p<0.0001$, Welch's unpaired t-test)), suggesting that TMEM16A is involved in the NOdCE in both AC CBs and CPs. To investigate the role of IP_3Rs and ryanodine receptors (RyRs) in NO-dependent Ca^{2+} signaling, IP_3R antagonist 2-APB (100 µM) and RyR inhibitor ryanodine (14 µM) are tested. Both are effective in suppressing Ca^{2+} elevations in ACs (CBs control, 132.1 ± 10.6 A.U., n=58; 2-APB, 65.3 ± 6.3 A.U., n=70; $p<0.0001$; CPs control, 23.0 ± 3.0 A.U., n=54; 2-APB, 8.5 ± 1.2 A.U., n=41; $p<0.0001$; CBs control, 99.5 ± 8.5 A.U.; n=73; ryanodine, 33.6 ± 4.2 A.U.; n=64; $p<0.0001$; CPs control, 14.0 ± 1.5 A.U.; n=79; ryanodine, 6.7 ± 1.5 A.U.; n=44; $p<0.001$; Welch's unpaired t-test). Together, these results indicate that TMEM16A, IP_3R, and RyR are involved in the NOdCE. These data expand the landscape of crosstalk between cytosolic Ca^{2+} and cytosolic Cl^- regulatory systems in retinal ACs.

Disclosures: L. Zhong: None. **E.L. Gleason:** None.

Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 296.26

Topic: D.06. Vision

Support: NIH Grant EY030155

Title: Component analysis of chromatic ERGs reveals photopic cone-rod pathway interactions

Authors: *C. W. TYLER[1], R. D. HAMER[2], M. LIANG[1], L. T. LIKOVA[1];
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Abstract: Purpose. Standard electroretinogram (ERG) protocols are designed to isolate the responses of the rod and cone retinal pathways, including the use of adapting backgrounds to suppress unwanted off-pathway responses. Here, the strategy was to record spectral ERGs in the photopic sensitivity range in the absence of any adapting background in order to assess interactions between the pathways. Method. The primary analysis of normative ERGs recorded at three levels of intensity (10, 100, and 1000 Td) and 4 color conditions (R, G, B, and W = R+G+B) was a principal components analysis (PCA), which showed significant contributions in only the first three 3 PCs. The structured rod-cone analysis was based on the assumption that the rod pathway response is 1) invariant in waveform and 2) dominant at lower intensities. These characteristics allowed the identification of the rod pathway response from the PCA across the set of mesopic/photopic ERG responses. This rod pathway response was validated by the fact that its amplitude matched the scotopic spectral sensitivity function at the lowest intensity. Since cone responses are expected to vary in waveform with intensity, the cone pathway response as a function of wavelength and intensity was derived by summing the remaining two significant PCs. This procedure was validated by the fact that its amplitude increased with intensity and matched the photopic spectral sensitivity function at the highest intensity, though saturating consistent with a gain-control mechanism. Results. While the putative rod (and cone) components showed the appropriate behavior at low (and high) intensities, respectively, their properties outside these canonical intensity ranges show remarkable anomalies suggestive of profound mesopic cone-rod interactions. At high intensities, where rods are expected to be silent, the rod component was weakly but significantly inverted, suggestive of inhibition of the rod pathway by the cone pathway. The match to the rod pathway time course suggests that this inhibition derived from cone input into the rod pathway by electrical gap junctions (Tsukamoto & Omi, 2017). The balance point occurred at medium intensities, where the rod pathway response was negative for R and positive for B and W. However, at low intensities the cone pathway response specific to white light (W) became inverted, suggestive of inhibitory input, perhaps from AII amacrine cells to OFF cone bipolar cells (Fain & Sampath, 2018). Conclusion. The results show that there are significant cone-rod interactions at mesopic levels, with unexpected properties consistent with recent knowledge of retinal physiological connectivity.

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Poster

296. Retinal Ganglion Cells and the Retina: Cell Types and Function

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Topic: D.06. Vision

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Title: Amacrine cells gate optic nerve regeneration through nitric oxide signaling

Authors: H.-Y. GILBERT[1], Y. LI[2], N. HANOVICE[2], K. OMURA[2], K. YUKI[2], M. WALTER[4], S. J. LIPPARD[4], *L. I. BENOWITZ[2], P. A. ROSENBERG[3];
[1]Neurosurg., Boston Children's Hosp, Boston, MA; [2]Neurosurg., [3]Neurol., Boston Children's Hospital/Harvard Med. Sch., Boston, MA; [4]Chem., MIT, Cambridge, MA

Abstract: Retinal ganglion cells (RGCs), the output neurons of the eye, cannot normally regenerate axons after optic nerve injury and soon begin to die as a result of both cell-autonomous and non-cell autonomous factors. In the CNS, nitric oxide (NO) acts as a gaseous signaling molecule in synaptic communication, in regulating vascular tone, and, after injury, as a critical intercellular messenger. Following injury to the mouse optic nerve, we found that elevating NO had a modest pro-regenerative effect by itself, but importantly, enabled other treatments to promote regeneration. Using the fluorescent sensor Cu_2FL2 or Cu_2FL2E, we observed a strong, sustained elevation of NO within an hour after optic nerve injury. Blocking NO elevation with L-NPA, an inhibitor of neuronal NO synthase (nNOS, NOS1), or knocking down the gene encoding NOS1 in amacrine cells (VGAT-Cre:$Nos1^{flx/flx}$ mice), suppressed the robust axon regeneration induced by PTEN deletion in RGCs combined with oncomodulin (Ocm) and the cAMP analog CPT-cAMP. The NMDA receptor antagonist D-APV eliminated NO generation, presumably by blocking glutamate-induced Ca^{2+} entry into NOS1-expressing amacrine cells, and decreased axon regeneration induced by the same combinatorial treatment. Likewise, inhibitors of the glutamate transporter GLT-1 (dihydrokainic acid [DHK] and WAY213613), as well as the Kv2.1 blocker RY796, disrupted NO generation. Conditional knockdown of GLT-1 in bipolar cells reduced axon regeneration. Taken together, these data show that optic nerve injury activates an intercellular signaling pathway in the retina that includes glutamate uptake dysregulation in bipolar cells, NMDA receptor-mediated NOS1 activation in a subpopulation of amacrine cells (ACs), and generation of NO that gates axon regeneration after optic nerve injury.

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Poster

297. Visual Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 297.01

Topic: D.06. Vision

Title: Normalization as a computational biomarker in genetic generalized epilepsy

Authors: G. S. PLUMMER, *J. J. TSAI;
Neurol., Univ. of Washington Sch. of Med., Seattle, WA

Abstract: Divisive normalization is a canonical neural computation in which a ‘normalization signal’ (NS) modulates an output via a divisive operation. We have previously shown that

changes to normalization model parameters account for differences in the *amplitude* of steady-state visual evoked responses (ssVEPs) in patients with genetic generalized epilepsy (GGE) compared to healthy controls. More recently, we found an increased variability of ssVEPs in GGE. However, it is unclear whether normalization accounts for changes in response variance. Coen-Cagli and Solomon (2019) extended the normalization model using a ratio of Gaussian random variables (RoG), allowing direct examination of response variability. We hypothesize that increased variability in ssVEPs in GGE is associated with decreased normalization. Data of 33 adult subjects (14 with GGE and 19 healthy controls) from Won et al. (2017) were used. SSVEPs were recorded with high-density scalp EEG. Stimuli consisted of a windowed 2 cycles per degree grating pattern contrast reversing at 7.5 Hz. Contrast was incremented from 1.3% to 94% in 10 equal log steps in each trial, for 20 trials. Fourier analysis extracted the 2nd harmonic complex response. We calculated a mean response vector for each EEG channel and contrast. The projected amplitude onto the mean vector, averaged across 7 occipital channels, constituted the response of each trial. Single trial NS was inferred by fitting response mean and variance to the RoG model. We found that the variability of human contrast VEPs is qualitatively similar to that observed in single neurons. Trial-to-trial variability of the response, expressed as Fano factor (FF), decreased with stimulus contrast ($F_{1,31} = 85$, $p < 10^{-9}$). Across contrasts, the FF of patients was significantly greater than that of controls ($F_{1,31} = 9.4$, $p < 0.01$). Single trial NS computed from the RoG model of 9 patients and 17 controls whose goodness of fit exceeded a threshold were used for subsequent analysis. In a linear mixed effect model of the NS with random effects of subjects on contrast and fixed effects of contrast and diagnosis, NS was greater in controls than patients ($F_{1, 24} = 9.56$, $p=0.005$). Although single-trial NS was correlated with response amplitude, a model including response amplitude as a covariate confirmed the greater NS in controls. Finally, trials with NS above the median had lower trial-to-trial variance than those below the median (paired *t*-test, $p<10^{-10}$). Thus, NS in GGE subjects is reduced compared to healthy controls and accounts for patients' greater response variability. These results further support the normalization model as a computational biomarker in GGE.

Disclosures: G.S. Plummer: None. **J.J. Tsai:** None.

Poster

297. Visual Contrast, Form, and Color

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Support: National Eye Institute of the National Institutes of Health Grant DP2EY032737
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Title: Connectome and functional imaging reveals visual features critical for navigation in Drosophila melanogaster

Authors: *Y. LAI, D. GARNER, S. KIM;
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Abstract: A fly's heading direction is represented by increased local activity in the population of compass neurons, which are in the ellipsoid body, a donut-shaped structure at the center of the brain. Compass neurons receive sensory inputs that are critical for navigation from the aptly named ring neurons, whose axonal branches form a ring shape. Some ring neuron populations are part of the fly's anterior visual pathway (AVP) and serve as the last relay in transmitting visual information to the compass neurons. Previous studies have demonstrated that distinct populations of ring neurons receive topographically organized visual inputs, but the functions of each type of ring neuron have not been fully characterized. To determine the visual features each ring neuron type processes, we densely reconstructed the AVP in the full adult fly brain electron microscopy dataset and traced entire connections that converge onto each ring neuron population at the synaptic level. We found that each ring neuron population receives signals from specific sets of upstream optic lobe neurons called MeTu neurons that are retinotopically arranged in the medulla and correspond to specific areas of the visual field. For example, MeTu input to ER4d ring neurons comes from both the upper and lower visual field, while MeTu input to ER4m ring neurons mainly comes from the upper visual field. We thus hypothesized that different spatial information is processed by different ring neuron populations. To test this, we mapped the visual space to which each ring neuron responded. We presented head-fixed flies with a random dot stimulus and recorded neural activity using *in vivo* 2-photon calcium imaging. As predicted from connectivity data, the visual space to which each ring neuron responded closely resembled the dendritic arrangement of their upstream MeTu neurons in the medulla. For instance, the ER4d ring neurons responded to vertically elongated visual stimuli in shape, which matched with the prediction based on the electron microscopy data. Overall, we have demonstrated that the architecture of the fly's brain supports the computation underlying visual information processing in navigation.

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Poster

297. Visual Contrast, Form, and Color

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Title: Effects of the GPR55 receptor on vision

Authors: *I. BACHAND, J.-F. BOUCHARD;
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Abstract: G protein-coupled receptor 55 (GPR55)'s principal ligands are lysophosphatidylinositols, but endogenous and exogenous cannabinoid could modulate it as well. It was previously shown that genetic deletion of cannabinoid type 2 receptor (CB2R) increases mouse visual acuity during development and later in adult animals. The addition of 2-AG, an endocannnabinoid ligand or of a CB2R synthetic ligand produces the same effect. Furthermore, electroretinographic analysis of *gpr55* knockout (*gpr55* KO) mice shows a modulation of cones and other retinal cells activity. In this study, the role of GPR55 on visual acuity was investigated comparing *gpr55* KO mice and wild types (*gpr55* WT) using the optomotor response assessed with the OptoMotry system from CerebralMechanics Inc. The finest bands or the lowest contrast triggering a reflex response were determined using an adaptative stairway method. No differences of spatial frequency threshold were found in adult animals. However, *gpr55* KO mice had a slower development of spatial frequency with lower values compared to the WT group from the opening of the eyes at day 15. The knockout group reached the same plateau as the control group at day 50 after the birth. *gpr55* KO mice also had a reduced contrast sensitivity for spatial frequencies going from 0.031 to 0.272 cycle/degree. In conclusion, GPR55 receptor modulates several different visual functions and could be the cause of some visual distortions observed during cannabis consumption. The results showed a decreased acuity during development, which adds further weight to a previous study that found that GPR55 modulates axon growth and guidance in that period. It is then legitimate to question the innocuity of cannabis consumption before adulthood.

Disclosures: I. Bachand: None. **J. Bouchard:** None.

Poster

297. Visual Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 297.04

Topic: D.06. Vision

Title: Assessing tree shrew high-level visual behavior using conventional and natural paradigms

Authors: *E. E. MEYER[1], W. S. ONG[2], M. BALBOA[2], M. J. ARCARO[2];
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Abstract: Closely related to primates, tree shrews (Tupaia belangari) are a promising model for studying a variety of visual functions. They have been extensively used for studying the eye (e.g. myopia, refractive development) and low-level visual function (e.g. contrast sensitivity, color and motion processing). Their high concentration of cones in the retina and expanded visual cortex could support high level visual representations. Here we evaluated their capacity to

recognize complex object forms using a conventional visual discrimination task and developed an analytic approach for assessing visual behavior in natural, freely moving contexts, independent of training.
In a series of experiments, three adult shrews were trained on a match-to-sample task using images of complex objects overlaid on mean gray and natural scene backgrounds. For each experiment, the tree shrews were trained to identify a specific target image across manipulations to position, viewpoint, and similarity to distractor images. To assess visual behavior during natural contexts, we recorded their movements and used a markerless pose estimation software, DeepLabCut, to track body position. We first applied this approach to video recordings during the match-to-sample task to validate accuracy and reaction time estimates with those from the experiment event logs. We then adapted this analysis to track visual behavior during in-cage exploration of familiar and novel objects.
From the match-to-sample experiments, the shrews were able to identify target objects across translation, scaling, and rotation when displayed on both mean gray and natural scene backgrounds. Further, they demonstrated a capacity to generalize across examples within an object category. Shrew choice and reaction time could be extracted from video recordings with high precision.
These results indicate that adult tree shrews have the capacity for visually recognizing and discriminating complex shapes. The ability to track and assess such visual behavior from video recordings in freely moving animals opens up new avenues for studying visual function in naturalistic environments. In particular, this approach may be useful for evaluating visual function across postnatal development. Since maturation in tree shrews is rapid, it is not feasible to assess visual capacities during critical periods of development with conventional paradigms, such as match-to-sample that require thousands of training trials to learn. Therefore, adapting methods for analyzing natural behaviors is critical in our investigation of high level vision throughout tree shrew development.

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Poster

297. Visual Contrast, Form, and Color

Location: SDCC Halls B-H

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Program #/Poster #: 297.05

Topic: D.06. Vision

Title: Alpha echoes in visual contrast processing originate in V1 and propagate up the visual system

Authors: *A. MORROW, A. PILIPENKO, S. SANKARAN, E. TURKOVICH, J. SAMAHA; Univ. of California Santa Cruz, Santa Cruz, CA

Abstract: Understanding how specific stimulus properties are maintained and distributed in the brain is critical to understanding how we are able to process and respond to varying perceptual

phenomena in our everyday environment. Using cross-correlation between EEG signals and random luminance sequences to estimate the brain's impulse response to luminance changes, it was found that a luminance change leads to a long-lasting "echo" in the alpha frequency range (Van Rullen & Macdonald, 2012). The neural origin of these echoes and the precise stimulus features that induce them have not been extensively studied. Here, we presented participants with checkerboard patches on a gray background which randomly modulated in contrast at 120 Hz and which could be presented in one of four visual quadrants. Our contrast manipulation held overall luminance constant to assess whether echoes were specific to luminance or contrast processing. Because of the known relationship between visual field location and the electric field polarity generated by primary visual cortex (V1) activation, we could also assess whether any echoes were confined to V1 or if they propagate through subsequent visual circuits. Using cross-correlation between EEG signals and the random contrast sequences, we observed alpha echoes that lasted 300ms on average. These echoes were highly correlated with each participant's individual alpha frequency (Spearman's $r = 0.7229$, $p = 0.0120$). Importantly, the initial wave of the echo showed a clear polarity reversal depending on the visual field location, but this phase difference quickly disappeared. These results suggest that contrast information is also eliciting a brief alpha-band echo and that the echo starts in V1 but propagates up the visual hierarchy.

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Poster

297. Visual Contrast, Form, and Color

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Program #/Poster #: 297.06

Topic: D.06. Vision

Title: Deep networks design "optimal stimuli" for early visual cortex

Authors: *M. SLAPIK, A. R. ANDREI, S. KHAN, V. DRAGOI;
Neurobio. & Anat., McGovern Med. Sch., Houston, TX

Abstract: Introduction:
Visual cortex builds a visual representation of our world, starting from basic features like lines and edges and leading up to more complex features like shapes and objects. However, these tunings are generally examined separately: it remains unclear how they come together when many visual features are combined in naturalistic settings. Using new machine learning techniques, we investigate tunings to these multi-faceted stimuli and demonstrate the encoding of naturalistic features in early visual cortex. In the process, we better capture the functioning of visual cortex in everyday life.
Methods:
We present visual stimuli to a macaque monkey on a computer monitor, and record from early visual cortex (V1) using a Plexon laminar electrode. Our machine learning algorithm was

pioneered by Ponce et al. (2019) and consists of two components: an image generator and an optimizer. The image generator, a generative adversarial network or "GAN," is trained on over a million natural images and can flexibly produce a wide range of stimuli that resemble textures, objects, and landscapes. Meanwhile, our optimizer, a co-variate matrix adaptation evolution strategy or "CMA-ES," uses neural feedback to iteratively evolve these stimuli and maximize the firing rate of a target neuron in early visual cortex.

Results:

Our results validate a new method of creating optimal stimuli in early visual cortex. By combining an image generator and an optimizer, we can consistently develop "optimal stimuli" that vastly outperform traditional stimuli such as oriented gratings or uniform colors. These optimal stimuli tend to incorporate preferred orientations and colors as measured by single-modality tasks, showing how these features optimally combine in naturalistic images. However, they also include suboptimal orientations and colors, as well as more complex features like textures and shapes not traditionally associated with early visual cortex. These findings reveal the diverse tunings of early visual cortex in response to naturalistic stimuli.

Discussion:

In this study, we demonstrate the power of using machine learning and neural feedback in stimulus design. Optimal stimuli vastly outperform traditional, single-modality stimuli and incorporate a diverse range of visual features such as color, shape and texture. This supports an overarching view of visual cortex as encoding multi-faceted, naturalistic features rather than simple, single-modality features like orientation or color alone. Furthermore, it shows how neural feedback can be used to guide stimulus design and discover new properties of neural circuits.

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Poster

297. Visual Contrast, Form, and Color

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Title: Memory recall-related information flow between the inferotemporal and prefrontal cortices through neural oscillations

Authors: T. ZHOU[1], X. XIA[1], K. KAWASAKI[2], I. HASEGAWA[2], *H. TANIGAWA[1]; [1]Interdisciplinary Inst. of Neurosci. and Technol., Zhejiang Univ. Sch. of Med., Hangzhou, China; [2]Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata, Japan

Abstract: The inferotemporal cortex (ITC) and the prefrontal cortex (PFC) are well known to contribute to memory recall, but the details of how information flows between two cortical areas during memory recall are poorly understood. Many studies have shown that communication between cortical areas is mediated through the synchronization of neural oscillations. In this study, we explored whether the information flow in bottom-up (BU) and top-down (TD) directions via neural oscillation between the ITC and PFC has specific spatial patterns for memory recall, where BU refers to the information flow from the ITC to PFC and TD refers to the opposite direction. We trained one macaque monkey for a picture-color association (color-recall) task and passive viewing tasks of picture or color stimuli used in the color-recall task. For the color-recall task, an achromatic picture was presented as a cue. After a delay period, when the corresponding color target was presented, the monkey needed to respond. To investigate the pattern of information flow between the ITC and PFC through neural oscillations in specific frequency bands, we used a high-density electrocorticogram (ECoG) to record the oscillatory activity in the ITC and PFC during the tasks and used non-parametric Granger causality (GC) analysis to the ECoG signals to calculate GC influences between channel pairs. By averaging the pairwise GC influences between each channel in the ITC or PFC and all channels in the other cortex (ITC or PFC), we visualized the average GC influence patterns between the two cortices as 2-D maps. By comparing GC influences in the color recall and passive-viewing tasks, we found regions of strong GC influences more related to color recall during the cue presentation and delay periods in the ITC and PFC. The GC influence patterns in the BU and TD directions in the theta band of the ITC and PFC during the color recall task showed higher correlations with those of the picture passive viewing task in the cue presentation period, but during the delay period, a higher correlation was observed with those of the color passive viewing in the TD direction. In addition, the recall-related GC influence patterns in the ITC and PFC differed depending on the color to be recalled. Our results indicate that during color memory recall, the spatial pattern of information flow via neural oscillations between the ITC and PFC changes over time and depends on the color to be recalled.

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Poster

297. Visual Contrast, Form, and Color

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Title: Emergence of visual symmetry perception in deep neural networks

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Abstract: A sense of symmetry, referring to the ability to detect the reflectional symmetry of visual objects, is considered a crucial function in animals for their survival under various conditions, such as mate selection or hunting. In primate brains, selective neural responses to visually symmetric objects are observed in extrastriate visual areas and are thought to provide a basis for symmetry perception. Nonetheless, these neural responses have been examined only at the population level using functional magnetic resonance imaging (Sasaki 2005) or electroencephalography (Bertamini 2014); thus, symmetry tuning at the single-cell level remains not fully understood. Here, using a model neural network of the ventral visual stream, we show that selective neural responses tuned to visual symmetry can emerge spontaneously from the training of natural images and that this selective activity contributes to the object classification performance of the network. First, we investigated single-unit responses to visual stimuli in a model deep neural network trained with natural images. Using controlled dot images with varying degrees of symmetry (DoS), we identified the units in the feature extraction layers whose responses varied depending on the DoS of the stimulus images. Interestingly, we found that the responses of certain units were highly correlated with the DoS of the input images, with these units defined as “symmetry units.” This suggests that symmetry sense in single units can emerge spontaneously from learning visual features for natural image classification. Next, we found that symmetry units play a crucial role in image classification. We estimated the classification performance capabilities of networks when symmetry units were ablated and found that this resulted in significantly decreased accuracy compared to that in the control group where non-selective units were ablated instead. In our additional analysis of the contribution of symmetry information to the classification performance of the network, the responses of trained classifiers were highly correlated with the DoS of the input images, implying that symmetry tuning is crucial for object recognition. Lastly, from an investigation of the weight matrices of feature extraction kernels, we found that the spatial distributions of weights relevant to symmetry units become more symmetric during training with natural images. We confirmed that this induces symmetry tuning in each unit, from the observation of significant correlations between the selectivity index of the symmetry units and the DoS of the weights. These results provide insight into visual symmetry perception for object recognition.

Disclosures: J. Lew: None. **M. Song:** None. **S. Paik:** None.

Poster

297. Visual Contrast, Form, and Color

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 297.09

Topic: D.06. Vision

Support: CIHR Project Grant

Title: A computational method to measure contrast sensitivity function in human visual cortex

Authors: *L. GOULET[1], R. ABBAS FARISHTA[2], R. FARIVAR-MOHSENI[3];
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Abstract: The processing of spatial frequencies and contrasts are basic building blocks of vision. The contrast sensitivity function (CSF) is one the most informative description of an observer's spatial vision abilities. Developed by Campbell and Robson (1968), the CSF describes the range of spatial frequencies (SF) that are detectable in an image with a fixed level of contrast. As it characterizes the boundary between the perceptually visible and invisible, the CSF informs us on the limiting factors of everyday visual functions. The CSF is therefore valuable to vision care providers as a non-invasive assessment tool. However, in clinical settings, measurements need to be quick, accurate and reliable. Yet, the complexity and duration of current CSF measurement methods limit their use to alert and cooperative subjects. We propose neuroCSF as a new method for measuring the CSF across the visual field directly from brain activity, and with minimal demand from participants. NeuroCSF is a computational model that estimates CSF parameters (i.e., peak contrast sensitivity, peak spatial frequency, spatial frequency bandwidth) from functional magnetic resonance imaging (fMRI) signals, under controlled visual stimulation conditions. The outcome is CSF estimates at each visual cortex location within 35 mins of scan time. The approach combined neuroimaging with a tightly matched dynamic stimulus, a canonical model of the CSF, and parameter optimization. Stimulus contents and timing were combined with the fMRI timeseries at each voxel to estimate the CSF parameters by iterating through many possible CSF models and evaluating their ability to explain a voxel's timeseries. The approach extends the population spatial frequency tuning (Aghajari, Vinke, & Ling, 2020) and population receptive field (Dumoulin & Wandell, 2008) methods, and provides the first complete characterization of a full CSF using neuroimaging data. We derived robust estimates of cortical CSF and show how CSF parameters vary across locations in the visual cortex. We observe that across early visual areas (V1, V2 and V3), the CSF peak spatial frequency is higher for foveal eccentricity and decreases at parafoveal eccentricities. Conversely, CSF spatial frequency bandwidth slowly increases with eccentricity. Thus, cortical CSF estimates vary systematically with eccentricity. The new neuroCSF approach enables fast estimates of cortical CSF parameters using fMRI neuroimaging. The procedure opens new perspectives for the study of cortical visual functions in various disorders where the CSF is impacted, such as amblyopia, traumatic brain injury, and multiple sclerosis.

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Poster

297. Visual Contrast, Form, and Color

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Topic: D.06. Vision

Support: European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 861423

Title: Identifying invariances in neural information encoding

Authors: *L. BARONI[1], M. BASHIRI[2], A. S. ECKER[3], J. ANTOLÍK[1], F. H. SINZ[3]; [1]Charles Univ., Prague, Czech Republic; [2]Univ. of Tübingen, Tübingen, Germany; [3]Univ. of Göttingen, Göttingen, Germany

Abstract: Visual sensory areas enable animals to identify objects regardless of their appearance and context. Such ability is thought to require neural mechanisms consisting of neurons selective to specific complex stimulus features while invariant to others (e.g., spatial location or orientation). To better understand biological vision, it is thus crucial to characterize which are the stimulus features that neurons in different visual areas are selective to, and which are the stimulus transformations leaving their response invariant. In the past, these transformations, called invariances, have commonly been identified by presenting carefully selected hypothesis-driven stimuli relying on the researcher's intuition. One example is the discovery of the phase invariance of V1 complex cells. The effectiveness of such hypothesis-driven approaches is, however, hindered by the nonlinear encoding of information and the high dimensionality of the stimulus space. To overcome this difficulty, in this work we propose a fully data-driven method to uncover invariances based on artificial neural networks. In recent years, artificial neural networks trained on large datasets of neural responses to natural images have proven to be powerful predictive models of neural responses. Due to their accurate predictions, they have already been used to identify stimuli that maximize the response of a given neuron. These results show the utility of these models as a digital twin of the biological brain, allowing neuroscientists to conduct analyses in-silico that are challenging to perform on the biological system, but whose predictions can be verified in-vivo. While previous work has focused on learning a single maximizing stimulus for a target neuron, in this study we present a novel technique to discover a continuous spectrum of stimuli that are different from each other but maximally excite the target neuron. That is, our technique learns directions in the stimulus space that the neuronal response is invariant to. Specifically, the approach consists in prepending to a response predicting model an image generating model (based on a CPPN architecture) that learns to map points from a low dimensional latent space to the entire manifold of images that maximize a neuron's response. We show that our technique successfully uncovers neural invariances and that, surprisingly, disentangles invariant directions in stimulus space if the neuron presents more than one. In contrast to previous hypothesis-driven strategies, our approach is fully data-driven, can be applied to different visual areas and identifies nontrivial neural invariance from recordings of neural responses to natural stimuli.

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Poster

297. Visual Contrast, Form, and Color

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Topic: D.06. Vision

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F31-EY031249

Title: Behavioral and neural sensitivity to simple shapes in developing macaques

Authors: *C. L. RODRIGUEZ DELIZ, G. M. LEE, N. J. MAJAJ, J. A. MOVSHON, L. KIORPES;
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Abstract: Shape discrimination improves throughout postnatal development; this cannot be wholly explained by changes in the early visual pathway, including primary visual cortex (V1). We propose that the maturation of downstream extrastriate areas underlies our ability to integrate lower-level contour information into global shape percepts. We focused on neural signals in V4 because V4 neurons - unlike those in V1 - respond selectively to object shape in adults. It is unknown when this selectivity emerges in early life, so we studied the development of form sensitivity in 4 macaques, aged 5 to 12 mo. We used radial frequency stimuli (RFS), circular targets whose radii are modulated sinusoidally, to track the development of form sensitivity. We tested monkeys' ability to discriminate RFS from circles as a function of the depth and frequency of modulation using a 4-alternative oddity task. As in humans, behavioral performance was best for higher radial frequencies. Performance improved both longitudinally and cross-sectionally across our subjects. We placed 96-channel "Utah" arrays in V4, and recorded single- and multi-unit neuronal responses to RFS from the same subjects during development. To measure the performance of neural populations, we used a classifier that learns an average population template, computes the Pearson correlation between responses on each trial and the template, and classifies each trial as circle- or RFS-driven. V4 populations showed shape-selective responses even at the earliest ages tested, and were able to reliably signal the presence of modulations near behavioral threshold. Neural decoding was optimal for shorter time windows in older animals than in younger ones, an effect related to the faster dynamics of responses in older animals. Using optimal time windows, we found no age-dependent differences in neural performance. Response changes in V4 therefore do not account for the development of behavioral form sensitivity, which presumably remains to be explained by changes in downstream areas.

Disclosures: C.L. Rodriguez Deliz: None. **G.M. Lee:** None. **N.J. Majaj:** None. **J.A. Movshon:** None. **L. Kiorpes:** None.

Poster

297. Visual Contrast, Form, and Color

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 297.12

Topic: D.06. Vision

Title: Brightness of disk-annulus stimuli is not due to edge integration plus contrast gain control

Authors: *O. B. KAVCAR[1], M. A. CROGNALE[1,2], M. E. RUDD[1,2];
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Abstract: Neural models based on the principle of edge integration are leading candidates for explaining brightness and lightness (grayscale appearance). According to these models, the early visual system encodes local contrast or luminance ratios at edges, and these local measurements are summed across space at a cortical level to compute brightness/lightness. This two-stage process distinguishes edge integration from alternative models of brightness/lightness based on contrast-normalized spatial channel outputs. In previous work, we combined edge integration (EI) with a contrast gain control (CGC) mechanism to account for brightness matches made with classical psychophysical stimuli comprising disks surrounded by annuli. Observers adjusted the luminance of a match disk to equate its brightness with that of a simultaneously presented test disk. The luminance of an annulus surrounding the match disk was fixed, while the luminance of an annulus surrounding the test was varied to induce brightness changes in the test (brightness induction). The model successfully accounted for matches made by different observers and for stimuli whose inner and outer annulus edges had all possible combinations of edge contrast polarities (that is, edge luminance either incremented or decremented in the direction of the disk). Here we tested our model in new experiments in which the annulus surrounding the matching disk was removed to simplify the theoretical analysis. To our surprise, the model failed to account for brightness matches made with our modified display. When the subject's matches are plotted against the test annulus luminance on a log-log scale, the model predicts that the plot will curve because changing the contrast on one edge automatically adjusts the gain associated with other edges. The curvature should correlate with the overall level of brightness induction from the surround. For our experiments, this relationship should depend only on the contrast polarity of the outer annulus edge. Instead, it depended only on the polarity of the *inner* annulus edge (whether the test was a luminance increment or decrement). The results are discussed in terms of a recent edge integration theory that incorporates neural data demonstrating different inherent gains for incremental and decremental stimuli in macaque LGN.

Disclosures: O.B. Kavcar: None. **M.A. Crognale:** None. **M.E. Rudd:** None.

Poster

297. Visual Contrast, Form, and Color

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Topic: D.06. Vision

Support: the Commissioned Research of NICT (1940101)
JSPS KAKENHI JP20K21803

Title: Decoding of material perception: analysis and synthesis from EEG via DNN model

Authors: *T. ORIMA[1,2], I. MOTOYOSHI[1];
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Abstract: It is widely known that the human visual cortex utilizes statistical structure of image inputs to perceive materials and properties of natural surfaces. To reveal the temporal processing of such textural information, on the basis of our previous findings (Orima & Motoyoshi, 2021; Wakita, Orima & Motoyoshi, 2021), we recorded visual evoked potentials (VEPs) for a variety of natural surface images, and analyzed reconstructed images and deep features derived from a multimodal variational autoencoder (MVAE). First, we measured VEPs for 191 natural surface images which contained 20 material categories (e.g., bark, fabric, glass etc.). Images were presented for 500 ms in random order with 750 ms blank, and EEG signals were recorded from 31 electrodes and 8 observers who viewed surface images foveally with no other tasks. Next, we developed MVAE inputting surface images and corresponding VEPs. In the training of MVAE model, the surface images and corresponding VEPs were split into 5 types of variants for each image, and one of those was assigned for test set. As a result, we found that the MVAE was able to reconstruct photorealistic surface images only by using VEPs in the test set, suggesting that the MVAE learned the relationship between surface images and corresponding VEPs successfully enough to reconstruct our perception. Moreover, deep features extracted from the MVAE could classify material categories on statistically significant level, and estimate the perceived surface quality (e.g., smoothness, heaviness, glossiness, etc.) that were rated by the observers in a separate psychophysical experiment. The classification accuracy improved as increasing time length of VEPs, and saturated at around 200 ms. These results suggest that VEPs reflect substantial information for our rich perceptual experience of natural surface materials, and further support the idea that global image features, which can be captured even by VEPs with poor spatial resolution, play critical roles in perception of textures and surfaces.

Disclosures: T. Orima: None. **I. Motoyoshi:** None.

Poster

297. Visual Contrast, Form, and Color

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Topic: D.06. Vision

Support: Australian Research Council ARC-FT180100458

Title: A five-primary Maxwellian-view display for independent control of melanopsin, rhodopsin and 3-cone opsins on a fine spatial scale

Authors: *T. W. NUGENT, A. J. ZELE;
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Abstract: Independent spatiotemporal control of the stimulation of the five photoreceptor classes requires a display with as many primary lights to probe their isolated spatial and temporal responses. No such system exists with suitable performance properties to simultaneously control melanopsin, rhodopsin and the three cone opsins in humans. Here we present the design and evaluation of a method of constructing a five-primary display from commercially available 3-primary digital light processing projectors (chosen projector performance: 360x640 pixels, 60Hz frame rate). We implement a computational algorithm to identify the optimal set of five primary lights from a set of available LEDs and narrowband interference filters (10nm FWHM). Each projector in the optical system displays an image created by one primary. These images are merged onto a common optical axis in the plane of the pupil of the observer in Maxwellian view. A single HDMI connection controls all projectors by splitting the data into five video streams using a custom designed algorithm implemented in an FPGA. Key to enabling this multiple display system is a novel control protocol in a deterministic FGPA controller; this allows nearly synchronous presentation of primary image data through multiple displays (maximum inter-primary delay of 1.4ms). Each pixel is controlled over 768 levels (9.5 bits) for each primary over a 60Hz frame for measurement of threshold level vision. The five primaries are optimised for melanopic photostimulation and in a silent substitution protocol produce a maximum Michelson contrast of 30% (silent rhodopsin, 3 cone opsins). In addition to a large contrast gamut, the Maxwellian view offers higher retinal illumination with a total primary radiance of 87 $W/m^2/sr$. This display supports the investigation of five opsin-based responses to spatiotemporal stimuli with a truly silent substitution protocol, while avoiding the confounding effects of uncontrolled photoreceptor excitations as occurs with four primary systems. The customisable primaries facilitate this display's translation to species with different photoreceptor spectral responses, and the optics are designed for integration into microscopes for use as a stimulus generator in physiological experiments.

Disclosures: T.W. Nugent: None. **A.J. Zele:** None.

Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

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NRF-2021M3E5D2A01023888
NRF2022R1A2C2007599
NRF2022M3E5E8081195

Title: An insect vision-inspired flight control algorithm with an auto-tuned efference copy

Authors: *A. CANELO[1], A. J. KIM[2];
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Abstract: Flying insects can process multiple visual features in parallel neural circuits and generate an appropriate action. Neural processing of singly presented visual patterns has been studied intensively in *Drosophila* for the past few decades. How do parallel visual circuits responding to different features presented concurrently are integrated to control a shared motor circuit? An influential theory proposed for combining multiple sensorimotor circuits is an efference copy mechanism, in which an intended action offsets other sensory circuits to prevent them from responding to reafferent sensory inputs caused by the action. Recent studies in *Drosophila* have identified efference copy-like signals in an array of motion-sensitive visual neurons that mediate visual stability reflexes. We used a classical approach to model visually guided flight control in *Drosophila* and captured its behavioral responses to elementary visual patterns. Through these responses and using a dynamical systems approach, we implemented two computational models that combine the stability reflex with spontaneous or other visually evoked flight controls such as object tracking and avoidance. The models combine the two neural pathways, one additively, and the other by the modulation of an efference copy, respectively. Furthermore, we included in the second model a simple multi-layered perceptron (MLP) that is used to auto-tune its efference copy enabling the modulation even in changing visual environments. The model demonstrates that the visual stability reflex dampens spontaneous as well as visual object-induced flight turns when combined additively and that the modulation of the stability reflex by an efference copy permits undamped, concurrent operation of multiple visual behaviors. Finally, the inclusion of the MLP auto-tunes the efference copy to match variations in sensory feedback associated with changes in internal or environmental variables. Our study provides an integrative model of vision-based flight control when multiple visual features are presented simultaneously in changing visual environments and may be extended to an adaptive flight control mechanism for artificial flying agents such as drones.

Disclosures: **A. Canelo:** None. **A.J. Kim:** None.

Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

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PIIF
A*STAR NSS(BS-PhD)

Title: Rodent Lateral Posterior (LP) thalamus input to Anterior Cingulate Cortex (ACC) tracks ongoing and post-factum perceptual uncertainty

Authors: *Y. LEOW[1], Y. OSAKO[1], A. R. BARLOWE[1], C. T. LUO[1], M. SUR[2];
[1]MIT, Cambridge, MA; [2]Dept. of Brain and Cognitive Sci., MIT Grad. Brain and Cognitive Sci., Cambridge, MA

Abstract: Ongoing uncertainty in our sensory environment is constantly being estimated in order to effectively guide attention and determine perceptual confidence. The pulvinar, or homologous rodent lateral posterior (LP) nucleus, is a higher order visual thalamic structure critical for regulating selective attention for behaviorally-relevant visual stimuli. Pulvinar interactions with the prefrontal cortices have been particularly implicated in regulating attentional processes. Here, we hypothesized that the rodent medial LP, by broadly integrating input from multiple visual areas as well as the frontoparietal cortices, is able to provide an ongoing estimate of the visual evidence, while the Anterior Cingulate Cortex (ACC) can utilize this information to guide sensorimotor decisions. We trained mice on a two-choice random dot (RDK) motion discrimination task, varying sensory certainty with the proportion of coherent target dot directions, while examining LP→ACC axonal calcium activity with two-photon microscopy. We found LP→ACC axons responded robustly during the task, not just during the stimulus period but also strongly upon reinforcement. We performed population analyses to determine the selectivity subspaces occupied by LP→ACC activity during the task. In the stimulus direction coding subspace, we found that a subset of LP→ACC axons represented the sensory uncertainty with reference to the dominant movement direction, while others represent absolute sensory uncertainty regardless of direction. Intriguingly, even after stimulus offset, the coherence of the stimulus appears to be represented even after reinforcement. This information persists in the LP→ACC axons across to the next trial which may potentially guide top-down allocation of attentional resources. Our results suggest LP involvement in decision-making beyond ongoing visual processing and perceptual evidence accumulation to include a potential involvement in conveying information on post-factum perceptual uncertainty.

Disclosures: Y. Leow: None. **Y. Osako:** None. **A.R. Barlowe:** None. **C.T. Luo:** None. **M. Sur:** None.

Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

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Title: Causal contributions of FEF, LIP and SC to rapid visual categorical decisions

Authors: *V. SHIRHATTI, O. ZHU, S. DAVID, S. CHANG, A. MEDOFF, D. J. FREEDMAN;
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Abstract: Rapid categorization of incoming sensory stimuli to guide timely motor responses is crucial to behavior. To understand the neural mechanisms in such a process we designed a novel rapid visual motion categorization task (RCT) that compels the subject to perform rapid categorization of a visual motion stimulus, and allows independent assessment of the visual, cognitive, and motor aspects of the subject's decisions. In this saccade-based RCT a monkey is required to rapidly categorize the direction of motion of moving dots under variable durations of coherent motion on each trial, referred to as the processing time (PT). To delineate the causal contributions of oculomotor areas lateral intraparietal (LIP), frontal eye field (FEF), and superior colliculus (SC) we reversibly inactivated local populations in these areas with a $GABA^A$ agonist, muscimol, during RCT (7 sessions/area). Either the category stimulus or one of the targets was placed in the inactivated visual field (IF) to separately test the impact of inactivation of visual and motor aspects of behavior. The strongest deficits in performance were produced by inactivation in FEF, followed by SC and LIP. Inactivating FEF caused a significant drop in mean accuracy for both spatial configurations, for nearly all processing times and motion directions. For PT>100ms the mean accuracy reduced by 15.98±0.35% (mean±s.e.m) and 8.95±0.33% compared to control conditions, for the cases with stimulus and target inside the IF respectively. Moreover, it slowed cognitive processing and required longer PT to reach peak performance across all conditions (PT increased by ~76ms and ~42ms to reach 85% accuracy). Weaker, but qualitatively similar, deficits were caused due to SC inactivation (~12.7±0.37% and ~7.07±0.16% drop in mean accuracy, ~58ms and ~24ms delay for 85% accuracy). In contrast, LIP inactivation impaired accuracy and produced longer PT only when the motion stimulus, but not saccade target, was placed in the IF (~8.65±0.45% drop in mean accuracy, ~38ms delay for 85% accuracy). Our results suggest a causal role for FEF across oculomotor and perceptual-cognitive components, whereas LIP appears to be more engaged in the cognitive evaluation of sensory stimuli during RCT. Importantly, SC seems to be causally involved even in the cognitive aspects of categorization, apart from its traditionally known roles in saccade planning and execution. We recorded neural signals simultaneously from multiple sites in FEF, LIP and SC during this task, and ongoing neural data analyses are directed towards elucidating the neural mechanisms underlying the causal contributions of these regions in rapid visual categorization.

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Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

Support: Howard Hughes Medical Institute at the Janelia Research Campus

Title: Interpretable behavioral features have conserved neural representations across mice

Authors: *A. SYEDA, R. TUNG, W. LONG, M. PACHITARIU, C. STRINGER;
Janelia Res. Campus, Ashburn, VA

Abstract: Recent studies in mice have shown that ongoing behaviors drive a large fraction of neural activity across the brain. However, the behavioral features used in these studies are hard to interpret, because they represent the principal components of the raw video recordings. We thus don't know what specific behaviors are encoded in the neural activity and comparisons across mice remain difficult. To address these issues, we developed new models of behavior and used them to predict neural recordings of ~50,000 neurons using new predictive models. To start, we used simultaneous five-camera video recordings to show that orofacial behaviors alone predict neural activity as well as features from the entire body. Focusing on the face, we tracked several keypoints using a new neural network model. The model was as accurate or more than the state-of-the-art pose estimation tools DeepLabCut and SLEAP respectively, while the inference speed was several times faster, making it a powerful tool for closed-loop behavioral experiments. Using the tracked keypoints, we derived pose relationships which in turn we used as features. These behavioral features predicted a much higher proportion of neural activity than the previous features based on principal components of the raw video. To make behavior models that can work across mice, we retrained the neural network on a new dataset of face camera videos recorded from many mice and from arbitrary camera angles. We then created a common behavioral framework that aligns facial keypoints to a single template. We used the aligned keypoints to predict neural activity using a single model across all mice. The universal mouse model predicted neural activity as well as a model fit to a single mouse, demonstrating that neural representations of behaviors are similar across mice. The resulting latent states shared across mice enabled the characterization of the behaviors represented in neural activity. We found that interpretable mouse behaviors drove distinct neural patterns. In summary, we developed a robust modeling framework for modeling neural activity based on orofacial behaviors and found that neural representations of behavior are shared across mice.

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Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

Support: Howard Hughes Medical Institute

Title: Brain-wide modulation of neural activity during behavioral passivity

Authors: *Z. WEI[1], J.-X. LIM[1], S. NARAYAN[1], X. MI[2], W. ZHENG[2], G. YU[2], J. E. FITZGERALD[1], M. B. AHRENS[1];
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Abstract: Animals can discern environmental context and adapt their behavior and change how their brain operates accordingly. For example, when actions continuously fail to produce the desired consequences, animals tend to ‘give up’ and fall into states of passivity. In larval zebrafish, this 'futility-induced passivity' is implemented through a cooperative mechanism of the noradrenergic system and brainstem radial astrocytes (Mu et al., Cell, 2019). However, little is known about how such changes of operating mode impact sensory and motor computations throughout the brain. Here we recorded brain-wide neural activity in larval zebrafish while they underwent state transitions between productive behavioral activity and futility-induced passivity as they swam against the flow in virtual reality environments. This revealed brain-wide modulations of neural dynamics encoding various aspects of the stimulus and behavior. Most simply, a passive state can last for tens of seconds, during which the fish’s behavioral response to optomotor response-driving stimuli was slow. We accordingly found that neural populations that encoded the visual stimulus and its integral were suppressed in a passive state, which could cause the slowed optomotor response. We also discovered more complex patterns of state modulation. For example, we identified a neural population that encoded sensory history after productive swimming but was inactive after futile swimming. Another population encoded swim vigor history only after futile swimming. These populations indicate that longer-timescale sensorimotor processing is brain-state dependent. Finally, besides astroglial dynamics, we also found widespread swim-evoked release of norepinephrine and dopamine during futile swimming. These neuromodulators persist for tens of seconds and may contribute to the various neuronal processing changes seen brain-wide. Overall, this study shows that state-dependent sensorimotor processing is surprisingly widespread and persistent, and modulates brain-wide information processing and computations in a logical, tractable way.

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Poster

298. Sensorimotor Behavior

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Title: Effects of locus coeruleus norepinephrine signaling on target regions during reinforcement learning

Authors: *G. DRUMMOND[1], J. SHIH[1], Y. OSAKO[1], J. PARK[1], V. BRETON-PROVENCHER[2], M. SUR[1];
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Abstract: The locus coeruleus (LC) is a small brainstem nucleus and the primary source of the neuromodulator, norepinephrine (NE), in the brain. Through a widely divergent set of projections, LC neurons release NE throughout the brain to regulate arousal and attention. However, recent work from our lab has also shown two distinct functions for LC-NE in reinforcement learning. In an instrumentally conditioned go/no-go task with graded auditory stimulus detection, phasic LC-NE activity is critical for task execution under high uncertainty conditions, and for optimization after surprising outcomes. These results suggest that spatiotemporal dynamics enable LC-NE circuits to facilitate task execution and to promote reinforcement encoding and performance optimization. Yet, it remains unknown how these signals alter target circuits to change behavior. Phasic LC-NE activity lasts only milliseconds, while the effect of a trial outcome on the next trial requires that the signal persists for several seconds. LC-NE has been shown to act on astrocytes, the major glial cell of the brain. Astrocytes can integrate and alter neuronal signals over diverse timescales, presenting a means by which the information from phasic LC-NE signals can be sustained through the next trial. Alternatively, neurons have been suggested to hold information in memory through recurrent network dynamics. Here, we explored the effects of LC-NE on cortical neurons and astrocytes in prefrontal cortex (PFC) and motor cortex (MC) during our reinforcement learning task. We used neuropixels probes to generate high density recordings of single units in PFC and MC in mice performing the task while silencing LC-NE neurons on a subset of trials. We then used population analyses to determine the effects of LC-NE on cortical population dynamics in each region during select task epochs. Using 2-photon calcium imaging, we recorded astrocyte and neuron calcium dynamics in MC and PFC in mice performing the task, and measured the effects of silencing LC-NE neurons on these cell types. Finally, by manipulating astrocyte calcium while recording MC and PFC neuronal activity, we explored the effects of astrocyte dynamics on behavioral performance and on neuronal population activity in our reinforcement learning task. We find that phasic LC-NE during pre-execution and post-reinforcement epochs alter neuronal population dynamics and astrocyte calcium to facilitate task execution and optimization.

Disclosures: G. Drummond: None. **J. Shih:** None. **Y. Osako:** None. **J. Park:** None. **V. Breton-Provencher:** None. **M. Sur:** None.

Poster

298. Sensorimotor Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 298.07

Topic: D.07. Visual Sensory-Motor Processing

Title: Refinement of visual responsive circuitry drives development of visuomotor behaviors

Authors: *M. LORING, M. NIKITCHENKO, E. THOMSON, E. NAUMANN;
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Abstract: Compensatory behavioral responses to visual motion stimuli are conserved across all vertebrates. Even the neural circuit underlying the 6-day old zebrafish optomotor response (OMR), an innate visual orienting behavior, is unexpectedly complex given the simple nature of the behavior and the organism, involving various brain regions and thousands of neurons with heterogeneous response properties. Yet, little is known about the developmental mechanisms of the behavior and associated functional neural circuit assembly. We find that fish proceed from broadly unresponsive to mature behavioral performance through an intermediate stage in which fish first respond to forward motion visual stimuli before all other directions. Using calcium imaging via volumetric two-photon microscopy, we demonstrate that visual representations in the retinorecipient pretectum mirror the stages of the behavioral development: progressing from weak, broadly tuned neuronal responses into mature, specific response classes. Together these studies lay the experimental foundation to delineate the functional development of a brain-scale complex neural circuit.

Disclosures: M. Loring: None. **M. Nikitchenko:** None. **E. Thomson:** None. **E. Naumann:** None.

Poster

298. Sensorimotor Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 298.08

Topic: D.07. Visual Sensory-Motor Processing

Support: Simon's Foundation
Wellcome Trust

Title: Basal ganglia and prethalamic pathways play disparate roles in sensory-guided decision-making.

Authors: *N. J. MISKA[1], T. D. MRSIC-FLOGEL[2], S. B. HOFER[1], I. BRAIN LABORATORY[3];
[1]Sainsbury Wellcome Ctr. (UCL), London, United Kingdom; [2]Sainsbury Wellcome Ctr. (UCL), London,, United Kingdom; [3]N/A, N/A, United Kingdom

Abstract: Both prethalamic and basal ganglia output pathways are implicated in goal-directed behaviours, but the function of these two pathways during decision-making influenced by prior knowledge and current sensory evidence is unclear. We trained mice on the international brain laboratory (IBL) task, a standardized and reproducible 2-alternative forced choice task in which the position of a visual stimulus of variable contrast must be indicated with a left or right wheel movement, and in which the probability of stimulus location changes in blocks of trials over time. In mice proficient in the task, we tested the behavioural consequences of optogenetic manipulation of two nuclei central to the basal ganglia and prethalamic pathways, respectively: substantia nigra pars reticulata (SNr) and zona incerta (ZI). While SNr and ZI both send overlapping inhibitory outputs to midbrain motor nuclei, we surprisingly found that unilateral optogenetic excitation of ZI versus SNr results in highly disparate motor effects during the task, with ZI excitation leading to immediate contraversive choices and SNr excitation leading to delayed ipsiversive choices, indicating that these projections differentially affect downstream circuits during the task. Strikingly, we found that optogenetic inhibition of SNr abolished animals' prior-mediated shift in psychometric curves while maintaining overall task accuracy, suggesting that this pathway is critically involved in the expression of animals' prior estimate but is not crucial for decision-making based on current sensory evidence. This result is reinforced by the fact that within the IBL brainwide map dataset, SNr contains among the highest percentages of units significantly modulated by the animals' prior. Ongoing and future experiments will look to elucidate both upstream and downstream circuit mechanisms of these behavioural effects.

Disclosures: N.J. Miska: None. **T.D. Mrsic-Flogel:** None. **S.B. Hofer:** None. **I. Brain Laboratory:** None.

Poster

298. Sensorimotor Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 298.09

Topic: D.07. Visual Sensory-Motor Processing

Title: The role of p11 in sensorimotor gating - behavioral and imaging investigation

Authors: *I. FLAIS[1,2,3], I. MANTAS[3], T. IONESCU[1,4], E. KIM[2], D. CASH[2], P. SVENNINGSSON[3], B. HENGERER[1];
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Abstract: Schizophrenia is a chronic debilitating mental disorder. A better understanding of its pathogenesis would facilitate improvements of pharmacological treatments. P11 is an adaptor protein, involved in the regulation of 5HT- and glutamate receptor trafficking and signaling. Recent evidence suggests that 5HT and glutamate neurotransmission are involved in schizophrenia.Sensorimotor gating impairment is a hallmark feature of schizophrenic patients. In this study we corroborated the role of p11 in psychosis, by testing its function in the pre-pulse inhibition (PPI) rodent paradigm. We also investigated the brain areas, where p11 expression might be crucial for the behavioral phenotype.We studied global p11 knockout (p11KO) mice and their wildtype (WT) littermates (female and male; 2-3 months). Behavioral testing involved PPI in the absence and presence of clozapine (Clz) (n=8). 3D T2w MR images were obtained from WT and p11KO mouse heads *ex vivo* and analyzed by tensor-based morphometry for structural differences (n=10). Functional Ultrasound (fUS) experiments at Bregma -1.5 to -2.5 were performed with WT and p11KO mice to measure cerebral blood volume (CBV) and connectivity changes between resting state conditions and following treatment with Clz, comparing genotype and treatment effect (n=10). We observed that p11 KO display an impaired sensorimotor gating, denoting a schizophrenia-like behavioral phenotype. Moreover, clozapine´s ability to improve PPI deficits was blunted in p11 KO mice.Structural *ex vivo* MRI scans demonstrated an enlargement of the hippocampus in p11KO mice, suggesting the importance of p11 expression for the morphometric development of the hippocampus. Accordingly, fUS imaging indicated lower resting state connectivity in p11KO mice in the hippocampal, region. Nevertheless, treatment with Clz resulted in a significant increase in hippocampal CBV of both, WT and p11KO mice.
Considering that p11 is enriched in medial septum/diagonal band (MSDB) neurons, we hypothesized that loss of p11 in MSDB might explain the sensorimotor gating deficit observed in p11 KO. Thus, we performed conditional KO experiments (p11 cKO) in medial septum (MS) by deleting p11 via AAV-mediated shRNA (n=9). Depletion of p11 in the MS resulted in decreased PPI in p11gKO mice compared to control mice.Conclusively, we hypothesize that p11 plays an important role in brain circuits known to be involved in regulating sensorimotor gating, via the septo-hippocampal pathway.

Disclosures: **I. Flais:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG. **I. Mantas:** None. **T. Ionescu:** A. Employment/Salary (full or part-time):; tudor_mihai.ionescu@boehringer-ingelheim.com. **E. Kim:** None. **D. Cash:** None. **P. Svenningsson:** None. **B. Hengerer:** A. Employment/Salary (full or part-time):; Boehringer Ingelheim Pharma GmbH & Co. KG.

Poster

298. Sensorimotor Behavior

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 298.10

Topic: D.07. Visual Sensory-Motor Processing

Support: JSPS KAKENHI Grant Number 19K11460
JSPS KAKENHI Grant Number 22H03492

Title: The effect of eccentricity on speed and accuracy in visual motion processing

Authors: *R. HIRANO[1], K. NUMASAWA[1], Y. YOSHIMURA[1], T. MIYAMOTO[3,4], T. KIZUKA[2], S. ONO[2];
[1]Grad. Sch. of Comprehensive Human Sci., [2]Hlth. and Sport Sci., Univ. of Tsukuba, Tsukuba, Japan; [3]Japan Society for the Promotion of Sci., Tokyo, Japan; [4]Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan

Abstract: The purpose of the current study was to clarify the effect of eccentricity on speed and accuracy in visual motion processing. Therefore, we measured time-to-contact (TTC) errors, light-on reaction time (RT) and motion RT at different eccentricities. TTC indicates the ability to accurately estimate the time to contact of a moving object, while visual RT reflects the speed of visual perception. Thirteen male subjects performed these three tasks. Visual stimuli were presented on a CRT monitor at a distance of 57 cm from the participants. The TTC task was to press a button when the moving target would arrive at the stationary goal. The target was occluded 500ms before the target arrival time. The light-on RT task was to press a button as soon as the target appeared, while the motion RT was to press a button as soon as the target moved. The visual stimuli were randomly presented at five different eccentricities (4, 6, 8, 10, 12 deg). In the TTC tasks and motion RT, the target moved on a circular trajectory at a constant tangent velocity (8 deg/s) to keep the eccentricity constant. Our results showed that TTC error in the occluded condition showed an earlier response as the eccentricity increased. In contrast, TTC error in the visible condition showed no effect of eccentricity. Furthermore, the light-on and motion RTs tended to become longer as the eccentricity increased. A significant positive correlation was found between TTC in the visible condition and light-on / motion RT. However, a significant positive correlation was not found between TTC in the occluded condition and light-on / motion RT. These results suggest that the speed and accuracy of motion perception using the TTC and RT tasks are affected by eccentricity in peripheral vision. This study showed a significant correlation between TTC error and RT only in the visible condition, where the target was presented throughout the task. Therefore, it is likely that participants did not predict the timing of arrival since the target was visible until coincidence. Rather, they responded when the target was reached visually, and thus it is considered that the result was dependent on the RT. In contrast, TTC error in the occluded condition did not show a positive correlation with RT. Previous studies have shown that TTC errors are affected by target speed, with higher target speed resulting in a delayed response. Moreover, it has been reported that peripheral vision perceives target velocity more slowly than central vision. Therefore, TTC error in the occluded condition may depend on the effect of eccentricity associated with speed perception.

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Poster

298. Sensorimotor Behavior

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 298.11

Topic: D.07. Visual Sensory-Motor Processing

Support: R15 EY027112/EY/NEI NIH HHS/United States

Title: Activity-dependent Plasticity of Structure and Function of Mauthner Cell Ventral Dendrites

Authors: *Z. TANVIR, D. RIVERA, K. E. SEVERI, G. HASPEL, D. SOARES;
New Jersey Inst. of Technol., Newark, NJ

Abstract: Activity-dependent Plasticity of Structure and Function of Mauthner Cell Ventral Dendrites
Two large hindbrain cells called Mauthner cells mediate escape in teleosts. The Mauthner cells have two modality-specific dendrites: a lateral dendrite that processes acoustic and lateral line stimuli and a ventral dendrite that is thought to process visual stimuli. While the ventral dendrite is less studied, we recently showed that blind Astyanax cavefish have truncated or missing ventral dendrites, compared to surface dwelling fish of the same species. On the other hand, raising Astyanax surface fish, as well as zebrafish, larvae in a light-deprived environment leads to longer, more elaborate ventral dendrites. This is an example of non-adaptive phenotypic plasticity, where the same condition leads to opposing outcomes in evolutionary and developmental timescales. We are currently studying synaptic changes that occur under these conditions, and their behavioral consequences. We are using a combination of immunohistochemistry and expansion microscopy to visualize chemical and electrical synapses on ventral dendrites; as well as behavior tracking and analysis of visually evoked escape responses to looming stimuli.

Disclosures: Z. Tanvir: None. **D. Rivera:** None. **K.E. Severi:** None. **G. Haspel:** None. **D. Soares:** None.

Poster

298. Sensorimotor Behavior

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Program #/Poster #: 298.12

Topic: D.07. Visual Sensory-Motor Processing

Support: Medical Research Council (MRC)
Wellcome Trust
Cancer Research UK

Title: Midbrain circuits for the execution of visual pursuit behaviours

Authors: *M. J. MOGLIE[1], Y. CHAN[2], X. CANO-FERRER[1], D. HUANG[2], M. F. IACARUSO[1];
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Abstract: Extraction of relevant stimulus features from the dynamic sensory scene needs to be coupled to the execution of appropriate adaptive responses to ensure survival. A predator will frequently need to evaluate its position with respect to that of moving prey, define an approach strategy and carry out the proper motor commands to execute it. The best strategy will require an estimation of the future position of the prey that accounts for sensorimotor processing delays, to make a predictive interception.
In the mammalian midbrain, the superior colliculus (SC) integrates visual motion information and plays a key role in mediating orienting behaviours towards or away from biologically relevant stimuli. We set out to investigate the role of the SC in predictive pursuit behaviours. First, we developed a behavioural paradigm in which mice were trained to pursue and intercept a moving target displayed on a touch screen to obtain a reward. To ensure the performance of behaviourally consistent pursuit approaches, we implemented a novel maze-like arena design and closed-loop stimuli presentation. Mice were able to perform ~350 trials per session and the proportion of successful interceptions was dependent on the target's initial location and speed. Mice adapted their pursuit strategy to the demands of the task by modifying their speed and/or the trajectory followed to reach the target. Tracking data was used to build a generative model of behaviour and assess the strategy employed to intercept the target. Our results indicate that mice can adapt their pursuit strategy in a predictive way.
The role of the SC was evaluated using a chemogenetic approach. An AAV was used to express the inhibitory DREADD hM4D(Gi) bilaterally in the SC. Performance was evaluated following suppression of SC neural activity resulting from intraperitoneal injection of the hM4D agonist Cyclopiazonic acid (CNO) (relative to that of vehicle (DMSO) treated controls). Suppression of activity in the SC reduced task performance, increasing the time required to capture the target and decreasing the accuracy of target interception. Moreover, analysis of the trajectory heading revealed that alignment to the moving target was delayed.
The reproducible presentation of moving stimuli allowed the consistent comparison of mice pursuit strategies to reveal predictive aspects of goal-directed interceptive approaches. We show that the SC plays a key role in this behaviour. Further experiments will aim to disentangle how the neural circuitry within the SC allows the integration of spatiotemporal features of moving sensory stimuli and links it to the execution of motor commands that guide the pursuit strategy.

Disclosures: M.J. Moglie: None. **Y. Chan:** None. **X. Cano-Ferrer:** None. **D. Huang:** None. **M.F. Iacaruso:** None.

Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

Support: NIH Grant U19NS104653
NIH Grant 1R01NS124017
NIH Grant IIs-1912293
Simons Foundation SCGB 542973

Title: Adenosinergic signaling in neuron-glial interactions underlying giving up in larval zebrafish

Authors: *A. B.-Y. CHEN[1], M. DUQUE[2], X. MI[4], S. NARAYAN[5], G. YU[4], F. ENGERT[3], M. B. AHRENS[6];
[1]Harvard Univ., Janelia Res. Campus, Ashburn, VA; [2]Mol. and Cell. Biol., [3]MCB, Harvard Univ., Cambridge, MA; [4]Virginia Tech., Blacksburg, VA; [5]HHMI- Janelia Res. Campus, Ashburn, VA; [6]Janelia Res. Campus / HHMI, Ashburn, VA

Abstract: Astrocytes have historically been considered primarily to play a supportive role in brain function. However, recent studies have revealed that astrocytes might play more direct roles in circuit computations by sensing neural activity and/or neuromodulators and communicating with downstream neurons via the release of gliotransmitters or other neuroactive substances. In the larval zebrafish, a population of hindbrain radial astrocytes integrates a noradrenergic futility signal due to visuo-motor mismatch and suppresses swimming. To date, the mechanisms and molecules through which these astrocytes signal downstream neurons to trigger passivity remain unknown. Here, I use fluorescence imaging of neuromodulator sensors, in situ hybridization, and behavioral pharmacology to reveal that astrocytes might release ATP during struggles leading to passivity. The ATP is then metabolized into adenosine, which acts on A2b adenosine receptors expressed by a subset of hindbrain neurons. Altogether, these experiments characterize how non-neuronal cells can play direct roles in behaviorally relevant circuit computations.

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Poster

298. Sensorimotor Behavior

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Program #/Poster #: 298.14

Topic: D.07. Visual Sensory-Motor Processing

Support: KCL Marin Studentship
Wellcome Trust Investigator Awards in Science 2016

Title: Types of projection neurons in the optic tectum of the larval zebrafish

Authors: *R. S. H. WILLIAMS, M. S. GRUBB, M. P. MEYER;
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Abstract: The optic tectum, analogous to the superior colliculus in mammals, facilitates the transformation of topographically organised visual information from the retina to appropriate behavioural responses. These responses include a diverse repertoire of goal-directed movements essential for prey capture and predator avoidance. Previous work in the larval zebrafish and other animals has shown that these movements can be elicited through stimulation of the optic tectum in a gradient along the anteroposterior axis. However, the features and distribution along this axis of tectal projection neurons targeting non-tectal brain areas is still yet to be described in detail.
Methods:At 6 days post-fertilization (dpf) larval zebrafish are small and translucent and so fluorescently labelled neurons can be visualized in their entirety in the intact, living brain. Tectal projection neurons were sparsely labelled by embryonic injection with FoxP2:Gal4/UAS:LYN-tdT. High resolution, 3-dimensional confocal images of 34 labelled neurons were acquired at 6dpf and registered to an anatomical atlas of the zebrafish brain. All non-overlapping, sparsely labelled cells that had a soma within the optic tectum and at least one neurite projecting out of the tectum were imaged and traced. Interneurons, defined as any cells without a neurite projecting out of the tectum, were excluded from analysis. These cells were compared with publicly available datasets volumetrically registered to standard space.
Results:Hierarchical clustering of these tectal projection neurons reveals cell types with distinct dendritic and axonal targets distributed along the anteroposterior axis of the optic tectum. Neurons targeting anterior regions, such as the arborisation fields of retinal ganglion cells, are located in the anterior tectum and smaller dendritic arbours. Cells located medially and posteriorly tend to have broad dendritic arbours and wider-ranging targets, from the rostral hypothalamus to the most caudal rhombomeres of the brain. Several cells identified send a single projection to the contralateral tectal hemisphere which crosses the tectum dorsally and terminates in a symmetrical location about the midline. All these cells separately target the hypothalamus, suggesting a mechanism for co-ordination between hemispheres in processing visual information from the two eyes.
Conclusion: The distribution of morphological cell types along the anteroposterior axis of the tectum creates a gradient of specialisation for the processing of visual information.

Disclosures: R.S.H. Williams: None. **M.S. Grubb:** None. **M.P. Meyer:** None.

Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

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Simons Foundation Grant SCGB 542973

Title: Investigating long-timescale dynamics of rheotaxis in larval zebrafish

Authors: *A. ZHANG, F. ENGERT;
Harvard Univ., Cambridge, MA

Abstract: Larval zebrafish are able to orient and swim against hydrodynamic flow as early as 5 days post-fertilization. This allows them to maintain constant position despite motion in their immediate environment, and can be either visually-driven (optomotor response) or visually-independent (rheotaxis). The dynamics of rheotaxis over short timescales, within a minute of flow onset, have been well studied, and previous work has shown that rheotaxis in the dark requires integration of flow information over both space and time (Oteiza et al 2017). However, behavior can also evolve on a longer timescale (e.g. adaptation), and how zebrafish respond to hydrodynamic flow over minutes or hours is not well-described. Here, we designed a free-swimming assay that allows us to measure the behavior of larval zebrafish in response to hydrodynamic flow over tens of minutes or longer, either in the dark or in combination with visual stimuli. We measure the orientation and swim speed of the larvae and find that behavior can be separated into contrasting behavioral regimes, i.e. active swimming to counter hydrodynamic flow, as previously described, versus a boundary-seeking behavior that allows fish to exploit low-speed regions of flow in the arena. We compare the swim statistics and proportion of time spent in these different behavioral regimes in the dark versus in the presence of visual cues. Here we find that fish behavior in the presence of hydrodynamic flow involves categorically different behaviors over the course of minutes.

Disclosures: **A. Zhang:** None. **F. Engert:** None.

Poster

298. Sensorimotor Behavior

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Topic: D.07. Visual Sensory-Motor Processing

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Simons Foundation SCGB 542973

Title: Sleep deprivation increases performance in larval zebrafish decision making through reduced bouting

Authors: *P. PFLITSCH[1], H. ZWAKA[1], W. JOO[1], K. KRISHNAN[1], N. OURY[1], A. BAHL[1], D. LYONS[3], J. RIHEL[3], F. ENGERT[2];

[1]Dept. of Mol. and Cell. Biol., [2]Ctr. for Brain Sci., Harvard Univ., Cambridge, MA; [3]Cell and Developmental Biol., UCL, London, United Kingdom

Abstract: Humans and most animals need sleep to function well. Lack of sleep has a multitude of effects on the body including a decrease of cognitive and physical performance. Zebrafish are a popular model to study sleep. Here we set out to investigate the effects of sleep deprivation on simple behavior and cognition in larval zebrafish. We show that in zebrafish larvae sleep deprivation downregulates bouting the day after sleep deprivation but does not compromise visual-motor integration. In contrast to expectation, sleep-deprived larvae that are faced with a random-dot motion discrimination task perform better than a well-rested control. Previous work has demonstrated that zebrafish larvae accumulate motion evidence over time and their resulting decision can be explained with a bounded leaky integrator model. We hypothesize that the reduced bouting gives them more time to integrate the random-dot-motion and hence leads to an increased performance. Finally, we artifically reduce bouting in zebrafish larvae using the drug melatonin and show that the decrease bouting in fact leads to an increased performance.

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Poster

298. Sensorimotor Behavior

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Swiss National Science Foundation Postdoc Mobility Fellowship to G. Schuhknecht (P500PB_203130)

Title: Ultrastructural analysis of a neural integrator in the larval zebrafish

Authors: *G. SCHUHKNECHT[1], M. PETKOVA[1], M. JANUSZEWSKI[2], A. HEBLING[1], M. STINGL[1], F. ENGERT[1], J. LICHTMAN[1];
[1]Dept. of Mol. and Cell. Biol., Harvard Univ., Cambridge, MA; [2]Google Res., Zürich, Switzerland

Abstract: When larval zebrafish experience whole-field visual motion, as in the random-dot task, they respond by swimming in the direction of motion, an innate behavior that enables animals to maintain position in a moving body of water. Intriguingly, this behavior is well-

explained by the same bounded drift-diffusion model that captures human and primate behavior in analogous random-dot paradigms. We previously proposed a functional circuit model that could implement this algorithm via a neural integrator circuit in the anterior hindbrain, which accumulates noisy visual motion evidence over time and excites downstream motor circuits after overcoming competitive inhibition from surrounding neurons. However, because the morphological cell types and connectivity of the anterior hindbrain are unknown, our circuit model remains hypothetical. Here, we use a sparse connectomics approach to systematically validate whether the anatomical requirements for our functional circuit model of evidence integration and swim-decision making are met in the anterior hindbrain, and to revise our model where needed. We analyzed data from an unpublished electron microscopy volume acquired from a seven-day old zebrafish larva, containing 170,000 neurons and glia cells from the central and peripheral nervous system. First, we used AI-assisted cell reconstructions in Neuroglancer of a random sample of 20 neurons in the left anterior hindbrain. A binomial power analysis showed that even sparse cell classes occurring with a frequency of only 15% could be detected at the 95% significance level. Neurons clustered into 3 distinct anatomical classes that corresponded well with the functional cell types we previously identified with calcium imaging: class 1 neurons extended dendrites into the cerebellar neuropil and formed synapses in the cerebellum, suggesting they may encode a novelty signal, or prediction error, which has been attributed to a population of 'dynamic threshold' neurons. Class II neurons formed axons and dendrites locally in the ipsilateral hindbrain neuropil, which may constitute the anatomical substrate of a recurrent integrator circuit. Likewise, dendrites of class III cells remained in the ipsilateral hindbrain, while their axons extended to the contralateral hemisphere. This may mediate interhemispheric push-pull inhibition, which was proposed to determine swim direction. In a next step, we will characterize pre- and postsynaptic partners of the reconstructed cells to test these hypotheses in more detail. In summary, our study provides one of the first examples for connectomics-based validation of existing functional circuit models in the larval zebrafish.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 299.01

Topic: E.02. Cerebellum

Title: Understanding cerebellum evolution through characterization of the cerebellar nuclei in two early branching vertebrates, Xenopus tropicalis and Protopterus annectens, the African lungfish

Authors: *D. FALTINE-GONZALEZ[1], J. M. KEBSCHULL[2];
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Abstract: The cerebellum, comprising the cerebellar cortex and the cerebellar nuclei, is an ancient hindbrain structure found in jawed vertebrates. Essentially all computations performed in the cerebellar cortex are processed in the cerebellar nuclei before they reach the rest of the brain via a large number of cerebellar nuclei projection targets. Cerebellar cortex-like structures exist in jawless vertebrates, while cerebellar nuclei have only been identified in cartilaginous fish, lungfish, and tetrapods. Interestingly, the number of nuclei varies among these organisms, with cartilaginous fish, lungfish, and amphibians possessing one nucleus per hemisphere; reptiles and avians possessing two, and mammals possessing three nuclei. This pattern suggests that all extant cerebellar nuclei have evolved from a single ancestral brain region. We recently characterized the cerebellar nuclei of chickens, mice, and humans and found a set of five conserved cell types that form an archetypal cerebellar sub-nucleus. This subnucleus appears to have duplicated and diverged during evolution, resulting in the evolution of the lateral cerebellar nuclei observed only in mammals and the avian-specific subnucleus IntX. We hypothesize that the single cerebellar nuclei observed in early branching vertebrates resemble the ancestral cerebellar nucleus and are composed of this same archetypal cerebellar sub-nucleus. To test this prediction, we characterized the cell type complements of the cerebellar nucleus of the amphibian *Xenopus tropicalis* and the African lungfish, *Protopterus annectens*, sister taxa to the tetrapods. We performed single-nucleus RNA sequencing and identified a set of excitatory and inhibitory cell types. Using STARmap in situ sequencing, we then located the discovered cell types in space to understand the spatial organization of the amphibian and lungfish CN. The combination of single-nuclei sequencing and in situ sequencing has allowed for an in-depth characterization of the cerebellar nuclei within early vertebrates for the first time.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 299.02

Topic: E.02. Cerebellum

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Title: Optimal routing to cerebellum-like structures

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Abstract: The cerebellar cortex is characterized by a vast layer of granule cells, which are believed to randomly mix their inputs to form high-dimensional neural representations. Theories have shown that such representations are well-suited to support functions, including associative and internal model learning. Other cerebellum-like structures, including the insect mushroom body, the electrosensory lobe of electric fish, and the mammalian dorsal cochlear nucleus, share this random expansion motif. However, in computational models the input to granule cell layers is typically described as random and uncorrelated, effectively neglecting the upstream pathways that route inputs to cerebellum-like structures. These pathways are characterized by a "bottleneck" motif, in which the representation is compressed prior to being expanded to the granule cell layer. While this motif is remarkably conserved across cerebellum-like structures, its function is not understood. Here, we develop a general theory of cerebellum-like structures in conjunction with their afferent pathways. When applied to the cortico-cerebellar pathway, our theory predicts that the pontine nuclei decorrelates and denoises cortical input to maximize the efficacy of the random expansion to the granule cell layer. We show that this function could be supported by Hebbian plasticity at cortico-pontine synapses. Using data analysis, we find signatures of pontine processing in cortico-cerebellar recordings that reconcile theories of nonlinear mixing with recent observations of correlated granule cell activity. When applied to the insect olfactory system, our theory predicts the glomerular organization of the insect antennal lobe. More broadly, our results show that structured compression followed by random expansion is an efficient architecture for flexible computation.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

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Title: Properties of cerebellar granule cells according to their projecting sublayers

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Abstract: Cerebellar granule cells (GCs) are the most numerous neurons in brain, composing approximately half of all neurons in brain. Although such large numbers and small size of neurons have been traditionally thought to be homogeneous, their non-uniform properties have been also reported regarding molecular expression, morphology, and physiological properties. One obvious diversity in GC network structure is the location of their axons, parallel fibers (PFs), in the molecular layer (ML), and morphological and functional differences of PFs according to their locations have been reported. However, the non-uniformity of GC somas or dendrites that are located in granular layer (GL) has not been linked with the locations of their PFs, and therefore the PF-location dependent GC information processing has not yet been entirely clarified. We took advantage of our adeno-associated viral vector-mediated technique that enables distinct GC labeling according to the locations of their PFs, and investigated the association between structural or functional properties of GCs in the GL and the locations of PFs in the ML. Specifically, we looked at synaptic connectivity with presynaptic mossy fiber (MF) terminals and GCs' responses to MF stimulations. Our anatomical observation and computational analysis revealed that overall MF-GC synaptic connectivity was mildly biased in a way that dendrites of GCs having PFs in proximity tended to connect with the same MF terminals. We also obtained results suggesting that the biased synaptic connectivity presumably arises from the synaptic formation between developmental stage-matched GCs and MFs. To compare responses to MF stimulations, we performed Ca^{2+} imaging, and detected significant differences in Ca^{2+} responses of GCs depending on the locations of their PFs, which were mainly attributable to varied distributions of $GABA_A$ receptors and NMDA receptors. Thus, our results indicate that there is structural and functional organization of cerebellar GCs according to the locations of their PFs, which could be partially made through developmental process. Such PF location-dependent organization of GCs may contribute to the information processing through GCs.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Title: The C4 Initiative: Cross-Species Cell Type Classification Of High-Density Recordings In The Cerebellar Cortex

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Abstract: Understanding the operation of a neural circuit requires that we dissect the contribution of its specific elements. It is therefore critical to develop classification tools that allow us to efficiently and robustly assign extracellularly recorded spike trains to defined cell types within neural circuits. The Cerebellum Cell type Classification Collaboration (C4) brings together a group of laboratories developing tools for rigorous and quantitative identification of cell types from high-density in vivo extracellular recordings in the cerebellum, a brain area where the cell type identification problem is particularly challenging due to the high density of neurons, their high firing rates, and the elaborately folded cytoarchitecture.
Our approach harnesses machine learning tools together with high-density silicon probe recordings in awake animals to build unsupervised and supervised classifiers that predict the most likely identity of each recorded neuron with a confidence score. Integration of datasets across labs is achieved with a unified pre-processing pipeline, available open-source (as part of NeuroPyxels). To obtain ground-truth neuron identification, we performed cell type-specific optogenetic tagging ('optotagging'). This ground-truth dataset was used to train classifiers to predict the identity of unlabelled cells based on the following features of each neuron: (1) the firing statistics, including those obtained from our new frequency-dependent 3D autocorrelogram, (2) the spatio-temporal profile of the extracellular waveform, and (3) the laminar location within the cerebellar cortex, which is automatically determined using Phyllum - our phy-based platform for identification of layers in the cerebellum. Classifier performance was evaluated with cross validation, as well as by direct comparison to the output of a classifier trained not with ground-truth optotagging data but with cell-identity labels assigned by cerebellar physiology experts.
We are currently testing the generalizability of the different classifiers across cerebellar recordings obtained with different probes (including Neuropixels), located in multiple regions of the cerebellar cortex, and across multiple species (including mice, rats, and monkeys). Our aim is to provide a fully integrated open-source toolkit for automatic cell type classification that will greatly facilitate a circuit-wide understanding of the contribution of different cell types to cerebellar-dependent behaviors.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

Support: NIH R01NS112917
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Title: Phyllum: a phy plugin for surveying high-density neural recordings in the cerebellum

Authors: *F. NAVEROS[1], A. SÁNCHEZ LÓPEZ[1], M. BEAU[2], D. KOSTADINOV[2], D. J. HERZFELD[3], M. E. HEMELT[3], M. OOSTLAND[2], S. OHMAE[1], N. J. HALL[3], D. COHEN[4], S. G. LISBERGER[3], M. HAUSSER[2], C. HULL[3], J. F. MEDINA[1];
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Abstract: New high-density neural probes like Neuropixels offer unprecedented access to the brain because they allow us to record the activity of thousands of neurons simultaneously. To facilitate analysis of such large datasets, it is critical to develop user-friendly tools for visualizing the spike activity of the recorded neural populations, search for specific cell types and assess their connectivity. Here, we introduce Phyllum, a software package optimized for analysis of high-density neural recordings in the cerebellar cortex. Phyllum provides multiple shortcuts to simplify the curating process and helps the user search for neurons with specific firing statistics, waveform properties and patterns of connectivity that are characteristic of different cell types in the cerebellum. In addition, Phyllum incorporates an intuitive 'Layer Tool' that can be used to identify the three layers of the cerebellar cortex (granular, Purkinje and molecular) and visualize the location of all the recorded neurons relative to them. We demonstrate Phyllum's performance using a series of Neuropixels recordings from different regions of the mouse cerebellar cortex in which ground-truth about the laminar organization was obtained by marking the probe location with a fluorescent dye. To speed up and simplify software adoption, we are making Phyllum available as an open-source plugin for the widely used Phy platform.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

Support: Wellcome Trust 224668/Z/21/Z
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Title: Identifying cell types in Neuropixels recordings from cerebellar cortex in awake mice using optotagging

Authors: *M. OOSTLAND[1], M. E. HEMELT[2], M. BEAU[1], D. KOSTADINOV[1], F. D'AGOSTINO[1], Y. CHUNG[1], G. MARTÍNEZ LOPERA[1], A. LAJKO[1], M. ZEDLER[1], D. J. HERZFELD[2], F. NAVEROS[3], A. SÁNCHEZ-LÓPEZ[3], S. OHMAE[3], N. J. HALL[2], D. COHEN[4], J. F. MEDINA[3], S. G. LISBERGER[2], C. HULL[2], M. HAUSSER[1];
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Abstract: Reliable identification of cell types in extracellular recordings is essential to define their contributions to neural circuit computations. This is difficult in any brain area, but is particularly challenging in the cerebellar cortex due to the high density of neurons, their high firing rates, and the elaborate folded cytoarchitecture. Here, we use a combination of in vivo 'optotagging' and pharmacology to identify the major cell types in the cerebellar cortex recorded with Neuropixels in awake mice. To identify genetically defined cell types, we expressed excitatory (ChR2) or inhibitory (GtACR2) opsins using both genetic crosses and viral strategies in specific subtypes of cerebellar neurons: Purkinje cells, molecular layer interneurons, Golgi cells, granule cells, and mossy fiber boutons. We validated the specificity of our expression strategies histologically. By optogenetically activating or suppressing specific populations of neurons and isolating direct optogenetic responses from synaptic responses using pharmacology, we were able to identify ground-truth cell identity for several major cell types. Optotagged units were identified by short-latency responses to optogenetic stimulation that persisted in the presence of pharmacological blockers of synaptic transmission. We further required that optically responsive units were located at depths where the pharmacological block could be verified. Additional validation was performed using *in vitro* patch-clamp recordings from identified neurons in cerebellar brain slices, allowing comparison of waveforms with *in vivo* recordings. We are using this strategy to build a library of the electrophysiological 'fingerprints' of the major cell types in the cerebellar cortex - including waveform shape, firing statistics, and layer identity - that we are leveraging to train a supervised machine-learning based classifier of cerebellar cell types. Together with recordings from cerebellar cortex using high-density probes in various species (mice, rats, and macaques), this will provide the basis for a more rigorous and comprehensive analysis of circuit computations in the cerebellar cortex.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

Support: NIH R01NS112917
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NIH K99EY030528

Title: Behavioral responses of expert-identified cerebellar cell types during smooth pursuit eye movements in primates

Authors: D. J. HERZFELD[1], N. J. HALL[1], M. E. HEMELT[1], M. J. BEAU[2], M. OOSTLAND[2], D. KOSTADINOV[2], F. NAVEROS[3], A. SÁNCHEZ LÓPEZ[3], S. OHMAE[3], D. COHEN[4], J. F. MEDINA[3], M. HAUSSER[2], C. HULL[1], **S. G. LISBERGER**[1];
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Abstract: The floccular complex of the cerebellum is crucial for the execution of smooth pursuit eye movements in primates. Leveraging the well-known circuit architecture of the cerebellum, our goal is to link large-scale activity of populations of identified cell-types to this exemplar motor behavior. We recorded >1,000 well-isolated floccular neurons using multi-contact silicon probes. Then, using machine learning techniques, we trained a probabilistic classifier to label extracellular recordings with their neuron identities. Training of the classifier used expert-labeled neuron identities established by examination of spike waveforms, cerebellar layer, and auto- and cross-correlograms. After training, the ability of the classifier to predict withheld expert labels via k-fold cross-validation was ~90%. Identified Purkinje cells (PCs) show a pause in simple-spike firing after a climbing fiber response and distinctive waveforms and auto-correlograms. Expert-identified molecular layer interneurons (MLIs) have distinctive waveform shapes and auto-correlograms that agree with those of optogenetically identified MLIs in mice; some show inhibition in MLI triggered cross-correlograms of PCs. Golgi cells are recorded in the granule layer, have waveforms of long-duration, and show low, regular spontaneous firing with distinctive interspike interval distributions. Identified mossy fibers have brief, frequently positive-negative waveforms with a slow after-potential, in agreement with recordings of optogenetically identified mossy fibers in mice. Different cell-types showed differential functional discharge properties during pursuit eye movements. Mossy fibers, the input to the cerebellum, respond preferentially to eye position signals during pursuit, while the primary output from the cerebellar cortex, PCs, preferentially encode eye velocity. MLIs respond more

strongly to eye velocity than eye position while unclassified elements recorded in the granule layer (including putative unipolar brush cells) respond more strongly to eye position. Thus, there is a striking transformation from eye position signals in the granular input layer to eye velocity signals in the molecular layer and PCs. Golgi cells showed little modulation in relation to eye position or velocity kinematic signals, implying that their inhibition of granule cells is unlikely to play a role in transforming eye position inputs into eye velocity outputs. The functional discharge of different putative cell types provides a database for asking what other cerebellar circuit mechanisms might transform eye position signals into eye velocity signals.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 299.08

Topic: E.02. Cerebellum

Support: FRQNT
Healthy Brains for Healthy Lives

Title: Purkinje cell inputs converge non-randomly onto neurons in the cerebellar nuclei

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Abstract: The connections between neurons form the foundations of brain circuits, and the spatial properties of these connections influence circuit function. In the cerebellum, Purkinje cells integrate extensive synaptic input and communicate this information to cerebellar nuclear neurons, the output cells of the cerebellum. The connections between Purkinje cells and cerebellar nuclear neurons thus represent a crucial component of the cerebellar circuit, but how Purkinje cells converge onto cerebellar nuclear neurons is poorly understood. To explore Purkinje cell - nuclear neuron connectivity, we performed whole-cell recordings from fastigial cerebellar nuclear neurons in acute slices from mice expressing ChR2-EYFP in Purkinje cells. We focally stimulated Purkinje cell axons with blue light in multiple locations and recorded evoked postsynaptic currents from cerebellar nuclear neurons. By identifying the locations that evoked synaptic responses, we developed connectivity maps of Purkinje cell convergence at the level of cerebellar lobules. We next grouped lobules into the four cerebellar transverse zones which exhibit distinct functional properties. We found that some cerebellar nuclear neurons received input from Purkinje cells located within a single zone, while other neurons received

input from Purkinje cells residing in multiple zones. Surprisingly, a subset of nuclear neurons received input from all four zones. To assess whether Purkinje cell - nuclear neuron connectivity exhibits convergence “motifs,” or non-random patterns, we compared our connectivity maps with a model assuming Purkinje cell convergence from each zone occurs independently. Motifs appearing more often than predicted by the model would suggest that a functional topography underlies Purkinje cell - nuclear neuron connectivity. Consistent with this, we found that the four-zone connectivity motif appeared significantly more frequently than predicted. This finding suggests that cerebellar nuclear neurons integrating Purkinje cell input associated with distinct functions is a feature of this circuit. Lastly, we performed viral labeling of Purkinje cells across multiple zones. We confirmed that Purkinje cell axonal convergence patterns are morphologically consistent with our functional connectivity maps since labeled Purkinje cell axons from multiple zones terminate in close proximity within the cerebellar nuclei. Our findings suggest that non-random connectivity motifs underlie the output synapse of the cerebellar circuit, and that cerebellar nuclear neurons integrate information from functionally distinct Purkinje cells.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

Support: NIH Grant R01-NS112518
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Title: Widefield Ca^{2+}imaging of the cerebellar cortex reveals networks of Purkinje cell dendritic and somatic activity that have distinct spatial and functional properties.

Authors: *M. L. STRENG[1], R. CARTER[2], K. TOGNERI[1], V. RAJENDRAN[3], S. B. KODANDARAMAIAH[4], E. KROOK-MAGNUSON[3], T. J. EBNER[2];
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Abstract: A major challenge in cerebellar physiology is determining how the highly conserved cytoarchitecture of the cerebellar cortex underlies its contributions to ongoing behavior. Clusters of cerebellar Purkinje cells can be characterized by parasagittial organization, common sources of climbing fiber input, and expression of various molecular markers, but it is unclear how the functional information conveyed by Purkinje cells is organized across the cerebellar cortex, as most observations of Purkinje cell activity have been restricted to individual cells or local populations. We recently developed a method for chronic mesoscale recordings of Purkinje cell

Ca^{2+} activity in head fixed, behaving mice using cerebellar windows. This technique allowed for the recording of individual Purkinje cells across multiple folia of both vermis and simplex regions of the cerebellar cortex. Single photon Ca^{2+} imaging in PcP2-GCaMP6s mice, which exhibit sparse expression in GCaMP6s in Purkinje cells, allowed us to identify Ca^{2+} modulation that could be attributed to somatic activity (presumably reflective of simple spike modulation) and dendritic activity, (presumably reflective of complex spike modulation) within the same recordings. To characterize organization of Purkinje cell dendritic and somatic information, we performed blind source separation using the spatial independent component analysis (sICA) algorithm JADER. At higher magnification, sICA was able to identify spatial independent components (ICs) corresponding to individual Purkinje cell dendrites or somata. At the mesoscale level, sICA identified local populations of Purkinje cells, in which dendritic ICs were organized in the parasagittal domain, whereas somatic ICs were organized more across a given folium in the medial-lateral domain. During a cued reaching task in which mice reach for a water reward, dendritic and somatic ICs in anterior lobules were highly activated during reaching compared to posterior ICs. Conversely, when water rewards were randomly omitted during a catch trial paradigm, posterior ICs showed stronger activation than anterior ICs. Together, these results shed important new light on the spatial and functional organization of the climbing fiber system and Purkinje cell outputs.

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

Support: Wellcome Trust DBT India Alliance Senior Fellowship (Dr Vatsala Thirumalai)

Title: Identifying bistable state from calcium imaging data using machine learning

Authors: *A. VARMA[1], S. UDUPA[2], M. SENGUPTA[3], P. K. GHOSH[2], V. THIRUMALAI[1]; [1]Natl. Ctr. for Biol Sci., Natl. Ctr. for Biol Sci., Bangalore, India; [2]Indian Inst. of Sci., Bangalore, India; [3]Washington University, Sch. of Med., Washington University, Med. Sch., Saint Louis, MO

Abstract: Purkinje neurons (PNs), the GABA-ergic principal neurons of the cerebellum, exhibit membrane potential bistability, firing either tonically or in bursts. Several studies have implicated the role of a population code in cerebellar function, with bistability adding an extra layer of complexity to the code. Calcium imaging is the method of choice to interrogate the activity of neuronal populations, however it does poorly at reporting the membrane potential state or the spiking patterns of these neurons. Nevertheless, we hypothesised that the calcium

signal might potentially contain information about the state of the PN.To bridge the gap between electrophysiology and imaging, we performed simultaneous patch-clamp electrophysiology and calcium imaging on larval zebrafish PNs. We used this ground truth dataset to develop a method of reconstructing the calcium signal given an electrophysiological trace. The calcium signal can be taken to be the convolution of spike timings with a GCaMP kernel. However, PNs have two sources of calcium - simple spikes (SS) and climbing fiber (CF) input. We treated each source as having its independent kernel and linearly summed their convolutions with corresponding event timings to produce a calcium signal reconstruction. The optimal combination of SS and CF kernels was found by minimizing the error between the reconstruction and the ground truth. We find that this method generalizes well across cells, and is independent of the state of the neuron.We then compiled electrophysiological recordings from PNs, grouped them by state, and applied this method on them to generate a large dataset of state-labelled calcium signal reconstructions (N=957 traces, from n=138 recordings). This data was used to train a 1D convolutional neural network with a long short-term memory (1-D CNN-LSTM) model to classify the state of a Purkinje neuron based on its calcium signal alone. This model trained well, with a median accuracy of 82.02% and median F-score of 0.8071 in the test phase. The network also performed equally well on other, independent challenges. Moreover, this network, which was trained only on reconstructions, identified PN state accurately even when presented with traces obtained from experiments. This tool opens up new avenues of research into understanding the role of Purkinje neuron bistability in cerebellar circuit function

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Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Topic: E.02. Cerebellum

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Title: Identifying the behavioral, genomic, and circuit-specific effects of Chd8 mutations in the developing and adult mouse cerebellum

Authors: *A. F. D'AMBRA[1], C. P. CANALES[1], N. SEBAN[1], S. JUNG[1], N. CHU[1], K. CICHEWICZ[1], S. A. LOZANO[1], E. SMITH[1], R. ORTIZ[1], J. ZHU[1], D. FIORAVANTE[1,2], A. S. NORD[4,3];
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Abstract: Despite the canonical view of the cerebellum (CB) as a motor control center, its contributions to social and cognitive functions have become increasingly more elucidated in the last several years. As such, its role in neurodevelopmental disorders, such as autism spectrum disorder (ASD), is an intriguing focal point for CB research. Mutations in the chromatin-remodeling factor CHD8 are strongly associated with ASD. Germline loss-of-function mutations in mouse *Chd8* gene impact neuronal connectivity and affect CB development, and mice with heterozygous *Chd8* mutations exhibit genomic, neuroanatomical, and ASD-relevant behavioral pathology. Here, we utilize germline *Chd8* haploinsufficient mice (*Chd8*$^{5bp\text{-}del}$), to investigate changes in circuitry and in gene expression in the CB of young (postnatal day 12) mice. We analyzed the cerebellar transcriptional landscape in *Chd8*$^{5bp\text{-}del}$ mice via single-nucleus RNA-seq, revealing altered gene expression in metabolic and synaptic pathways in specific CB cell populations. Additionally, mutant mice showed sex-specific differences in inhibitory synaptic transmission within the CB. Finally, we targeted viral-induced, cre-mediated *Chd8* deletion to the deep cerebellar nuclei (DCN) of the adult mouse CB, to investigate the functional influence of this manipulation in DCN-relevant circuits. Adult transient cre-mediated manipulation of *Chd8* in the CB led to sexually dimorphic behavioral phenotypes in learning and social tasks, but not in anxiety or motor coordination. These sex-specific changes are an interesting avenue for continued ASD research and could contribute to the general understanding of the genotype-by-sex interaction previously linked to pathology in the adult mouse brain and in the clinical ASD population. Current efforts focus on connecting cell-type specific differential gene expression with observed changes in electrophysiological and behavioral phenotypes. All in all, these data will expound upon the role of *Chd8* in CB connectivity and development and help elucidate the CB's role in ASD.

Disclosures: A.F. D'Ambra: None. **C.P. Canales:** None. **N. Seban:** None. **S. Jung:** None. **N. Chu:** None. **K. Cichewicz:** None. **S.A. Lozano:** None. **E. Smith:** None. **R. Ortiz:** None. **J. Zhu:** None. **D. Fioravante:** None. **A.S. Nord:** None.

Poster

299. Cerebellum: Cell Types and Circuit Physiology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 299.12

Topic: E.02. Cerebellum

Support: NIH Grant R01 NS088257
NIH Grant R01 NS117745

Title: Role of defective cerebellar ryanodine receptor type 1 and endoplasmic reticulum calcium leak in essential tremor pathophysiology

Authors: R. T. MARTUSCELLO[1], M.-L. CHEN[2], S. REIKEN[3], D. S. RUFF[2], E. D. LOUIS[4], M.-K. PAN[5], A. R. MARKS[3], S.-H. KUO[2], *P. L. FAUST[1];
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Abstract: Essential tremor (ET) is a chronic, highly prevalent neurological disorder characterized by a progressive 8-12 Hz action tremor, predominantly of the arms. Clinical and neuroimaging studies have confirmed that the cerebellum plays an important role in ET tremor circuitry. In extensive morphological studies of postmortem human cerebellum, we have demonstrated that the Purkinje cell (PC) incurs significant cellular damage in ET. Our recent cerebellar cortex transcriptome studies identified gene expression alterations in ryanodine receptor type 1 (*RyR1*) in ET. RyR1 is a novel target that has not been studied in ET previously. The RyR1 protein is an intracellular calcium-sensing-calcium release channel located on the endoplasmic reticulum (ER), and, notably, is exclusively expressed in PCs in the cerebellar cortex. Under stress conditions, RyR1 undergoes several post-translational modifications (PKA phosphorylation, oxidation, nitrosylation), coupled with depletion of the calcium stabilizing binding partner calstabin-1, which collectively characterize a “leaky channel” biochemical signature. In postmortem human cerebellum we found strikingly increased levels of PKA phosphorylation at the RyR1-S2844 site, increased levels of RyR1 oxidation and nitrosylation, and depletion calstabin-1 from RyR1 in ET vs. controls. Remarkably, this “leaky” RyR1 signature was specific to ET and not seen in control or Parkinson’s disease cerebellum. Both tremor duration and PC loss correlated with extent of calstabin-1 depletion from RyR1 in ET. Analysis of microsomes isolated from postmortem cerebellum directly demonstrated excessive calcium leak in ET *vs.* controls. We further studied the role of RyR1 in tremor by examining a mouse model with a point mutation in RyR1 that mimics constitutive site-specific PKA phosphorylation (RyR1-S2844D mice), recapitulating the ‘‘leaky’’ channel phenotype. We found that RyR1-S2844D mice develop a 10Hz action tremor, similar to ET patients, and cerebellar physiological recordings in these mice demonstrate robust abnormal cerebellar oscillatory activity. Intra-cerebellar microinfusion of an RyR1 agonist or antagonist respectively increased or decreased tremor amplitude in real time in RyR1-S2844D mice, supporting a direct role of cerebellum for tremor generation. Last, treating RyR1-S2844D mice with a novel RyR1 channel stabilizing compound, RyCal, effectively dampened cerebellar oscillatory activity, reduced tremor and normalized cerebellar RyR1 calstabin-1 binding. Collectively, these data support a novel role of stress-associated abnormal ER calcium handling in PCs for tremor pathophysiology.

Disclosures: R.T. Martuscello: None. **M. Chen:** None. **S. Reiken:** None. **D.S. Ruff:** None. **E.D. Louis:** None. **M. Pan:** None. **A.R. Marks:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ARMGO Pharma, Inc.. **S. Kuo:** None. **P.L. Faust:** None.

Poster

299. Cerebellum: Cell Types and Circuit Physiology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 299.13

Topic: E.02. Cerebellum

Support: Wellcome Trust 209453/Z/17/Z

Title: The role of Golgi cells in cerebellar cortical transformation

Authors: E. R. PALACIOS[1], C. HOUGHTON[2], *P. CHADDERTON[1];
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Abstract: The cerebellar cortex converts patterns of mossy fibre activity into Purkinje cell outputs that encode transformed behavioural variables. This conversion is regulated by different elements of the cerebellar cortical circuit, including local inhibitory interneurons. Here we focus on the role of one class of interneuron, Golgi cells, which are located in the input layer of the cerebellar cortex and integrate local feedforward and feedback signals. We selectively reduced Golgi cell activity *in vivo* using an inhibitory chemogenetic receptor (hM4Di) in the lateral cerebellum, in a region that encodes whisker movements (lobule Crus 1). Under normal conditions, Purkinje cells and molecular layer interneurons in Crus 1 robustly encode current and future whisking trajectories via linear changes in firing rate. We explored the extent to which downregulating Golgi cell activity disrupts these representations. To do this, we performed Neuropixels recordings in awake whisking mice to broadly sample population activity from all cerebellar cortical layers, while tracking whisker dynamics with a high-speed camera. Targeted expression of inhibitory hM4Di receptors was achieved by injection of Cre-dependent adeno-associated virus (AAV-hSyn-DIO-hM4Di-mCherry) in mice selectively expressing Cre recombinase in Golgi cells (GlyT2-Cre; N = 10). To determine the influence of Golgi cell inhibition upon circuit computation, we reduced their firing rates by applying clozapine-n-oxidase (CNO; 30uM) onto the brain surface exposed during electrophysiological recording. Our results show that selective reduction of Golgi cell activity produces an overall mild increase in neural activity compared to the control groups. The change in neural activity is not extreme; this may suggest that Golgi cells preferentially fine tune excitatory responses to incoming patterns of mossy fibre activity, rather than controlling overall excitability levels. We are quantifying how impaired Golgi cell inhibition impacts neuronal activity in different layers of the cerebellar cortex and representation of motor-related behavioural variables. Thus we can determine the contribution of Golgi cells to transformation of movement-related information.

Disclosures: E.R. Palacios: None. **C. Houghton:** None. **P. Chadderton:** None.

Poster

299. Cerebellum: Cell Types and Circuit Physiology

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Program #/Poster #: 299.14

Topic: E.02. Cerebellum

Support: NIH 1DP2MH122398-01
Autism Research Institute Grant

Title: Light sheet mapping of parvalbumin subtypes of Purkinje cells using in vivo CRISPR strategies

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Abstract: Although long used as a general PC marker, the calcium binding protein parvalbumin labels a subpopulation of PCs based on high and low/no expression, with a conserved distribution pattern across animals examined. The parvalbumin subtypes show differences in spontaneous firing that can be modified by altering calcium buffer content. These subtypes also show differential responses to potassium and calcium channel blockade, suggesting a mechanistic role for variability in PC intrinsic firing through differences in ion channel composition. Since antibody titrations for immunohistochemistry had varying results in quantifying parvalbumin expression, we use Cas9 fusions delivered to Purkinje cells via *in utero* electroporation to insert a fluorescent marker in the endogenous parvalbumin locus and quantify parvalbumin expression differences across Purkinje cells. CRISPR/Cas9 is used to knockout the parvalbumin gene in a small population of Purkinje cells to confirm appropriate antibody titrations for subsequent light sheet mapping of detailed anatomical positions of the subtypes within cerebellar stripes.

Disclosures: C. Brandenburg: None. **G.W. Bunce:** None. **C. Robertson:** None. **A. Romanowski:** None. **A. Poulopoulos:** None.

Poster

299. Cerebellum: Cell Types and Circuit Physiology

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 299.15

Topic: E.02. Cerebellum

Support: South-Eastern Norway Regional Health Authority (Grant No 2021040)

Title: The genetic architecture of human cerebellar morphology

Authors: *T. MOBERGET[1,2], S. BHARAMI[1], D. ROELFS[1], D. VAN DER MEER[1,3], O. FREI[1,4], T. KAUFMANN[1,5], O. A. ANDREASSEN[1,6], L. T. WESTLYE[1,6,7];
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Abstract: The cerebellum contains ~80% of all neurons in the human brain and has rapidly expanded in volume over recent primate evolution. Moreover, variation in the relative volumes of cerebellar subregions has been associated with variation in behavioral repertoires in several species. In humans, a growing number of neuroimaging and clinical studies link cerebellar structure and function to a wide range of cognitive and affective functions, as well as to a number of heritable developmental and psychiatric disorders. However, compared to other brain structures, such as the cerebral cortex and the hippocampus, the cerebellum has so far been relatively neglected in research mapping genetic influences on human brain structure. In order to bridge this current knowledge gap, we here first 1) used a data-driven approach (non-negative matrix factorization) to parcellate MRI-based maps of cerebellar grey matter volume from 28,212 UK Biobank participants into robustly reproducible sub-regions. 27,302 unrelated individuals were retained for the genetic analyses. We next 2) estimated the SNP-heritability of these morphological features; 3) identified genetic variants associated with cerebellar morphology; 4) explored regional effect patterns associated with significant SNPs; 5) functionally characterized the genetic signal using gene-expression and gene-set analyses; and 6) tested for genetic overlap with selected mental disorders. Data-driven decomposition of cerebellar grey matter maps revealed highly reproducible and moderately heritable morphological features, which only partially overlapped traditional cerebellar parcellations. Multivariate GWAS (MOSTest, developed in-house at NORMENT) on these cerebellar features yielded 351 genome-wide significant genetic loci associated with cerebellar morphology, 229 (65%) of which are novel. For all cerebellar features we observed high genetic correlations (mean rg: .96) with an independent sample (n=8,896), and 219 (62%) of the genetic loci from the multivariate analyses were replicated, indicating robust results. Genetic variants showed spatially heterogeneous effects across the cerebellar cortex, thus influencing regional as well as overall volume. Gene level analyses revealed selective expression in cerebellar and neonatal human brain tissue, as well as significant enrichment for curated gene sets associated with neurodevelopment and altered cerebellar morphology in mouse gene-perturbation experiments. Finally, we observed genetic overlap between cerebellar morphology and five mental disorders, with the strongest evidence for schizophrenia and bipolar disorder.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.01

Topic: E.04. Voluntary Movements

Support: NINDS R01-NS114046

Title: Bilateral parietal cortex supports control of drawing performance with the non-dominant hand

Authors: *T. KIM[1], A. R. CARTER[2], I. G. DOBBINS[3], L. LIU[4], M. P. MCAVOY[5], B. A. PHILIP[1];
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Abstract: Motor compensation refers to the use of the non-dominant hand after unilateral impairment of the dominant hand. Compensation is critical for patient groups including individuals with stroke or peripheral nerve injury, because approximately 40% of these patients never adequately recover dominant hand function. However, compensation does not occur in daily life or traditional therapy, even with an intact brain: patients with nerve injury continue to use their injured dominant hand, even when their unaffected non-dominant hand is more dexterous. However, it remains unknown what neural mechanisms drive non-dominant hand compensation and performance, even in the healthy brain.

To address these issues, we conducted a functional MRI study with healthy right-handed volunteers (currently n = 6). Participants underwent fMRI scanning while the Precision Drawing Task, which involves using a pen stylus to draw a line inside the path of pre-determined geometric forms. Movement performance was quantified as endpoint (pen tip) velocity smoothness. The task was delivered in a block design, alternating between 20 sec drawing and 20 sec rest, one hand per run. Our primary analysis identified BOLD activity that was hand-specific and correlated with each hand's average performance.

We hypothesized that bilateral parietal networks would be involved in drawing with the left non-dominant hand only, due to interhemispheric processes wherein left-hemisphere mechanisms (typically for the dominant hand) support drawing with the ipsilateral left non-dominant hand. As expected, preliminary results indicate that bilateral parietal areas show performance-correlated activity during left non-dominant hand drawing (vs. right dominant hand drawing). Specifically, these posterior parietal areas include left inferior parietal lobule, left superior parietal lobule, and right intraparietal sulcus. Conversely, we found no clusters of performance-correlated activity in posterior parietal cortex during right dominant hand drawing (vs. left non-dominant hand drawing). Therefore, drawing only involved performance-related bilateral parietal activation when drawing with the left non-dominant hand.

This left hand drawing network is consistent with previous theories suggesting that interhemispheric parietal connections play a critical role in supporting skilled movement with the non-dominant hand. Ongoing experiments are aimed at determining whether the same neural mechanisms also support compensatory hand movement after chronic injury to the dominant hand.

Disclosures: **T. Kim:** None. **A.R. Carter:** None. **I.G. Dobbins:** None. **L. Liu:** None. **M.P. McAvoy:** None. **B.A. Philip:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PlatformSTL (St. Louis, MO).

Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.02

Topic: E.04. Voluntary Movements

Support: Italian multiple sclerosis foundation (FISM) (Grant 2016/R/2 and 2019/R-Multi/009)

Title: Seed-based resting-state functional connectivity of hand motor networks using the individual hand motor hotspot identified by TMS

Authors: ***L. BONZANO**[1], M. BORTOLETTO[2], A. ZAZIO[2], C. IESTER[1], A. STANGO[2], R. GASPAROTTI[3], C. MINIUSSI[3], M. BOVE[1];
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Abstract: In the seed-based method for studying functional connectivity (FC), seed selection is relevant. Here, we propose a new methodological approach for resting-state FC analysis of hand motor networks using the individual hand motor hotspot (hMHS) as seed, for the dominant and non-dominant hemispheres. Nineteen right-handed healthy volunteers underwent a transcranial magnetic stimulation (TMS) session and resting-state fMRI (rs-fMRI). For each subject, the hMHS in both hemispheres was detected by TMS with contralateral abductor pollicis brevis (APB) muscle as target, as the site eliciting the highest and most reliable motor evoked potentials. A neuronavigation system allowed to co-register the individual anatomical MRI to head position; once the coil was positioned on the scalp and the APB hotspot was identified, its exact location was recorded, and the coil focus was projected perpendicularly to the coil plane on the grey matter surface of the individual MRI. This procedure provided by the neuronavigation system produced as output the corresponding coordinates in the MNI space. Separately for each subject, seed regions were built as spheres centered on the coordinates on the cortex corresponding to the individual left and right hMHS. For comparison, the left and right Brodmann's area 4 (BA4) masks extracted from a standard atlas were used as seed for all subjects. For each participant, the averaged time course during the rs-fMRI period was extracted from the identified seed regions, and the correlation analysis was performed with every other voxel in the brain, to assess the connectivity between these regions and the rest of the brain. The left and right hMHS showed FC patterns at rest mainly including sensorimotor regions, with bilateral connectivity only for the left hMHS. The statistical contrast BA4>hMHS, for both hemispheres, showed different extension and lateralization of the functionally connected cortical

regions. On the contrary, no voxels survived the opposite contrast (hMHS>BA4). This suggests that the detection of individual hand motor seeds by TMS allows to identify functionally connected motor networks that are more specific with respect to those obtained starting from the a priori atlas-based identification of the primary motor cortex.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.03

Topic: E.04. Voluntary Movements

Support: NSF Grant EEC-2127509

Title: Repetitive practice attenuates EEG spectral features associated with upper-limb movement

Authors: *A. Y. PAEK, S. PRASHAD;
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Abstract: Neural efficiency suggests that as individuals become well-trained in a motor skill, the brain can perform the skill more efficiently with less cortical engagement. However, this notion is rarely accounted for in neuroimaging studies that explore motor behavior. This idea has been supported by EEG studies, where weaker neural activity was found in experts when they perform a skilled motor task, but not in novices performing the same task. Thus, while this observation has been found with brain rhythm comparisons between novices and experts, it is uncertain if it can occur within a single session where participants perform very repetitive motor tasks. This may have implications for studies that aim to identify EEG patterns associated with movement, since it is generally assumed that these neural features will be consistent across trials throughout the entire dataset. This assumption is particularly critical in neural decoding studies that use machine learning algorithms to predict limb movements from EEG signals. In this work, we explored if movement-related EEG features can change from early to late trials as participants performed a repetitive task. We used an open access dataset called WAY-EEG-GAL (Luciw, Jarocka, & Edin, 2014), where 12 right-handed participants performed a grasp and lift task while grip force, muscular activity (EMG), and brain rhythms (32-channel EEG) were recorded. In this dataset, 10 blocks of data were collected, each of which contained approximately 30 trials. We compared the EEG features from the first block to one of the last blocks for each participant. Spectral power in the alpha (8-13 Hz) and beta (20-30 Hz) frequency bands were extracted in 1 second time windows during the rest and movement periods for each trial and for each EEG sensor. We calculated event-related desynchronization (ERD) as the ratio of the spectral power during movement compared to rest. A paired t-test was used within participants to compare ERDs between the first and late block. We found significant attenuation in alpha band ERD in

central parietal areas ($p<0.05$ for sensors FC2, FC6, Cz, T8, CP1, CP2, CP6, P3, Pz, and P4). We also found significant attenuation in beta band ERD in the ipsilateral central area ($p<0.5$ for sensors C4, CP2, and Pz). These results suggest that repeated practice of a motor task can reduce motor-related EEG features over time. Future work will explore if other similar datasets corroborate this finding.

Disclosures: **A.Y. Paek:** None. **S. Prashad:** None.

Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.04

Topic: E.04. Voluntary Movements

Support: NIH R01 NS120226

Title: Dual joint coordination increases interhemispheric and ipsilateral connectivity with increasing shoulder abduction loads

Authors: *E. N. A. ADJEI, K. M. WRIGHT, J. P. A. DEWALD, J. YAO;
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Abstract: Despite the complexity of natural movements, involving multi-joint coordination and simultaneous engagement of both proximal and distal joints, little is known about the cortical communication during motor planning even in able bodied individuals. We, therefore, studied the communication between motor cortices in able bodied individuals prior to a simultaneous arm lifting and hand opening task (dual task) against various shoulder abduction loads, compared to the communication prior to pure lifting (single task L) and pure hand opening (single task O) tasks. The motor cortex sends motor commands more contralaterally to motor- and inter-neurons at the spinal cord via corticospinal tracts (CSTs), and bilaterally via the cortico-reticulospinal tracts (C-RSTs) that originate at the reticular formation in brainstem and receive more projections from ipsilateral premotor cortex. The CSTs project more to distal than proximal muscles and are responsible for dexterity and improve accurate task execution, while the C-RSTs are non-specific, projecting to multiple groups of muscles with preference to proximal muscles and are responsible for postural control and multi-joint movements. Thus, we hypothesize that increasing shoulder abduction loads will require more engagement of the C-RSTs and will be evident as increased ipsilateral communication in the premotor cortex. However, since this dual task also requires hand opening, which involves more CST engagement, we anticipate that there will also be interhemispheric communication to modulate the increase in C-RST engagement and ensure accurate dual task execution. To quantify this communication, we investigated the cortico-cortical connectivity (CCC) using electroencephalographic and electromyographic data from 5 subjects (2 females) participating in the dual task and the single L task at 25%, 39%, and 53% of their max shoulder abduction (SABD) force, and the single O task. For increases in

SABD load, we saw an increase in contralateral and interhemispheric connectivity in the dual task as compared to the single L task. We also saw an increase in ipsilateral and interhemispheric connectivity when comparing dual task to the single O task. Comparing the dual task to both the single L and single O tasks, we saw an increase in interhemispheric connectivity. This change in CCC prior to the dual task, may allow for increased interhemispheric connectivity to modulate C-RST engagement and allow for accurate task execution via the CST track.

Disclosures: E.N.A. Adjei: None. **K.M. Wright:** None. **J.P.A. Dewald:** None. **J. Yao:** None.

Poster

300. Mapping Sensorimotor Function in Upper Limbs

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.05

Topic: E.04. Voluntary Movements

Support: Brain and Spinal Cord Injury Research Trust Bridge Funds, Center for Respiratory Research and Rehabilitation (CRRR) and the Trauma, University of Florida (SV)

Title: Fmri in mice during learning and performance of a forelimb force control task

Authors: *V. JINDAL[1,2], D. J. COMPTE[1,2], C.-L. CHIANG[1,2], D. W. WESSON[3], D. E. VAILLANCOURT[1,2], S. VAHDAT[1,2];
[1]Applied Physiol. and Kinesiology, [2]McKnight Brain Inst., [3]Pharmacol. and Therapeut., Univ. of Florida, Gainesville, FL

Abstract: Background: Controlling the level of upper limbs force is crucial for performing daily activities. The neural mechanisms involved in learning appropriate force control are not well understood. Functional magnetic resonance imaging (fMRI) in rodents allows unbiased tracking of whole-brain activation maps during learning to pinpoint key cortical, subcortical, and brainstem structures. Yet the need for anesthesia to suppress head motion during scanning has limited its applications to study behavioral underpinnings in rodents. Here, we developed and tested a novel MR-compatible head-fixation apparatus for awake mouse fMRI during an odor-cued forelimb force control task, minimizing noise and motion artifacts, and powerfully harnessing behavior.
Methods: We built an accurate (resolution 0.005 N) MR-compatible miniature force transducer, as well as a 3D-printed head fixation system to shape and allow mice to engage in the forelimb force control task. We also designed and built a saddle linear MRI coil to fit our head fixation system (with an opening for fiber-optic cannula for opto-stimulation). The training paradigm involves wild-type water-deprived mice undergoing a reward-based forepaw press task. In initial shaping, the mice were shaped to press the force transducer in a water-motivated press/no-press task, cued by an odor. After the initial training days, the mice are further trained to press the force transducer at a required force level. Mice underwent an event-related awake SE-EPI fMRI

scan in an 11T Bruker scanner while performing the forepaw press task (resolution 0.35x0.35x0.5 mm^3, TR = 2s). T2w anatomical scans (resolution 0.1x0.1x0.35 mm^3) were also acquired for registration to template.
Results: Our fMRI results showed significant activation clusters ($p < 0.05$, corrected) related to forelimb force control in several structures, including the primary and secondary motor cortices, somatosensory cortex, thalamus, striatum, and piriform cortex, after removing the effects of odor presentation and licking. The average motion during functional scans was minimal (less than 0.25 mm in all 3 directions), and additional motion correction parameters from FSL software package were included as confound in the regression model to ensure decoupling the effects of body motion from the activation maps.
Conclusion: Our study shows the feasibility of awake mouse fMRI in forelimb motor control and provides evidence for a widespread network of cortical and subcortical areas activated during force control. Future work can use this paradigm alongside optogenetics and/or fiber photometry to target specific neuronal circuits.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.06

Topic: E.04. Voluntary Movements

Title: Somatotopy of Arm and Legs representations in primary motor cortex using functional magnetic resonance imaging

Authors: *S. SALEH[1,2], C. JIANG[3], A. ALIVAR[4], J.-H. GAO[5], **G. H. YUE**[1,2];
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Abstract: The somatotopic representation of arms and legs movement in the primary motor cortex (M1) are defined based on previous research on lesions and brain stimulation studies. To our knowledge, no one yet examined the difference in functional connectivity of upper extremity (UE) and lower extremity (LE) M1 during isolated movements of the arms and legs versus walking-like movements of the UEs and LEs. In this project, we acquired fMRI data during alternating movement of the UEs and LEs legs, and movements of simulated walking (SIMwalk). Our objective was to understand how the M1 controls isolated UEs and LEs movements compared to SIMwalk, and whether the connectivity networks differ among these tasks. We used localized activation in M1 in each task to examine the functional connectivity

map of M1 (M1FC) with other cortical regions. Data of 20 healthy and young subjects was acquired after signing informed consent approved by the local institutional review board. A 3T MRI (Simmons) scanner was used to acquire the data. UE or LE task was practiced by alternating the UE and LE movements for 30 sec (12-30 sec rest). In SIMwalk, movements were performed by lifting one arm and contralateral leg simultaneously (e.g., left arm and right leg) followed by the other two limbs (right arm and left leg) and continue the walking-like movements for 30 seconds. Each condition was practiced in 40 trials in separate fMRI scan. The fMRI data and T1-weighted images were acquired using EPI and MPRAGE sequences respectively. The fMRI data was preprocessed using conventional methods. Paired t-tests were used to compare between conditions and gPPI was adopted to examine the FC map of seeds within M1 identified in each of the 3 tasks with other network regions.Relative to UE movement, SIMwalk task showed lower activation in the orbitofrontal, anterior cingulate, and inferior frontal cortices, and M1FC during SIMwalk showed weaker connectivity between M1 and middle frontal, inferior parietal, and inferior occipital cortices. Relative to LE movement, SIMwalk showed higher activation in the proximal part of M1, and M1FC during SIMwalk showed lower connectivity among the M1 and anterior cingulate cortex, posterior parietal cortex, and Cerebellum. The M1FC during SIMwalk showed positive connectivity with posterior cingulate cortex. The results show higher activation and stronger M1FC with frontal and parietal cortices during UE movement. This is not surprising knowing the complexity of voluntary arm movements. M1FC during SIMwalk relative to UE and LE isolated movements shows weaker connectivity with frontal and parietal regions suggesting less cognitive demand for controlling walking-like movements.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 300.07

Topic: E.04. Voluntary Movements

Support: NIH R01 NS120226

Title: Ipsilateral cortico-cortical connectivity increases during preparation for shoulder abduction against increased shoulder loads

Authors: *K. M. WRIGHT, E. N. ADJEI, J. P. DEWALD, J. YAO;
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Abstract: Previous research has shown there is an increase in ipsilateral (IPSI) cortico-cortical connectivity (CCC) for simultaneously shoulder abduction (SABD) and hand opening tasks compared to solely hand opening tasks (Wilkins 2020). The increased IPSI connectivity could

reflect the recruitment of IPSI descending motor tracts, such as the reticulospinal tract (RST), to drive the shoulder muscles during lifting. In this study, we extend our previous research and hypothesize that, when lifting against heavier SABD loads, a participant will display increased IPSI CCC during the preparation phase to facilitate the shoulder muscle recruitment via RST.
To test our hypothesis, we recruited six able-bodied participants with right-hand dominance to perform SABD against shoulder loads for 25% and 53% of their maximum voluntary force. Each loading condition was assigned to a trial-block of 50 trials. At the beginning of each trial a sound signaled subjects to prepare for a self-initiated SABD in 5-6s.
EEG activity was recorded using a High-Density EEG system (BrainVision, LLC, NC, USA). The electrodes' positions and three facial markers were digitized using an NDI Krios scanning system. This allowed for co-registering the EEG electrodes with each subjects' anatomical MRI data collected in a 3-Tesla Siemens Prisma scanner. EEG recordings were preprocessed using Statistical Parameter Mapping MATLAB toolbox (UCL Queen Square Institute of Neurology). Dynamic causal modeling for induced responses was used to uncover the within- and cross-frequency interactions between 5 sources: a merged bilateral supplementary motor area, contra- and ipsilateral- premotor cortex, and contra- and ipsilateral primary motor cortex.
Our results showed more IPSI CCC in the 53% lift condition compared to the 25% lift condition. Specifically, for the 25% condition we saw a majority of CCC happening on contralateral cortices, with little to no CCC in the IPSI hemisphere. However, for the 53% condition, there was an increase in CCC, with the connections being more heavily weighted to the IPSI hemisphere, supporting our hypothesis. In the future, we will combine measuring brainstem activity to further test that the increased IPSI CCC facilitates the use of RST via brainstem during SABD against a heavier load.

Disclosures: **K.M. Wright:** None. **E.N. Adjei:** None. **J.P. Dewald:** None. **J. Yao:** None.

Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

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Title: Tuning short-latency digit-to-motor cortex interaction in the human primary motor hand area

Authors: *L. CHRISTIANSEN[1], M. T. BONNESEN[1], M. M. BECK[1], H. R. SIEBNER[1,2,3]; [1]Danish Res. Ctr. for Magnetic Resonance, Copenhagen Univ. Hosp. Amager and Hvidovre, Hvidovre, Denmark; [2]Dept. of Neurol., Copenhagen Univ. Hosp. Bispebjerg and Frederiksberg, Copenhagen, Denmark; [3]Dept. of Clin. Med., Univ. of Copenhagen, Copenhagen, Denmark

Abstract: Background Sensorimotor integration can be investigated with transcranial magnetic stimulation (TMS) by measuring short-latency afferent inhibition (SAI). SAI indicates the inhibitory effects of peripheral nerve stimulation of the hand on the response magnitude to a TMS pulse delivered ~25ms later to contralateral primary motor cortex (M1). Repeated pairing of the peripheral and transcranial stimulus (i.e. Paired Associative Stimulation, PAS) can produce facilitatory after-effects on corticomotor excitability. Since conventional PAS protocols involves mixed nerve stimulation, after-effects might be mediated by both proprioceptive and cutaneous afferent signals. Here, we investigated the after-effects of a 5Hz PAS protocol that selectively pairs cutaneous inputs from a single digit with TMS of the motor cortical hand representation ($M1_{HAND}$).
Material and methods On two separate days, we mapped corticomotor representation of three hand muscles in fourteen healthy volunteers (18-29, 6 females) by delivering single-pulse TMS to seven anatomically defined targets in $M1_{HAND}$ and record motor evoked potentials before and after one of two PAS interventions. $M1_{HAND}$ sensorimotor integration was assessed through SAI maps in which TMS pulses to the left $M1_{HAND}$ were conditioned by cutaneous electrical stimulation of the right index ('Homotopic') and little finger ('Heterotopic').Both PAS interventions combined TMS over the hotspot of the first dorsal interossei and electrical stimulation of the index finger, but they differed in terms of the temporal relationship between the peripheral and transcranial stimuli: temporally coupled PAS ($PAS_{COUPLED}$) using a fixed inter-stimulus interval and temporally uncoupled control protocol with varying intervals between peripheral and transcranial stimuli.
ResultsOnly $PAS_{COUPLED}$ increased corticomotor excitability immediately after PAS. Furthermore, SAI mapping revealed a selective reduction in homotopic (but not heterotopic) short-latency afferent inhibition after $PAS_{COUPLED}$. Neither of the two PAS protocols affected the spatial distribution of corticomotor excitability or SAI along the precentral gyrus.
ConclusionsCombining contralateral electrical stimulation of cutaneous afferents with temporally coupled 5Hz rTMS of the M1-HAND cause pathway-specific effects on sensorimotor integration. The $PAS_{COUPLED}$ protocol enables experimental modulation of cutaneous-motor integration with unprecedented mechanistic specificity and might be suited to study aberrant sensorimotor integration associated with certain neurological disorders such as focal dystonias.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

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Title: Linking regional brain metabolism and excitability in human primary motor hand area - a combined 7T MR spectroscopy and TMS study

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Abstract: Introduction: Magnetic resonance spectroscopy (MRS) at 7T is increasingly used to probe the regional levels of neurochemicals in the human brain due to its improved signal-to-noise ratio relative to MRS at 3T. However, little is known about how the regional metabolic profiles obtained with 7T MRS relate to the regional excitability profile. **Aim:** Focusing on the human sensorimotor hand area (SM1-HAND), we examined the relationship between metabolite concentrations of glutamate and GABA as measured with 7T MRS and regional excitability of the brain tissue covered by the MRS voxel. **Methods:** 20 healthy volunteers (mean age 43.3 ys., range: 24-68 ys.) underwent single-voxel 7T MRS of the right and left SM1-HAND to measure regional concentrations of glutamate and GABA. Structural MRI was acquired to estimate partial volumes of the MRS voxel. We used transcranial magnetic stimulation (TMS) to obtain various metrics of regional cortical excitability, including resting motor threshold (RMT), the maximal amplitude of the motor evoked potential (MEP_{max}), short-latency intracortical inhibition (SICI) intracortical facilitation (ICF), the cortical silent period (CSP), and cortico-motor conduction time (CMCT). We tested for relationships between regional glutamate and GABA levels and MEP amplitudes of SICI, ICF and 'test' stimulations at 120% RMT in a single mixed linear model. In separate models we also investigated relationships between neurotransmitter concentrations and RMT, CSP, MEP_{max} and the CMCT. **Results:** Regional glutamate concentration scaled positively with the grey-to-white matter ratio of the MRS voxel ($p<0.001$), but GABA did not. There were no associations with age or gender for either glutamate or GABA. In the SICI/ICF model, increased glutamate levels were associated with larger MEP amplitudes ($p=0.023$) and increased GABA levels were associated with lower MEP amplitudes ($p=0.006$). However, these effects were less pronounced in the ICF condition (i.e. decreased contribution of glutamate to larger MEPs ($p=0.026$) and of GABA to smaller MEPs ($p<0.001$). For the CSP we found an interaction between glutamate and GABA levels ($p=0.024$) such that increased GABA levels were associated with a prolonged CSP only at low glutamate levels. There were no effects of GABA or glutamate on the MEP_{max}, CMCT or the RMT. **Conclusion:** Regional GABA and glutamate concentrations, as measured with 7T MRI, reflect the regional excitation-inhibition balance in the cortex volume covered by the MRS voxel.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

Location: SDCC Halls B-H

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Program #/Poster #: 300.10

Topic: E.04. Voluntary Movements

Title: Effect of hand dominance on decoding reach-to-grasp motor intent from non-invasive human EEG

Authors: *K. HOOKS[1], Q. FU[1], K. KIANI[2];
[1]Univ. of Central Florida, Orlando, FL; [2]Biomed. engineering, Univ. of Central Florida, orlando, FL

Abstract: Effect of hand dominance on decoding reach-to-grasp motor intent from non-invasive human EEGAuthors Kevin A. Hooks, Kimia Kiani, Qiushi Fu Department of Mechanical and Aerospace Engineering University of Central Florida, Orlando, FL Disclosures Kevin Hooks: None. Kimia Kiani: None. Qiushi Fu: None Abstract Fundamental to human movement is the ability to interact with and manipulate objects in our environment. Although the neural control of reach-to-grasp movements have been studied extensively, many knowledge gaps still remainremains due to the complexity of hand-object interactions. It has been shown that electroencephalography (EEG) contains rich information regarding the motor planning of reach-to-grasp actions, such that grasp type and reach directions can be decoded from EEG signals. However, it is unclear the extent to which decoding performance may differ between dominant and non-dominant hands. In this preliminary study, we investigated the decoding of motor intent from either hands before the movement onset of a flexible object interaction task. We asked right-handed participants (n=6, 4F, 2M, 26.5 +/- 4.9 years) to perform 8 blocks of 40 randomized trials to either reach-grasp-lift or reach-to-touch a novel object that affords different actions. Specifically, the object had 3 cylindrical handles attached to a long base, and lifting the object requires different wrist actions that depends on which side of the object was reached to and grasped. The participants would alternate which hand they used between blocks. A visual cue of required motor action was presented 1.5 second before the action start cue. Therefore, for each hand, we quantified the decoding accuracy during this action cue presentation period for two

factors: reaching direction and intended action on the object. Linear discriminant analysis (LDA) with 10-fold cross validation was used with spatial temporal feature vectors from delta band (1-4 Hz) of 14 electrodes over the sensorimotor and parietal regions. Accuracies were calculated in 200 ms time windows with 100 ms overlap. Overall, we found that two hands demonstrated similar levels of decoding accuracy for both factors (direction: ~70%, action: ~60%), but they showed different timing. For the dominant (right) hand, the highest decoding accuracy occurred 200 to 400 ms for direction and 300 to 500 ms for action after visual cue. For the non-dominant (left) hand, the highest accuracy occurred at 400-600 ms after visual cue for both factors. This resultsThese results suggested that hand dominance may play a role in the temporal evolution of the visuomotor transformation of hand-object interactions.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: DoD Grant W81XWH1810530

Title: Clinical and neurophysiology biomarkers of upper limb rehabilitative gains in persons with chronic cervical spinal cord injury

Authors: *J. LIU[1], S. WANIECK[2], T. ARORA[3], K. O'LAUGHLIN[1], G. F. FORREST[4], S. PUNDIK[5], K. KILGORE[6], A. BRYDEN[6], S. KIRSHBLUM[4], E. B. PLOW[1];
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Abstract: Introduction: Rehabilitation is a standard care to improve upper limb functions in persons with cervical spinal cord injury (SCI). However, therapeutic outcomes are variable. Identifying biomarkers that predict treatment effects is important for clinical decision making and customizing rehabilitation programs. The purpose of this study is to determine clinical and neurophysiologic biomarkers of upper limb rehabilitative gains in this population. **Methods:** Fourteen individuals from an ongoing clinical trial (NCT03892746) with chronic (>1 year) cervical (C1-C8) SCI and American Spinal Injury Association impairment scale (AIS) grades of A-D were included in this analysis. At baseline, these participants had residual sparing of triceps (0<muscle strength =< 3) relative to biceps (>= 3) based on medical research council scale. Each participant received upper limb rehabilitation and a double-blinded, randomized assignment of real or sham transcranial direct current stimulation (tDCS) in 15, 2-hour sessions over 3-5 weeks. At baseline and post-treatment, we measured motor function using the upper extremity motor scale (UEMS) and calculated the pre-post rehabilitative gains as α UEMS. Also, we measured

corticomotor physiology using transcranial magnetic stimulation (TMS), including corticomotor excitability indexed as active motor threshold (AMT), corticomotor inhibition tested as cortical silent periods, and corticomotor gain studied as recruitment curve (area under curve, slope, S-50). Recruitment curve S-50 was defined as the TMS intensity that produced a motor evoked potential (MEP) response half the size of maximal MEP; higher values of S-50 indicate higher physiologic responsiveness of corticomotor pathways. **Results:** While we are currently statistically underpowered to determine biomarkers associated with tDCS treatment effects, results of pooled data showed that participants with lesser SCI severity (greater grades of AIS, $r=0.63$, $p=0.016$) and greater neuro-responsiveness of triceps (higher values of S-50, $r=0.76$, $p=0.002$) at baseline achieved larger rehabilitative gains (α UEMS). AIS grades and triceps S-50 together highly explained the variance in α UEMS (adjusted $R^2=0.78$, $p<0.005$). Larger rehabilitative gains (α UEMS) were also associated with greater reduction in AMT of biceps ($r=-0.52$, $p=0.04$). **Conclusion:** Clinical severity of SCI and corticomotor responsiveness of a weaker muscle (triceps) can serve as composite biomarkers to predict therapeutic outcomes, while change in corticospinal excitability of a spared muscle (biceps) can be a dynamic biomarker to index that a biologic change has occurred during rehabilitation.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

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Program #/Poster #: 300.12

Topic: E.04. Voluntary Movements

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Title: Severity-specific characterization of ipsilateral motor evoked potentials in chronic stroke survivors with moderate to severe upper limb impairment

Authors: *A. MOHAN[1], D. A. CUNNINGHAM[3], X. LI[1], J. S. KNUTSON[3], M. WIDINA[1], K. O'LAUGHLIN[1], X. WANG[2], E. B. PLOW[1];
[1]Biomed. Engin., [2]Quantitative Hlth. Sci., Cleveland Clin. Lerner Res. Inst., Cleveland, OH; [3]Physical Med. and Rehabil., MetroHealth Syst., Cleveland, OH

Abstract: Introduction: Ipsilateral motor evoked potentials (iMEPs) represent the excitability of uncrossed motor pathways to the paretic upper extremity (UE) in stroke survivors. It is believed these pathways can serve as an alternate resource to support paretic UE motor recovery in individuals with severe loss of the (crossed) ipsilesional substrate. However, iMEPs have not

been characterized among severely impaired stroke survivors to establish there is indeed greater uncrossed pathway contribution. The purpose of this study was to compare iMEP features across severely and moderately impaired stroke survivors. **Methods:** Thirty-one chronic (>= 6 months post) stroke survivors with moderate and severe UE hemiplegia were enrolled. Participants were categorized as moderate or severe based on UE Fugl Meyer (UEFM) score. Those with UEFM score <=19 are severely impaired (n=19, mean (SD): 15.6 (3.0)), while those with UEFM score between 20 and 47 (included) are moderately impaired (n=12, mean (SD): 28.7 (7.8)). IMEPs were collected from paretic biceps brachii muscle by delivering suprathreshold TMS to contralesional motor cortices (at least 30 pulses given to different sites) while participants maintained maximal contraction of the paretic biceps and the (opposite) non-paretic triceps muscles. Participants also turned their neck to the paretic side to increase cervical propriospinal activity, thought to enhance the likelihood of facilitating ipsilateral pathway terminations. Following features of iMEPs were compared (peak-to-peak iMEP amplitude normalized to maximal voluntary activation of muscle, normalized area, onset, and offset) using independent t-tests. **Results:** IMEPs were evident in both moderate and severely impaired stroke survivors. The characteristics however were not different between the two severity subgroups. An exploratory correlation analysis revealed there was a significant association between normalized area of iMEPs and paretic grip strength in severe survivors, meaning those severely impaired survivors who had larger normalized iMEPs also had stronger paretic grip (ρ=0.46, p=0.048). **Discussion:** These early findings do not reveal a difference in ipsilateral pathway excitability across moderate and severely impaired stroke survivors, though severely impaired who have higher ipsilateral excitability to proximal flexors show stronger UE flexor muscle strength. Studies in a larger number of stroke survivors in each impairment category, including the mildly impaired, are underway and a comparator sample of aged able-bodied participants is also being studied.

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Poster

300. Mapping Sensorimotor Function in Upper Limbs

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Program #/Poster #: 300.13

Topic: E.04. Voluntary Movements

Support: KAKENHI 22H03456
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Title: Cerebello-rubral tract is related to the recovery of forced-limb use after cerebral hemorrhage

Authors: S. UENO[1], T. SHIMIZU[1], K. KOBAYASHI[2], N. TAJIRI[1], ***H. HIDA**[1];
[1]Nagoya City Univ. Grad Sch. Med. Sci., Nagoya City Univ. Grad Sch. Med. Sci., Nagoya, Japan; [2]Natl. Inst. for Physiological Sci., Natl. Inst. For Physiological Sci., Okazaki, Japan

Abstract: Intensive forced-limb use (FLU) after intracerebral hemorrhage (ICH) results in the recovery of disturbed forelimb function in rats, showing a causal relationship between the cortico-rubral tract and the recovery (J Neurosci 36:455-67, 2016). However, the contribution of motor regulatory system in the recovery of forelimb function by FLU after ICH remains unclear. In order to analyze the changes in motor regulatory system caused by FLU, we focused on the cerebellar output system in the cerebellar lateral nucleus to the parvocellular red nucleus (cerebello-rubral tract), using selective blockade of the tract by double-virus infection method. ICH model was made by the injection of Type IV collagenase (15 units/ml, 1.4µl) into the internal capsule of male rats, followed by FLU from 1 day after the lesion (D1) for 7 days and subsequent assessment of skilled reaching test. We revealed that rehabilitative training by FLU after ICH significantly induced better recovery in skilled forelimb reaching (single pellet reaching test). To evaluate the contribution of cerebello-rubral tract in the recovery of forelimb motor function, DREADD system was used: AAV-DJ-EF1a-DIO-hM4D(Gi)-mCherry and FuG-E-MSCV-Cre were injected into the cerebellar lateral nucleus and the parvocellular red nucleus, respectively. The blockade of cerebello-rubral tract with clozapine-N-oxide (CNO) significantly resulted in the reduction of the success rate, suggesting that the cerebello-rubral tract are involved in the improvement of impaired motor function by intensive use of the upper limb in rats.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Title: Posture and motor signals are organized in primary motor cortex

Authors: *P. MARINO[1], L. BAHUREKSA[2], C. FISAC[3], E. OBY[1], A. MOTIWALA[3], E. GRIGSBY[1], A. L. SMOULDER[2], A. D. DEGENHART[4], W. M. JOINER[5], S. M. CHASE[2], B. M. YU[3], A. P. BATISTA[1];
[1]Univ. of Pittsburgh, Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., [3]Carnegie Mellon Univ., Pittsburgh, PA; [4]Starfish Neuro, Seattle, WA; [5]Univ. of California, Davis, Univ. of California, Davis, Davis, CA

Abstract: Motor cortex (M1) receives convergent inputs about body posture and movement goals. How do these signals interact to shape neural population activity during motor tasks? We examined neural activity during both overt (reaching and isometric force) and covert (brain-computer interface, or BCI) tasks and identified organizing principles of this interaction. First, we found that the arm's posture drove neural population activity in a space that is separate from the space that accounts for neural tuning to intended movement direction. Second, we found that neural tuning to posture was consistent across tasks. Third, we found the interactions between posture and target signals reflected task demands. How do posture and target signals separately influence neural activity in M1? It is difficult to answer this question with overt tasks, because moving the arm changes its posture. We leveraged a BCI task to decouple target and posture signals. A BCI does not require overt movements, leaving posture fixed while M1 is active. In our task, Rhesus monkeys modulated M1 activity to drive a computer cursor to a target. Before each block of trials, we placed the monkey's arm in a different posture and calibrated a new decoder. We found that responses of individual neurons changed in complex ways with posture, but that the population response was organized: posture and target signals modulated separate neural dimensions. Changes in posture caused large changes in the starting points of neural trajectories in the BCI task, but only minimal reshaping of the trajectories. We next asked how posture and target signals shaped neural population activity during tasks requiring arm muscle activation. Monkeys engaged in isometric force and delayed center-out reaching paradigms from multiple initial postures. We found that neural tuning to posture was consistent across all overt and covert tasks conducted on the same day, even as overall neural activity was starkly different. Target signals still modulated separate neural dimensions from the posture signal. Interestingly, interactions between target and posture signals were larger in overt tasks than in the BCI task. This likely reflects task demands: in overt tasks, different initial postures required different movements for success. Taken together, these results suggest organizing principles underlying the integration of posture and target signals in M1: postural signals are task-invariant, modulate separate neural dimensions from target signals, and interact with target signals in ways that reflect task demands. A BCI enabled us to see this structure, and behaviors involving the muscles then allowed us to see how these signals interact.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Topic: E.04. Voluntary Movements

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Title: Multiplexing Neurons and Multiple Overlapping Cell Assemblies Active During Motor Behavior

Authors: *A. STELLA[1,2], P. BOUSS[1,2], G. PALM[3], A. RIEHLE[4], T. G. BROCHIER[4], S. GRÜN[1,5];
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Abstract: The cell assembly hypothesis [1] postulates that neurons coordinate their activity through the formation of repetitive co-activation of groups, called cell assemblies. We assume that spatio-temporal spike patterns (STPs) occur as an expression of active neuronal assemblies, at the resolution of a few milliseconds.
In order to test this hypothesis, we used the SPADE method [2,3,4,5], which detects significant STPs in parallel spike trains. We analyzed experimental data recorded by a 10x10 electrode Utah array in the pre-/motor cortex of macaque monkeys performing a reach-to-grasp task [6,7]. The task comprised four different trial types of grasping and pulling an object by combining two grip types and two force levels.
We find significant STPs in 19/20 recording sessions (of 15min) from different days. They occur in all phases of the behavior and across all trial types. Their size ranges between 2 and 6 neurons, with a maximal temporal extent of 60ms. The STPs show a high behavioral specificity, suggesting that different cell assemblies are active in the context of different behaviors. Moreover, we observed that pattern spikes are only a small fraction of the total recorded spiking activity, which may be explained by downsampling due to the recording. A surprising finding is that STPs overlap on different levels: 1) the same neuron may be involved in a different STP during another behavioral epoch during an individual session, which may indicate overlapping assemblies; 2) in 85% of the sessions with patterns at least one neuron participates in many

patterns, which may be interpreted as a hub neuron linking assemblies; 3) even individual spikes take part in more than one STP.
Concluding, our results indicate that STPs occur frequently in parallel spike trains. Quantitative analysis of their properties suggests that STPs are functionally related to behavior and specific to it, and may be an indication of the presence of assemblies being activated during the task. The assemblies may include tens or even hundreds of neurons, however, given the sub-sampling of our experimental setting, we may capture their activation in the form of patterns composed of a few neurons.
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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.03

Title: WITHDRAWN

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.04

Topic: E.04. Voluntary Movements

Support: NIH Grant RO1NS121409

Title: A framework for differentiating neural dynamics associated with cognitive and motor processes.

Authors: *J. BIRNBAUM[1], M. HASNAIN[2], M. ECONOMO[2];
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Abstract: During learned behavioral tasks, mice frequently perform spontaneous movements unrelated to explicit task demands. Further, representations of these ‘uninstructed’ movements often dominate brain-wide neural activity. The ubiquitous nature of uninstructed movements -

and the neural dynamics associated with them - presents a challenge in isolating and interpreting cognitive signals of interest during learned behaviors. This problem is made particularly acute when uninstructed movements are correlated with cognitive processes or aligned with task variables in ways not immediately obvious to the experimenter. How does one isolate the neural dynamics associated with cognitive processes of interest in the presence of task-correlated uninstructed movements expressed throughout the brain?
We simultaneously captured high-speed video and performed silicon probe recordings in the anterolateral motor cortex (ALM) while mice performed a delayed-response task to determine whether uninstructed movements confound estimates of motor planning signals. In this paradigm, a perceptual decision informs a motor action that is separated in time by a delay epoch during which animals must withhold their response. During the delay epoch, signals that predict upcoming movements are typically interpreted as neural correlates of motor planning, but the potentially confounding effects of ongoing uninstructed movements remain unclear. We found that delay-epoch uninstructed movements were highly predictive of choice and that the neural dynamics commonly associated with motor planning encoded the timing and amplitude of uninstructed movements on a trial-by-trial basis. Decomposing ALM dynamics into movement-related and movement-unrelated subspaces of neural activity revealed that established methods for isolating motor planning signals in mice often spuriously identified activity within a movement-related subspace. Restricting analysis to only the movement-unrelated subspace revealed a distinct set of dynamics encoding upcoming movements. These results show that neural dynamics previously associated with motor planning may be largely encoding ongoing, choice-correlated uninstructed movements and present a general procedure for differentiating dynamics associated with cognitive and motor processes.

Disclosures: **J. Birnbaum:** None. **M. Hasnain:** None. **M. Economo:** None.

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: E.04. Voluntary Movements

Support: Robert and Janice McNair Foundation
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Title: Stable neural dynamics in premotor cortex during motor planning

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Abstract: Motor planning is a fundamental brain function that give rise to volitional movements. However, it remains unclear whether the underlying neural dynamics that produce the same movement are stably maintained or flexibly reorganized over long time periods. Neurons in mouse anterior lateral motor cortex (ALM) exhibit preparatory activity that instructs future movements. Using longitudinal two-photon calcium imaging, we measured activity from the same ALM populations for over one month in mice performing a tactile-based delayed response task. Mice discriminated object location using their whiskers and reported choice using directional licking after a delay epoch. Mice were well-trained in the task with high performance before the imaging commenced. We found that task-related activities of ALM neurons were remarkably stable (Pearson's correlations (r) for task-aligned activity across sessions, 0.66 ± 0.07, mean ± SD across sessions). To assess the stability of preparatory activity at the population level, we trained a linear decoder to read out future lick directions from ALM delay activity. A decoder trained in one session can reliably predict movement direction when applied to other sessions regardless of the time interval between sessions (decoding accuracy, 81.18 ± 10.00%, mean ± SD across sessions). Taken together, these results suggest that preparatory activity under the same task context is conserved over at least one month.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.06

Topic: E.04. Voluntary Movements

Support: PAPITT

Title: Facial palsy alters the cortical motor planning of facial expressions in a murine model

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Abstract: Facial palsy is the partial or complete loss of function of some or all of the structures innervated bythe facial nerve, a fundamental structure for the communication of emotions. Interestingly, rodents use their orofacial musculature to express long-lasting internal states and convey emotions through facial expressions. In murine models, it has been observed that neurons of the anterolateralmotor cortex (ALM) project subcortical structures to control facial movements. It is considered a structure that anticipates, prepares, plans, and executes movements. Facial palsy alters the face musculature;in murine models, angles formed by the vibrissae movements are lost; at the cortical level, it is represented as electrophysiological changesin the primary motor and somatosensory cortex; specifically prolonged disinhibition in bothhemispheres and a decrease in the firing rate of pyramidal neurons. As can be seen, facial palsyhas cortical effects that control the movement of the facial muscles, resulting in a loss of

thegeneration of facial expressions, which are voluntary movements that are anticipated, planned, andsend information to executing centers through the ALM.However, alterations in these processes have not been studied in depth when there is experimentalfacial palsy and how it correlates with the loss of the correct planning and execution of facialexpressions. Extracellular neuronal activity of ALM is evaluated; at thesame time, the facial expressions and whiskers movements of mice were video-recorded whilethey were subjected to a behavioral predictive consumption of a solution rewarding (sucrose) task. The results show that reversible facial palsy is maintained from the time of injury up to 11 days later. In the case of the irreversible model, loss of motion persists throughout the experimental protocol. This loss of vibrissae movements correlates with the inability to plan and generate facial expressions related toconsuming a rewarding substance. But the, facial palsy does not alter the execution of the behavioralparadigm.When comparing the neuronal activity before and after sucrose stimulation, ipsi- and contra-lateral(to the injury) activity of ALM do not change their firing rate. On the other hand, the neuronal activityin both ALM increases their firing rate when mice consume sucrose and generate a facial expressionof pleasure. In the case of facial palsy, the subjects cannot generate facial expressions on the injuredside. This suggests that facial palsy affects a premotor structure and the preparation, planification,and execution of facial movement.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Program #/Poster #: 301.07

Topic: E.04. Voluntary Movements

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Title: Nonlinear dynamical modeling of behaviorally relevant neural dynamics for discrete and categorical neural-behavioral data

Authors: *O. G. SANI[1], B. PESARAN[2], M. M. SHANECHI[1];
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Abstract: Neural activity exhibits diverse nonlinear dynamics that simultaneously relate to multiple brain functions and behaviors. Thus, a significant challenge when studying the neural basis of a specific measured behavior is that the dynamics related to that behavior may be masked or confounded by all the other neural dynamics that coexist. Recently, we developed a new method termed recurrent neural networks (RNN) preferential system identification (PSID), or RNN PSID (bioRxiv 2021.09.03.458628) for joint dynamical modeling of neural-behavioral data. We showed that RNN PSID learns nonlinear dynamical models of neural population

activity with low-dimensional latent states that predict both behavior and neural activity. Further, RNN PSID preferentially prioritizes the learning of behaviorally relevant neural dynamics. However, RNN PSID has so far focused on modeling of continuous behavioral and neural signals. Here, besides providing additional validations and results for RNN PSID, we also extend RNN PSID for modeling of intermittently measured data, as well as diverse neural-behavioral modalities including Poisson neural data and categorical behavioral data. We validate the method in numerical simulations and in multiple monkey datasets with different behavioral tasks and with categorical behavioral and Poisson neural distributions. We find that RNN PSID successfully extends to these diverse cases; it extracts low-dimensional latent states from neural activity that more accurately predict behavior compared to linear preferential modeling methods (linear PSID) and nonlinear dynamic modeling approaches that do not prioritize behaviorally relevant information. Finally, we demonstrate that models learned by the new method achieve better neural decoding of behavior compared with other methods, both for gaussian and non-gaussian distributed neural and behavioral data. Overall, this novel method enables nonlinear dynamical modeling of neural-behavioral data, dissociates and prioritizes behaviorally relevant neural dynamics, extends to discrete or categorical neural and behavioral signals, allows the modeling of intermittently measured data, and enables causal real-time behavior decoding.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.08

Topic: E.04. Voluntary Movements

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JSPS KAKENHI 19K03377
JSPS KAKENHI 16K17369
JSPS KAKENHI 25780453

Title: Evolving contralateral dominance of hand movements from the caudal cingulate motor area to the primary motor cortex via the supplementary motor area in monkeys

Authors: *Y. NAKAYAMA, O. YOKOYAMA, E. HOSHI, Y. NISHIMURA;
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Abstract: The primary motor cortex (M1), supplementary motor area (SMA), and caudal cingulate motor area (CMAc) send axons that project to the ventral horn (lamina IX) and intermediate zone (laminae V-VIII) of the spinal cord. Although most of the corticospinal terminations from the three regions are on the contralateral side of the spinal cord, a certain proportion of them is on the ipsilateral side. These anatomical evidences indicate that these three areas control both contralateral and ipsilateral limb movements. Therefore, it is of great interest

to directly compare the laterality representation of neurons in the three areas. In the present study, we examined the laterality representation of the M1, SMA, and CMAc underlying contralateral and ipsilateral hand movements by investigating neuronal activity while monkeys were performing a button-press task with the right or left hand. Neuronal representations in M1 were strongly biased toward contralateral hand movements, those in CMAc represented contralateral and ipsilateral hand movements to the same degree, and those in SMA were biased toward contralateral but the degree of contralateral bias was smaller than M1. These indicate that the three areas control contralateral and ipsilateral movements in a different manner. We then tested the influences of neuronal activity on initiating contralateral and ipsilateral hand movements by calculating the correlation coefficient between spike count at a certain time and reaction time. We found that the activity of M1 neurons for contralateral movements expressed a strong correlation with reaction times, whereas that of SMA and CMAc neurons did not. By contrast, the correlation coefficient of M1 neurons for ipsilateral movement did not express a notable change and was not stronger than that of SMA and CMAc neurons. These suggest that among the three areas, M1 is closest to the output stage of the movement of the contralateral body part. Taken together, the present findings suggest that the contralateral dominance of neuronal representation in terms of hand movements evolves from CMAc to M1 through SMA.

Disclosures: Y. Nakayama: None. **O. Yokoyama:** None. **E. Hoshi:** None. **Y. Nishimura:** None.

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01 NS105697

Title: The spatiotemporal organization of M1 activity that supports reach-to-grasp movements

Authors: *N. CHEHADE, O. A. GHARBAWIE;
Dept. of Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Primary motor cortex (M1) is central to coordinated arm and hand movements. In monkeys, M1 motor outputs are spatially organized as a nested somatotopy of the hand zone and arm zone. Nevertheless, the spatial relationship between M1 functional encoding and the motor map is not known. We sought to understand how the temporal dynamics of M1 activity that support reach-to-grasp movements are spatially organized with respect to M1 outputs. We trained two rhesus macaques on an instructed reach-to-grasp task while head-fixed. Monkeys were cued to: reach, grasp, and lift a (1) small or (2) large sphere, (3) reach out and cover a photocell, or (4) maintain the start position. A chronic optical window implanted in each monkey provided access to sensorimotor cortex. We used intracortical microstimulation to map (n = 211

& n = 158) the cortical surface to establish M1 output topography. Then we recorded single unit activity in acute penetrations (n=94 & n = 44) throughout arm and hand zones with linear electrode arrays. In total, we recorded from 908 single units in the M1 arm representation and 620 in the M1 hand representation. In each zone, 75% of the units were considered task modulated as their firing rate increased significantly during movement as compared to baseline. The profiles of the average peristimulus time histograms (PSTHs) appeared to be zone-specific. Namely, the average PSTH from the arm zone indicated increased firing rate during arm transport and object contact. In contrast, the average PSTH from the hand zone indicated a peak with movement onset and another larger peak during object contact and manipulation. An unsupervised clustering algorithm on all recording sessions returned 3 PSTH profiles. Two of the profiles (i.e., two clusters) were consistent with the average PSTH profiles from the arm and hand zone. However, each cluster was comprised of recording sites that were present throughout the M1 arm and hand zone. Our results suggest a complicated spatial relationship between M1 motor outputs (i.e. anatomical organization) and neural activity that support movement (i.e., functional organization).

Disclosures: **N. Chehade:** None. **O.A. Gharbawie:** None.

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

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Topic: E.04. Voluntary Movements

Support: KU Leuven grant C14/18/100
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Title: Effective connectivity between mirror neurons in the ventral premotor cortex and the primary motor cortex

Authors: *S. DE SCHRIJVER, T. DECRAMER, P. JANSSEN;
Neurosciences, KU Leuven, Leuven, Belgium

Abstract: The premotor cortex is known to play a pivotal role in the processing of visuomotor actions. More specifically, mirror neurons, which respond to both action execution and action observation, were discovered in area F5c of the ventral premotor cortex (PMv). We have previously shown that it is possible to evoke a behavioral effect during grasping (i.e. increased grasping times) when delivering subthreshold microstimulation to F5c sites, implying that F5c mirror neurons play a causal role in visually guided grasping. A similar effect was observed when stimulating non-mirror sites in F5c. It is however unknown how F5c mirror neurons influence other motor areas, such as the primary motor cortex (M1). To investigate the effective connectivity of F5c neurons, we implanted two 96-channel Utah arrays, one in area F5c and one in M1, in two macaque monkeys that were trained to perform a visually guided grasping (VGG)

task and to fixate videos of actions. We tested mirror activity during passive fixation of videos of a human or a monkey hand performing the same grasping task. In 8 sessions, we investigated the responses of M1 neurons when performing subthreshold intracortical microstimulation (ICMS) in both mirror and non-mirror sites in F5c. Stimulation trains of biphasic pulses (duration 1 ms) were delivered at 16 Hz for 685 ms during the delay period of a VGG task, with an intensity below the motor threshold (ranging from 10 to 70 μA). ICMS at 16Hz allowed us to measure spiking activity in 50 ms intervals between the pulses while discarding 15 ms around each pulse due to the stimulation artefact. We observed that, on average, ICMS on one F5c electrode elicited significant inhibitory responses in 24% of the recorded sites in M1 and excitatory responses in 10% of M1 sites ($p<0.05$). Overall, M1 responses were more frequently inhibited during stimulation (195 sites out of 277 significantly modulated sites with inhibition, compared to 82 sites with excitation, χ^2 (1,277) = 92.2, $p < 0.001$). For all sites, the response was modulated immediately after the first pulse of the stimulation train and maintained elevated or reduced (for the excitatory and inhibitory responses, respectively) until the last pulse of the stimulation train. The mainly inhibitory response in M1 may explain the behavioral deficit when stimulating F5c mirror sites. Surprisingly, stimulation in F5c mainly elicited strong inhibitory responses in other F5c sites (recorded from electrodes on the same F5c array). On average, 41% of the recorded F5c sites ($p<0.05$) showed an inhibitory response when stimulating a single F5c site. Together, these results suggest causal evidence for connectivity between ventral premotor area F5c and M1.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Title: Evolution of movement-related neural population dynamics following focal ischemic injury

Authors: D. T. BUNDY[1], F. BARBAN[3,4], H. M. HUDSON[1], M. NISHIMOTO[5], S. BARBAY[1], M. CHIAPPALONE[3,4], R. J. NUDO[1,2], ***D. J. GUGGENMOS**[1];
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Abstract: Stroke impacts ~ 800,000 people annually in the U.S., often leading to chronic motor impairments due to a combination of damage at the injury site and disrupted communication across spared regions. After focal cortical injury, large-scale reorganization occurs within and between spared cortical areas, potentially supporting recovery. Because it is possible to record from large numbers of neurons in multiple areas simultaneously, dimensionality reduction techniques have been used recently to extract low-dimensional neural trajectories, allowing isolation of task-relevant dimensions. While stereotyped neural trajectories have been observed in normal animals, it is unknown what impact focal ischemic infarcts have on these dynamics. We sought to determine 1) if neural population dynamics are disrupted by a focal ischemic lesion within the sensorimotor network, and 2) if behavioral recovery is associated with a restoration of neural population dynamics. We tested these questions in two related experiments utilizing male Long-Evans rats (*Rattus norvegicus*). In a within-subject design, 12 rats were trained to perform a skilled pellet retrieval task prior to receiving chronic electrode implants into premotor cortex and primary somatosensory cortex. Neural data were recorded during task performance prior to and one week following a focal ischemic infarct to the primary motor cortex. In a longitudinal study, 14 rats trained on the same task were randomized to receive a cortical infarct, subcortical infarct, or no infarct. Neural activity was recorded from premotor cortex, primary motor cortex, and primary somatosensory cortex twice per-week until 8 weeks post-infarct. In all rats, neural population dynamics were estimated using Gaussian Process Factor Analysis (GPFA). The variance explained by the entire model, and specifically in task-relevant dimensions, were compared across lesion conditions and time. The lesion induced an immediate behavioral deficit accompanied by a disruption in the variance explained by the model and an absence of task-relevant dimensions. During spontaneous behavioral recovery, we observed a restoration of stereotypical neural trajectories. These results show that ischemic injuries alter the normal population dynamics associated with skilled movements. Importantly, the disruption of neural dynamics following either cortical or subcortical infarcts shows that neural dynamics represent important features of neural activity necessary for the execution of skilled movements. The restoration of neural population dynamics following recovery suggests the importance of spared cortical networks for motor recovery.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.12

Topic: E.04. Voluntary Movements

Support: NIH 1R01NS092894-01

Title: Influence of reward and effort context on intra- and inter-cortical (S1, M1, and PMd) phase-amplitude coupling during arm reaching movements

Authors: T. YADAV[1], O. MAGAÑA-TELLEZ[2], D. J. FRANCIS[2], ***J. T. FRANCIS**[2];
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Abstract: Growing evidence from multiple studies over the last decade has established the role of primary somatosensory (S1), primary motor (M1), and premotor (PMd) in processing task-relevant cues besides motor planning and execution. Among these, we have previously reported robust modulation of alpha and beta-band local field potentials recorded from the S1, M1, and PMd cortices with reward and effort levels associated with arm movements. Yet, it is unknown whether the processing of such task contextual information influences communication within and between these sensorimotor regions before movement onset. To address this gap, we studied changes in directional communication within and between S1, M1, and PMd cortices by computing phase-amplitude coupling (PAC) to be followed by more direct measures of information flow. One non-human primate (female, Macaca Mulatta) was trained on three planar center-out arm reaching task variations: reward-only, effort-only, and reward-effort tasks. Task context, reward and effort, were cued using color and shape, indicating the expected juice reward (3 levels) and expected effort (velocity-dependent viscous force field with varying viscosity, 4 levels) of a trial. Only successfully completed trials were rewarded with the cued amount of juice drops. While no viscous field was active for the reward-only task, a fixed reward (1 drop) was delivered in the effort-only task, whereas both reward and effort levels were varied in the reward-effort task. PAC was computed using the Kullback-Leibler-based modulation index (MI) between the amplitude of gamma oscillations (60 to 100 Hz) from one region and phase of alpha/beta oscillations (10 to 20 Hz) from the same or a different region. This analysis was restricted to the cue processing period (0.7 s in reward-only task and 1.5 sec for others), devoid of arm movement or motor planning. Statistical significance of PAC was tested by generating 200 surrogate MIs for each amplitude-frequency pair, z-scoring, and determining the threshold for significant MI ($p<0.01$) assuming a normal distribution. PAC was considered significant if the observed MI > threshold MI at a given phase-amplitude frequency pair. Our initial results indicate increased communication from M1 (phase) to PMd and S1 (amplitude) during reward-only processing, which decreased during effort-only processing. During combined reward and effort processing, an increased communication directed from PMd to M1 and S1 was observed. These findings highlight the differential role of S1, M1 and PMd cortices in processing different task contexts relevant to the same movement.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Program #/Poster #: 301.13

Topic: E.04. Voluntary Movements

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Title: Detecting multi lag spiking patterns within motor cortical neural recordings in non-human primates

Authors: *H. AKOLKAR[1], H. MAO[1], A. B. SCHWARTZ[2], R. BENOSMAN[3];
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Abstract: Advances in recording technology has made it possible to record action potentials from many neurons simultaneously. Current methods for analyzing these data primarily rely on binned spike count activity of neural populations without consideration of the timing between action potentials in different neurons.
Here, we demonstrate a novel unsupervised multi-layer paradigm using individual action potentials to detect multi-lag correlations and spike-time interaction in groups of neurons. We introduce a hierarchical network of temporal layers that maps the inter-neuron spike-time delays onto a normalized temporal feature space. Clusters of features within the temporal space reveal unique recurring spiking patterns. Through simulations, we show that the method could reveal patterns are formed by consistent changes in firing rates across unconnected neurons and/or causal activity of anatomically or functionally connected neurons. The multi-layer network can detect interaction at different time scales and successively group smaller short-scale patterns into larger clusters with longer timescale correlation.
We applied the method to motor cortical recordings from non-human primates performing voluntary motor tasks such as center-out target reaches and object grasping. The network found patterns of M1 activity at different time-scales in an unsupervised manner in the absence of task-related information. For the center-out task, we found a specific order of spiking activity during repeated reaching tasks. Temporal feature maps were used to decode hand trajectories (Pearson correlation coefficient $R > 0.9$). In a grasping task, the temporal features were able to discriminate between different task conditions such as no-object, only touch, pinch grasp or power grasps with accuracy of up to 95%.
This method is useful for describing consistent neuron-neuron interactions taking place at a multiple time scales. Initial results show that these interactions form patterns that are clearly related to ongoing behavior.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Topic: E.04. Voluntary Movements

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Title: Longitudinal bihemispheric reorganization of motor encoding in rats after a primary motor cortex lesion

Authors: *P. HAYLEY[1], F. BARBAN[3], D. T. BUNDY[2], D. J. GUGGENMOS[2], R. J. NUDO[2]; [1]Mol. and Integrative Physiol., [2]Rehabil. Med., Univ. of Kansas Med. Ctr., Kansas City, KS; [3]Inst. Italiano di Tecnologia, Genoa, Italy

Abstract: New and recurring ischemic stroke represents a significant proportion of acquired injuries leading to motor impairment. Because ischemia is most often unilateral in nature, research on the spared motor areas of the opposite, homotopic hemisphere as potential substrate for restitution of motor ability is ongoing; however, these studies have often been cross-sectional and lack direct insight into the functional consequences of reorganization. Additional evidence suggesting that motor map reorganization and behavioral recovery are asynchronous prompts the need for detailed investigation into how important spared regions with interhemispheric connections such as the premotor cortex are actively shaped during the recovery process. Here, we report on the novel application of chronic microelectrode arrays (MEAs) implanted in both hemispheres using a within-animal design that allows for combined insight into the longitudinal reorganization of somatotopic representation and neural recruitment during movement in a model of ischemic injury. After reaching baseline performance in a single pellet retrieval task, a total of 6 male Long-Evans rats were bilaterally implanted with two-32 channel MEAs in rat premotor cortex identified with intracortical microstimulation (ICMS) mapping of the hemispheres during surgery. The arrays were fixed into place while leaving access to primary motor cortex. Following implantation, the rats continued with assays for the pellet retrieval task in combination with neurophysiological recordings of the MEAs until baseline performance was obtained. In situ motor maps were obtained by delivering ICMS pulse trains through the array sites with increasing current up to 80μA at each electrode site under light anesthesia. Following these baseline measures, rats underwent a second surgery to induce a photothrombotic lesion using Rose-Bengal injection and illumination unilaterally over primary motor cortex. Rats were tested weekly to monitor changes in neurophysiological, motor map, and behavioral outcomes. During analysis, multi-unit activity recorded at each electrode site was aligned with the behavioral task using video recordings. We then related the evoked motor output at the electrode site to its task-aligned neural activity, finding that the combined metrics helped resolve the interhemispheric contribution of premotor cortex to motor activity. Ultimately, this knowledge can be added to a growing compendium of the emergent post-ischemic motor network foundational to rehabilitation efforts.

Disclosures: P. Hayley: None. **F. Barban:** None. **D.T. Bundy:** None. **D.J. Guggenmos:** None. **R.J. Nudo:** None.

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.15

Topic: E.04. Voluntary Movements

Support: NSF IOS Award 1558151

Title: Effects of a tactile arm position cue on motor cortical activity when reaching in a virtual environment

Authors: K. PHATARAPHRUK, *C. BUNEO;
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Abstract: Although psychophysical experiments have shown that tactile cues can provide information about arm position that is useful for guiding reaching actions, the neural mechanisms underlying this phenomenon are not well understood. As a result, we are currently investigating the effects of providing or withholding a tactile cue regarding the initial position of the arm on static positional and reaching related activity in dorsal premotor and primary motor cortex (M1). Here we focus on activity in M1. The activity of ~150 units was recorded from two 32-channel floating microelectrode arrays while a rhesus macaque performed goal-directed reaches in four directions within a semi-immersive virtual reality (VR) environment. Tactile feedback was modulated in blocks by adding or removing a tactile cue (3 cm diameter plastic button) at the starting position. Visual feedback of the arm was modulated on a trial-by-trial basis by rendering either a veridical or perturbed (6cm shift towards the body) representation of a virtual monkey arm at the start position. On each trial, the start position was cued visually but no visual feedback of the arm was provided. After acquiring the start position, the start cue was extinguished and the animal was required to maintain position without arm visual feedback for one second. The virtual monkey arm was then presented in either its veridical or perturbed position for another second, followed by the presentation a visual target cue, which instructed the animal to reach. Visual feedback of the arm was removed prior to movement onset. The effects of the tactile and visual cues were assessed behaviorally by comparing mean reach directions at peak tangential acceleration. Although we observed target-dependent differences in these initial reach directions, they tended to be more accurate (i.e., less deviated from cued target directions) with tactile cueing than without and were also more accurate in the veridical arm vision condition. However, although the firing rates of some individual neurons did differ between trials with and without the tactile arm position cue, at the population level no clear differences between tactile conditions were observed in either the veridical or perturbed visual conditions. More robust neural correlates of the observed behavioral effects may be evident in frontoparietal areas more directly involved in sensorimotor transformations and/or in signals known to reflect convergent inputs into a given cortical area, i.e., local field potentials.

Disclosures: K. Phataraphruk: None. **C. Buneo:** None.

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.16

Topic: E.04. Voluntary Movements

Support: Jean Sheng

Title: The impact of prior experience on tactile-motor corrections

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Abstract: Depending on our experiences, we may not always immediately use sensory cues to adjust our actions. While a novice tennis player may constantly adjust their stroke style after every single miss, a well-practiced tennis player may stick to the same stroke style unless misses are consistent. Here, we leverage kilohertz framerate imaging and a deep neural network to image and track the 3D kinematics of the tongue as mice learn to lick from a water spout. To investigate how error correction depends on past experience, we created a novel lick paradigm where spout positions switch between two possible locations: a center location and either a left or right location. After a random number of licks at one position, the spout is displaced to the other location before the next lick in the lick bout. The positions are chosen such that the side of the tongue "nicks" the spout on the first lick following this displacement. In concurrent work (Ito et al, in prep), we find that well-trained mice in a similar paradigm react to nicks with immediate re-aiming on the subsequent lick - if the left (right) side of the tongue nicks the spout, the next lick is aimed left (right). To study the effects of prior experiences on these tactile-guided corrections, we compare a group of naïve mice never exposed to water spouts with a group of experienced mice having been trained to lick a water spout at a fixed location in this task. Preliminary results suggest experienced mice already trained to lick a fixed spout may not immediately adjust their tongue re-aiming after partial tongue-spout contacts relative to their naïve counterparts. This novel behavior paradigm may lay the foundation for understanding the neural mechanisms of how prior experiences may regulate fast corrections in response to errors in predicted sensory feedback.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.17

Topic: E.04. Voluntary Movements

Title: Co-expression Patterns of Convergently Regulated Genes across Diverse Vocal Learning Taxa

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Abstract: Vocal learning is a rare trait in the animal kingdom, present in a select few mammalian and avian lineages. Efforts to characterize the neurobiology underlying the trait have primarily utilized the zebra finch as an experimental model. Human and zebra finch have specialized vocal learning circuits, which show convergent gene expression patterns not seen in surrounding non-vocal brain regions. The highest degree of molecular convergence is between the human laryngeal motor cortex (LMC), and the songbird HVC and robust nucleus of the arcopallium (RA) vocal motor nuclei. Close relatives of these species who are incapable of vocal learning show no genetic specialization in the anatomically analogous area of the cortex and pallium. While LMC, HVC, and RA broadly share convergent expression patterns, mammals and birds differ in the cellular organization of the cortex, with the neurons of the mammalian cortex stratified into distinct layers, while those of the bird are organized into large nuclear clusters.Preliminary data from our group suggest that specific cell types could show convergent specialized transcriptome functions. This study seeks to identify if the LMC/HVC/RA specialized gene set is uniformly expressed across these regions or if distinct co-expression patterns mark cell populations and types within each area. Utilizing a multiplexed spatial imaging approach, we are performing spatial transcriptomics for the expression of genes specialized in vocal learning circuits and well-established cell type markers across the vocal learning regions of the finch and human. Additionally, we are screening the motor cortex of the bottlenose dolphin, a mammal whose vocal learning ability is comparable to humans, for these genes to see if any subregion shares the specialization seen across the LMC/RA/HVC. Doing so will allow us to identify the extent to which the advanced mammalian learners are uniquely marked by their co-expression of vocal learning genes. This project will be a significant step in determining the evolution of human spoken language.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Topic: E.04. Voluntary Movements

Support: NIH Grant RO1 NS108424
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Title: Holographic Manipulation of Premotor Neuronal Ensembles in the Zebra Finch Using Two-Photon Optogenetics

Authors: *H. PANCHOLI, M. TRUSEL, T. F. ROBERTS;
Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: Drawing causal links between patterns of neuronal activity and behavior remains a major research challenge. The stereotyped courtship song of zebra finches is encoded by a precise pattern of premotor neuron activity. Premotor neurons in the cortical vocal region HVC that project to the motor area RA (HVC-RA neurons) exhibit precise sequential activity during song and current evidence suggests that this activity is necessary for song production. However, it is not known how this apparent sequence causally relates to the vocal-motor actions underlying song performance. New cellular-resolution methods for optically manipulating activity patterns in the brain provide a potential approach for testing the causal interactions between neuronal ensemble activity and behavior. Here we describe our progress in using two-photon optogenetics for manipulating the neural sequences associated with birdsong production. This technique permits cellular-resolution optogenetic stimulation of neurons in a 3-d volume of brain tissue using holographic patterns generated by a spatial light modulator. All-optical experiments that combine this technique with two-photon calcium imaging can non-invasively read-out and write-in activity patterns in the brain. To establish these methods in zebra finches we first used an intersectional viral strategy to express the red-shifted soma-targeted opsin ChrMine exclusively in HVC-RA neurons. We found that two-photon holographic stimulation of individual HVC-RA neurons can elicit bursts of action potentials like those observed during singing. We next used viral expression of ChrMine and the calcium indicator jGCaMP8m in HVC-RA neurons to test if we could conduct all-optical experiments *in vivo*. We found that holographic stimulation permits cellular-resolution manipulations of neuronal activity and that we can play-in sequences of activity across ensembles of HVC-RA neurons. Having established this method in the zebra finch, future experiments will test the role of HVC neurons in song production. We envision two-photon optogenetics as a powerful method for better understanding neuronal connectivity and how neuronal ensembles in HVC function in song memory, sensorimotor learning, and the production of adult song.

Disclosures: H. Pancholi: None. **M. Trusel:** None. **T.F. Roberts:** None.

Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Support: NIH grant UF1NS115821
NIH grant R01NS108424

Title: Defining a minimal circuit for adult song production

Authors: *M. TRUSEL[1], D. ALAM[1], H. PANCHOLI[1], B. G. COOPER[2], T. F. ROBERTS[1]; [1]Neurosci., UT Southwestern Med. Ctr., Dallas, TX; [2]Psychology, Texas Christian Univ., Fort Worth, TX

Abstract: It is commonly assumed that neuronal activity within cortical circuits is essential for the generation and control of skilled behaviors. However, recent studies indicate that, once learned, some skilled behaviors can be performed independently of cortical circuits, raising questions about whether and how motor cortical areas and their input structures control skilled motor behaviors. The adult zebra finch song is an example of a learned skilled behavior that is dependent on forebrain structures analogous to mammalian motor cortical regions. The motor cortical analog HVC (letters used as proper name) is critical for song production and learning, but the contributions of its afferents and efferents to song have not been investigated. HVC receives input from at least four nuclei spanning the thalamus and sensory or sensorimotor regions of the forebrain and has anatomically separable projection pathways onto the descending song motor pathway, the vocal basal ganglia, and the auditory forebrain. Yet, the precise synaptic connectivity of these HVC afferents and their contribution to song is still poorly understood. Using patch-clamp recordings of HVC projection neurons in concert with optogenetic excitation of individual afferents, we now provide a synaptic wiring diagram of the various inputs to HVC. We then tested the role of these various circuits in song motor control. Strong optogenetic stimulation of terminals reaching HVC does not disrupt ongoing song in adult birds. In line with this observation, the contemporaneous bilateral lesion of four forebrain afferents to the song motor pathway leaves the adult song intact. In contrast, we find that even brief 10 to 25 ms optogenetic excitation of projection neurons in HVC at any moment in the song immediately halts and rapidly resets the song back to the beginning of the motif. Our data indicate that the projection neurons originating in HVC function as a conductor, controlling the moment-to-moment progression of song autonomously of their extrinsic synaptic inputs. Together, our findings help delineate a minimal circuit for adult song production and highlight circuit architecture intrinsic to HVC that leads to the sequential structure of the skilled behavior.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Title: Primate vs rat divergence in the corticospinal projectome

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Abstract: Restoring upper limb and hand function is one of the highest priorities for individuals with tetraplegia resulting from spinal cord injury. The corticospinal tract (CST) is the main pathway controlling skilled arm and hand function in primates and humans. The aim of this study is to map all projections of CST axons (the 'projectome') related to hand function in the intact primate (rhesus monkey) CST and compare it to the CST projectome in the rat.
We used a set of viral intersectional tools to trace all projections of CST neurons associated with hand and forelimb function. To date, five intact primates have been injected with an adeno-associated virus encoding Cre-recombinase (rAAV2-retro-Cre) at 10 sites spanning the caudal C7, C8, and T1 spinal cord segments (which contain motor neurons controlling hand muscles). This resulted in efficient retrograde transport of rAAV2-retro-Cre to the motor cortex. In the same surgical session, *Cre-dependent* tracer injections were made into the region of motor cortex controlling the right arm and hand. Specifically, AAVdj-CAG-DIO-gCOMET, which, *when activated by Cre recombinase*, encodes an enhanced, membrane-targeted Green Fluorescent Protein (GFP), was injected at 28 sites in the left motor cortex. Because only corticospinal neurons that project to the C7-C8-T1 spinal cord segment will receive both Cre and the Cre-dependent tracer, only those neurons will express GFP. This allows us to map not only the corticospinal axon terminals that project to C7-C8-T1, but *all other branches (arising from those same neurons) which terminate in any region of the brain and spinal cord*. In rats, we found an extraordinarily diverse set of collateral projections from corticospinal neurons to 23 different brain and spinal regions. Remarkably, the vast weighting of this "motor" projection was to sensory systems in both the brain and spinal cord, confirmed by optogenetic and trans-synaptic viral intersectional tools. In contrast, primates exhibited far heavier and narrower weighting of corticospinal outputs toward spinal and brainstem motor systems. Unique structural-functional correlations can be achieved by mapping and quantifying a single neuronal system's total axonal output and its relative weighting across CNS targets. There is distinct evolutionary divergence: corticospinal systems in primates primarily constitute a final output system for fine motor control, whereas in rats the same system exerts a multi-modal integrative role that accesses broad CNS regions. By understanding this pathway's unique properties and changes after injury, we may be able to identify new targets to enhance recovery after primate spinal cord injury.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.21

Topic: E.04. Voluntary Movements

Support: CIHR 201803PJ8

Title: Complementary contributions from transcortical and spinal feedback pathways during postural control

Authors: *H. GUANG[1], J. Y. NASHED[1], J. A. PRUSZYNSKI[2,3,4], H. T. KALIDINDI[5,6], G. BLOHM[1], S. H. SCOTT[1,7,8];
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Abstract: In biological motor control multiple feedback pathways simultaneously contribute to online control. For voluntary control of the arm, spinal and transcortical feedback pathways begin contributing at 25 and 60ms, respectively. The former scales with muscle stretch magnitude, and increases with the level of background muscle activity, termed gain scaling. Does transcortical feedback alter its contribution to counter how spinal feedback contributes to feedback corrections? To address this issue, three monkeys performed a posture perturbation task when maintaining their hand at a spatial target and countering mechanical disturbances. We altered pre-perturbation background loads to alter the spinal reflex response. Muscle activity in the high background load condition showed a significantly larger increase from baseline during the short-latency reflex epoch (180% increase from 25-50 ms post-perturbation) than in the low background load condition, and EMG responses were roughly similar throughout the rest of the motor correction. As predicted, recordings from 280 M1 neurons showed a significantly lower response from baseline between the high vs. low background load, starting from the long-latency epoch (11% and 29% decrease from 50-100 ms and 150-200ms post-perturbation). Motor corrections were faster with less arm displacement for the higher vs. lower background load. However, such differences in limb kinematics were not observable until ~100 ms; thus, reductions in M1 responses with large background loads were not due to sensory feedback. We developed a model with both a spinal level feedback pathway (with gain scaling) and a

transcortical feedback pathway modeled as a recurrent neural network (RNN). The model was trained to perform a posture perturbation task where the goal was to keep the limb within a specified target location, while countering randomly applied loads to the limb. The model captured the reciprocal response patterns where the M1 responses decreased to counter the increase in the spinal response with higher background loads leading to a faster corrective response. This highlights how the higher-level transcortical feedback pathway must consider lower-level spinal feedback contributions to generate goal-directed motor corrections.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 301.22

Topic: E.04. Voluntary Movements

Support: UCSC

Title: Distributed computation in a Drosophila motor command microcircuit

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Abstract: When conflicting signals converge in a motor command pathway, downstream neurons must arbitrate between these conflicts to produce coordinated behavior. How this arbitration occurs is unknown. Recent work identified a network in the brain of the fruit fly, *Drosophila melanogaster*, that likely arbitrates between motor command signals to control behavior. This motor control network connects olfactory and navigation-related signaling pathways to a motor control neuron associated with turns (the DNa02 neuron, *Rayshubskiy* et al., 2020). We used a computational approach to determine how this putative motor command circuit might arbitrate between motor command signals. We first analyzed the network structure in the publicly available hemibrain dataset from Janelia. While computations to select between or modulate signals are typically thought to rely on distributed networks of neurons, our analysis suggested that DNa02 dendrites are well positioned to perform these computations within their arbors. Due to their small size, fly dendrites rapidly reach iso-potential regardless of synaptic input location. However, DNa02 dendrites have extensive arborizations, covering ~60% of the anterior-posterior brain axis. We therefore tested if DNa02 dendrites have electrically isolated compartments using a passive conductance model. We measured the voltage decay between input synapses at a site near the purported axon initial segment (AIS). If the dendrites function as

part of a single compartment, all sub-groups of synapses would display uniform voltage decay. Instead, we found that simulated EPSPs that originate from an upstream excitatory partner (PFL3) that directly connects to DNa02 far from the AIS decay by a factor of 19.0, significantly greater than the average decay (10.6) for EPSPs from an arbitrary input synapse (p value=0.0016, Mann-Whitney U). We also asked how indirect connections from the PFL3 neurons might alter the voltage in DNa02 dendrites. To determine if individual interneurons that receive input from PFL3 and output to DNa02 are excitatory or inhibitory, we utilized a machine learning classifier to determine their likely neurotransmitter (per synapse true-positive rate of 0.91 and 0.83, and a per-neuron true-positive rate of 1 and 0.9 for GABA and acetylcholine respectively). Both the putative excitatory and inhibitory indirect synapses are widely distributed across DNa02's dendritic arbors, suggesting that they may influence signals coming from other brain regions. Together, these data suggest that this motor control network in the fly may compute its motor output using dendritic computations and not just through network interactions.

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Poster

301. Premotor and Motor Cortex Dynamics During Movement Planning and Execution

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Institut TransMedTech
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Natural Sciences and Engineering Research Council of Canada

Title: Invasiveness-efficacy trade-off for motor cortex neuroprosthetics in healthy and spinal cord injured rodent and feline models.

Authors: *M. BONIZZATO, M. REGNIEZ, C. LANDRY, M. DUGUAY, A.-C. CHOUINARD, D. BERGERON, H. DELIVET-MONGRAIN, R. DRAINVILLE, M. MARTINEZ;
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Abstract: Cortical neurostimulation is a powerful tool for causal investigation of motor function. We recently proposed it as an intervention to alleviate locomotor deficits after spinal cord injury (SCI). In a rat model of incomplete SCI, intracortical microstimulation delivered in synchrony with foot lift increases leg flexion and alleviates dragging. Daily stimulation therapy accelerates and improves rehabilitation outcomes.
To facilitate clinical translation, we next investigated the trade-off between invasiveness of implantation and efficacy of neurostimulation. The intracortical stimulation approach is

clinically invasive, requires penetrating the cortical tissue, but is extremely effective in activating cortico-spinal circuitries. Conversely, surface (epidural) positioning of cortical leads is a more common practice in neurosurgery, but the effects of epidural cortical stimulation on motor control are unexplored.
We assessed the invasiveness-efficacy trade-off of cortical neurostimulation in two animal models, the rat and the cat. We compared intracortical and epidural stimulation with respect to their:
1) Immediate efficacy to modulate locomotor output. Gait analysis showed an inferior modulation of leg kinematics for epidural stimulation in rats and cats. In severe SCIs, only intracortical stimulation was effective in alleviating leg deficits. Movement selectivity was also much limited for epidural stimulation.
2) Effect on long-term recovery of locomotion. Supplementing motor training with each of the two cortical neuroprosthetic approaches improved recovery compared to training with no stimulation in the rat.
3) Biocompatibility. Histological analysis of brain tissue showed increased neuroinflammation and scar tissue formation for the intracortical probes. These effects were independent from the stimulation dosage. We achieved lesser tissue response by reducing intracortical wire diameter.
Notwithstanding better biocompatibility, epidural probes achieve largely inferior immediate modulation of cortico-motor transmission. These experiments can inform the design of translational cortical stimulation approaches towards human studies, to help effectively incorporate these techniques into the clinical toolbox for rehabilitation after SCI.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Topic: E.06. Posture and Gait

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5U01NS086607-05

Title: Muscle Stimulation as a Probe to Explore Spinal Heteronymous pathways in Stroke Survivors.

Authors: *C. J. CUADRA[1,2], S. L. WOLF[3,4], M. A. LYLE[1];
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Abstract: Coupled joint movements arising from muscles abnormally active at the same time is a common impairment after stroke. A possible explanation for this synergistic muscle activations is altered heteronymous spinal reflex circuits, which normally provide excitatory and inhibitory feedback between muscles. For example, femoral nerve stimulation (FNS) normally produces short latency excitatory followed by inhibitory feedback onto soleus in healthy adults. Recently, Dyer et al. found markedly increased excitation with minimal inhibition in persons after stroke which could contribute to excitatory coupling of knee and ankle extensors. However, the reason for the marked heteronymous excitation (i.e., larger excitation or reduced inhibition) cannot be determined using FNS, because nerve stimulation activates excitatory muscle spindle afferents and inhibitory pathways. Based on our recent work showing that quadriceps muscle stimulation can evoke heteronymous inhibition with minimal excitation onto SOL, this study aims to determine the fate of heteronymous inhibitory feedback after stroke by comparing the effects of femoral nerve and quadriceps muscle stimulation onto SOL ongoing EMG in stroke survivors and matched controls.We hypothesized: a greater magnitude and frequency of heteronymous excitation onto SOL from FN compared to Q stimulation, and larger excitation on the paretic compared to nonparetic limb and to healthy control limbs; and that heteronymous inhibition onto SOL will be greater with Q compared to FN stimulation and largest in healthy control limbs and nonparetic limbs compared to the paretic limbs. Participants sat wearing an ankle immobilizer boot. Heteronymous feedback was examined by stimulating FN and Q while participants held 20% SOL MVIC. FN and Q intensities (~1.5 vs ~2x motor threshold) were selected to match knee extension torque between stimulation conditions. Bilateral lower extremities for stroke participants and one lower extremity for the control subject were tested. Preliminary results indicate a trend of reduced inhibition in stroke survivors compared to controls. No differences in heteronymous inhibition were observed between limbs in stroke survivors (paretic vs non-paretic) or between FN and Q stimulation. Additionally, inhibition magnitude was larger in FN compared to Q stimulation only in controls. Contrary to prior findings, heteronymous excitation was not larger on the paretic limb compared to the nonparetic.A better understanding of heteronymous circuitry would help clarify their functional role during movement and their role after stroke.

Disclosures: C.J. Cuadra: None. **S.L. Wolf:** None. **M.A. Lyle:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.02

Topic: E.06. Posture and Gait

Title: Comparison and evaluation of MVC measurements from selected reference tasks

Authors: *K. GLOECKLER[1], N. MRACHACZ-KERSTING[2], A. K. THOMPSON[3], J. R. WOLPAW[4], U. KERSTING[1];
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Abstract: Operant conditioning of reflexes can influence motor function; thus, it is being applied to rehabilitation after CNS injury (e.g., spinal cord injury, stroke (Prog Brain Res 218: 157-72, 2015)). To analyze and compare EMG data of reflexes, a normalization method is needed. The standard method uses the maximum M-wave to normalize EMG data from electrically evoked muscle reflexes. In laboratory setups, this is a highly reliable method, but it is elaborate and cannot be used with some patients (e.g., those with heart diseases or cardiac pacemaker). In order to simplify the normalization method, this study evaluates the repeatability and relation between different maximum voluntary contraction tasks and the maximum M-wave. Methods: four reference tasks were defined for which maximum EMG was extracted from soleus muscle and compared to Mmax. Between-day and within-day repeatability were assessed. MVC measures were derived from two dynamic tasks (counter movement jumps (CMJ), plyometric drop jumps (DJ) from an elevation of 30 cm) and two isometric tasks in seated position by pressing the toes against a pedal (maximum voluntary contraction of the soleus were measured with an ankle joint angle of 0° and a knee angle of 0°. Three repetitions were completed as a standard 5-s isometric contraction (Hold). Additionally, three fast isometric contraction were performed without an extended holding (Fast). To find the Mmax, an input-output curve was generated during upright standing. One stimulation electrode (anode) was positioned above the knee, while the cathode was placed on the back of the knee at the popliteal fossa. Initially, the stimulation intensity was manually increased until the H-reflex disappeared and the M-wave reached its maximum. Then a stimulation protocol applied 20 X 3 stimulation intensities (randomized, 5-7 s inter-stimulus intervals) controlled by the Mr. Kick III Software, enabling a Boltzmann fit to the input-output data. Accordingly, the relationship between Mmax and MVC tasks was evaluated. Calculations on 22 preliminary measurements showed significant correlations between Mmax and CMJ ($r = 0.623$), Mmax and Fast ($r = 0.655$) as well as Mmax and Hold ($r = 0.606$). There was no significant correlation between Mmax and DJ ($r = 0.281$). Conclusion: Results indicate that combinations of MVC measures from several tasks may be able to replace the time consuming and, in some cases, non-applicable electrical stimulation protocol. Data from more subjects are needed to confirm this multifactorial prediction of Mmax amplitude.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.03

Topic: E.06. Posture and Gait

Support: NWO VENI talent program 17351

Title: Revealing reflex modulation during goal-directed movement using continuous perturbations

Authors: *M. VAN DE RUIT, W. MUGGE, F. C. VAN DER HELM, A. C. SCHOUTEN; Delft Univ. of Technol., Delft Univ. of Technol., Delft, Netherlands

Abstract: Reflex strength modulation plays a vital role during movement. The ability to rapidly modulate reflex strength determines the steadiness of our movements during our interaction with the environment when faced with unexpected changes. In this study we aimed to develop a new paradigm to assess reflex strength modulation during a goal-directed movement using continuous perturbations.
Participants were comfortably seated with their right hand holding the handle of a robotic wrist manipulator. Participants made rapid goal-directed wrist flexion movements in a constant force field (directed opposite to the movement) over 0.32 rad in 200-500 ms. During the main experiment, they received continuous pseudo-random binary sequence position perturbations (peak-to-peak amplitude: 0.06 rad, switching rate: 150 ms). In a validation experiment participants received transient ramp-and-hold perturbations (ramp: 40 ms at 2.0 rad/s) before and after the movement, and at three positions during the movement. Position of, and torque applied to, the handle together with electromyography (EMG) from the flexor and extensor carpi radialis muscles were recorded. Reflex strength was quantified using time-varying system identification (short data segment method) in the main experiment, while for the validation experiment the mean EMG amplitude during the short and long-latency reflex response window was used.
Using continuous perturbations, reflex strength was found to be significantly reduced during the movement when compared to before and after the movement. The validation using transient perturbations revealed a significant reduction in the short-latency reflex response during movement, whereas no significant change in the long-latency reflex response was found.
We successfully developed a paradigm to reveal reflex modulation during movement using continuous perturbations. The paradigm enables us to get information on reflex modulation with a higher temporal resolution and less data than when using transient perturbations. Using the developed paradigm we will be able to gain fundamental new insights in the pathways underlying (impairments in) reflex strength modulation during movement, and hence to truly understand movement disorders and improve sensorimotor rehabilitation.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.04

Topic: E.06. Posture and Gait

Support: Lockheed Martin

Title: H-reflexes stabilize more slowly than kinematics and muscle activity during locomotor adaptation on a split-belt treadmill

Authors: *O. REFY[1], O. MO[1], Y. SHI[1], B. BLANCHARD[1], A. MILLER-PETERSON[1], E. H. BEDOY[2], H. GEYER[1], D. WEBER[1];
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Abstract: Historically, the spinal cord has been thought of as a hard-wired system that encodes reflexes necessary for quick execution of locomotor tasks. Throughout the past few decades, however, there has been overwhelming evidence that challenges this simplistic view of spinal cord function. The spinal circuitry exhibits dynamic changes in reflex gains throughout the step-cycle during steady-state walking, and is also capable of adapting to environmental changes, such as terrain, and internal changes following neurological injury. Split-belt treadmill studies provide a useful paradigm to induce and study adaptations in locomotor control. However, the focus has been to infer neurological changes from biomechanical parameters. Explicit probing of nerve response changes during split-belt adaptation has not been reported before. In this work, we recruited 7 subjects to walk repeatedly on a split-belt treadmill during repetitions of a fixed speed change sequence. We used a motion-capture system to measure the subjects' leg kinematics, a force plate sensor to measure ground reactions forces and a set of EMG electrodes to measure the muscle activity of their gastrocnemius, tibialis, vastus and hamstring muscle groups. Prior to data collection, M-H recruitment curves were obtained during walking at the self-selected walking speed to find the stimulation intensity corresponding to Hmax. Stimulation pules (1ms duration) were delivered at mid-stance (around 50 +/- 5% of stance duration). We also used a control loop to measure H-reflex responses every 10 seconds at Hmax (normalized to Mmax) during mid-stance. H-reflexes of the ipsilateral and contralateral legs were measured in separate trials. We found that H-reflexes of ipsi- and contralateral legs follow an opposite trend during adaptation, where ipsilateral drops then recovers after a split-belt speed drop, while contralateral H-reflexes increase. All measured parameters were declared stabilized when their change over time settled at zero. We found that EMGs, leg joint angles and ground reaction forces typically stabilize within 10 steps, while H-reflexes can take up to 40-50 steps to stabilize. We argue that this is only possible by a significant reshuffling of motor control between supraspinal and spinal contributions following the speed transitions. The method presented in this work can be used to indirectly probe and quantify supraspinal contributions to locomotor adaptation in humans.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 302.05

Topic: E.06. Posture and Gait

Support: NIH

Title: Modulation of cutaneous reflexes during quadrupedal and hindlimb-only locomotion in intact adult cats

Authors: *J. HARNIE, S. MARI, S. YASSINE, P. JEHANNIN, J. AUDET, F. SOUCY, O. EDDAOUI, C. G. LECOMTE, R. ALARAB, C. IORIO-MORIN, A. FRIGON;
Univ. of Sherbrooke, Sherbrooke, QC, Canada

Abstract: Proper sensorimotor integration between cutaneous afferent feedback and central neural circuits is important for executing various motor tasks including locomotion. In quadrupeds, such as cats, the forelimbs play an important role in modulating the hindlimb pattern and evidence suggests a role of arm swing in modulating reflexes in the legs during bipedal walking in humans. However, few studies have investigated the impact of forelimb movements on sensorimotor interactions controlling the hindlimbs during locomotion. Here, we evaluated cutaneous reflexes in hindlimb muscles during quadrupedal (Intact4) and hindlimb-only (Intact2) locomotion with the forelimbs placed on a stationary platform in 3 intact cats. We implanted electrodes to chronically record muscle activity and to electrically stimulate the right or left superficial peroneal nerve (innervation of paw dorsum, n = 5) to evoke cutaneous reflexes. As we recently showed, cycle and stance durations were shorter in Intact2 compared to Intact4 (Harnie et al., 2022). We observed similar phase-dependent modulation of homonymous and crossed reflex responses in both locomotor conditions. Differences in homonymous responses included smaller amplitude short-latency (P1) and medium-latency (P2) excitatory responses in the semitendinosus and smaller P2 responses in the soleus in Intact2 compared to Intact4. We also observed a consistent short-latency inhibition (N1) in the homonymous sartorius throughout the cycle in Intact2 compared to Intact4. The incidence of crossed responses between hindlimbs decreased from 68% in Intact4 to 32% in Intact2. The present results suggest that forelimb movements strongly influence hindlimb cutaneous reflexes during locomotion.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.06

Topic: E.06. Posture and Gait

Title: Rate-dependent depression of hoffman reflex is not affected by anodal and cathodal gvs in healthy subjects

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Abstract: The vestibulospinal tract (VSt) participates in the control of posture through ipsilateral projections to the cervical and lumbar cord to facilitate the action of extensor muscles and inhibit flexor muscles. Galvanic Vestibular Stimulation (GVS) applied has been used to study the influence of the VSt on lumbar motor pools through the Hoffman reflex (H reflex). H reflex exhibited an increase in excitability when GVS is applied in condition-test intervals between 80-140 ms. The Rate-Dependent Depression of the H reflex (RDDH) occurs when a mechanical or electrical stimulus is applied at frequencies above 1 Hz, representing an inhibitory process in the spinal cord. The effect of GVS on the RDDH has not been tested. The aim of this study was to evaluate the effects of GVS on the RDDH in lower limbs during cathodal and anodal GVS in healthy subjects. The protocol was approved by the local IRB of the Faculty of Medicine of the Autonomous University of the State of Mexico. Subjects signed informed consent. Fourteen subjects (median 22, range 18-22 y/o, 8 M/6 F) participated in the study. The H reflex was evoked by monophasic (1 ms) squared pulses delivered at the fossa popliteal of the left limb and recorded (10 KHz) ipsilaterally in the gastrocnemius muscle. Ten consecutive pulses were applied at 0.1, 1, 5 and 10 Hz to evaluate the RDDH. GVS was applied by electrodes placed at the mastoid process. The intensity of the current was determined for each subject according to the movement sensation threshold (2.1 ± 0.6 mA). GVS -cathodal or anodal- direct current was delivered during the total duration of each stimulation frequency in a random sequence. Subjects were lying in a prone position during the protocol. The amplitudes of the 2^{nd} to 10^{th} pulses were averaged and normalized for each stimulation frequency and reported as percentages. RDDH was expressed as the ratio of the mean of the 2^{nd}-10^{th} pulses divided by the 1^{st}. Compared to the control condition, the normalized amplitude of the H reflex increased with the GVS cathode contralateral (CC), and with the cathode ipsilateral (CI) to the recording electrode (0.1 Hz CI = 31% CC= 41%, 1 Hz CI = 63% CC = 67%, 5 Hz CI = 45% CC = 63%, 10 Hz CI =85% CC=78%). The RDDH did not change significantly during GVS at any stimulation frequency (0.1 Hz CI = 9.4% CC=11.4 %, 1 Hz CI =19.8% CC = -0.8%, 5 Hz CI = 0.3% CC = 8.8%, 10 Hz CI = -1.2% CC = 2.4 %, One Way Repeated Measures ANOVA). In conclusion, GVS induces an increase in excitability of the H reflex but does not suppress the RDDH in the spinal cord. We suggest that GVS in combination with RDDH could be used for vestibulospinal excitability evaluation in pathological conditions.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Program #/Poster #: 302.07

Topic: E.06. Posture and Gait

Support: Deutsche Forschungsgemeinschaft (DFG) - 500615768
NSF 2015317

Title: Biomimetic strain sensors in fly-like robot legs

Authors: *G. F. DINGES, W. P. ZYHOWSKI, C. A. GOLDSMITH, N. S. SZCZECINSKI; Dept. of Mechanical & Aerospace Engin., West Virginia Univ., Morgantown, WV

Abstract: Insect locomotion achieves adaptability through limb sensory organs that monitor motor output. Their dynamic feedback onto central network components both modifies and reinforces limb movements. A group of sensory organs, called campaniform sensilla (CS), are found on the legs of *Drosophila melanogaster* and comprise 42 neurons, each associated with a cuticular cap embedded within the cuticle. Found throughout the surface of insect exoskeletons, they encode highly dynamic strains that occur in the cuticle. Scanning electron microscopy has shown interindividual differences in the relative position and arrangement of CS caps in the trochanteral and femoral fields. Although flies exhibit idiosyncrasies during walking, the overall strain that spreads throughout each leg during stepping should be similar. To investigate the relevance of sensor location for the motor network, we will implement strain sensors that mimic the different orientations previously described in animals in robotic legs. Preliminary experiments in legs based on those of stick insects, with two simplified groups of strain sensors, have shown a clear relationship between sensor location, strain sensing, and the step cycle. Testing sensor arrays with more realistic and detailed morphology will allow us to analyze how the interplay of sensor orientation and arrangement affects discrete measurements of cyclic strain. Expanding on these experiments, we will test sensor placement in a fully intact, six-legged walking robot. These experiments will illuminate which strains are present on the leg and can be measured during each step, in addition to highlighting which strains are detected by which CS. Further, we will analyze how the CS that show leg type-specific positions differ functionally. In the context of species-specific biomechanics, connecting CS morphology with behavior requires an interdisciplinary approach. This work will help elucidate the importance of sensor morphology in the context of limb movements and, thus, will aid in the understanding of how the nervous system integrates sensory information.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.08

Topic: E.06. Posture and Gait

Support: NSF BCS Grant 1748986
NIH GMS Grant P20GM103408

Title: Development and recovery of motor behavior in neonatal spinal-transected rats: trajectory of hindlimb reflex recovery

Authors: *A. C. MARTES[1], J. MEREDITH[1], A. L. BOZEMAN[1], T. L. ROTH[2], M. R. BRUMLEY[1];
[1]Psychology, Idaho State Univ., Pocatello, ID; [2]Psychology, Univ. of Delaware, Newark, DE

Abstract: Background: Spinal cord injury often leads to loss or impairment of sensorimotor function. However, neonatal animals tend to recover more function after such an injury compared to adults. Research has shown that rats can exhibit hindlimb reflexes following a neonatal spinal cord transection. The purpose of this study was to examine the trajectory of reflex development and the recovery of reflexes following a complete low-thoracic spinal cord transection. Methods: On postnatal day 1 (P1), female and male rats underwent a complete T8/T9 spinal cord transection or a sham surgery. On P7, P14, or P21, rats were tested for three reflexes: surface righting, hindlimb placing, and hindlimb crossed-extensor response. To test the righting reflex, subjects were placed in a supine position and their latency to turn to the prone position was recorded. For the placing response, a metal spatula was used to brush the top of the subjects' hind paws and the latency to withdraw that paw and place it on top of the spatula was recorded. For crossed-extensor testing, a hind paw was lightly squeezed with metal forceps and the latency to withdraw the pinched paw and extend the opposite paw was recorded. Ten subjects per group were tested. Results: There were no sex effects on any of the reflexes. Spinal-transected rats showed significantly longer surface righting latencies compared to shams. Additionally, P7s showed longer latencies compared to P21s. For the placing reflex, P21s showed shorter latencies than P7 and P14 subjects. There was no effect of surgery on placing latency. However, seven spinal-transected rats showed no response, while all shams showed a response. For the crossed-extensor reflex, there were no effects of age or surgery on latency. All subjects showed a crossed-extensor response. Conclusions: Hindlimb reflexes can recover over time following a spinal cord injury, though some reflexes (i.e. crossed-extensor) appear to not be as impacted by the loss of communication with the brain. Several spinal-transected rats showed no response to the light-touch stimulus used to elicit the placing reflex but did respond to the more intense stimulus used for the crossed-extensor reflex. This shows that the hindlimbs are capable of responding to sensory input, but the type and intensity of this input are important factors. This study indicates that the lumbar spinal cord can respond to environmental stimuli and exhibits neural plasticity independently from the brain.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Topic: E.06. Posture and Gait

Support: NSF BCS Grant 1748986
NIH GMS Grant P20GM103408

Title: Development and recovery of motor behavior in neonatal spinal-transected rats: changes in hindlimb weight-bearing locomotion

Authors: *L. D. HERRERA[1], K. WEEDN[1], A. L. BOZEMAN[1], A. C. MARTES[1], T. L. ROTH[2], M. R. BRUMLEY[1];
[1]Psychology, Idaho State Univ., Pocatello, ID; [2]Psychology, Univ. of Delaware, Newark, DE

Abstract: Introduction: When a spinal cord injury occurs, it disrupts bidirectional signals between the brain and the spinal cord. If the spinal injury occurs during early development, research has shown that there is an increased possibility for recovery of motor function due to greater plasticity in the developing spinal cord. The purpose of this study was to investigate the effect of a neonatal spinal cord transection on the development of spontaneous weight-bearing locomotion in developing rats. We hypothesized that hindlimb weight-bearing locomotion would be reduced in spinal-transected rats, but that partial recovery would occur by weaning age. Methods: On postnatal day 1 (P1), forty rats were placed into four groups (2 sexes x 2 surgery conditions x 10 subjects per group) and received either a low-thoracic spinal cord transection or sham surgery (control). On P21, spontaneous locomotion was recorded in an open-field chamber for 20 minutes. The duration of full-, partial-, and non-weight bearing hindlimb activity was scored and then analyzed with a series of two-way ANOVA tests. Results: Sham subjects showed significantly more full weight-bearing hindlimb activity compared to spinal-transected subjects, whereas spinal-transected subjects showed significantly more partial and non-weight bearing hindlimb activity compared to shams. Few spinal-transected rats showed full hindlimb weight-bearing activity, though it was for very brief durations. When all categories of hindlimb weight-bearing were combined together for a total duration of locomotion, there were no differences between spinal-transected and sham subjects. There were no differences in weight-bearing locomotion between males and females. Conclusions: The spinal cord can develop and support some weight-bearing locomotion following a neonatal spinal cord transection. Because the total duration of spontaneous locomotion was not different between the sexes or surgery groups, this suggests that the spinal cord supported comparable levels of activity in both the intact and neonatally-injured conditions. Overall, our results provide evidence that the immature spinal cord is capable of learning and supporting substantial hindlimb locomotion, even when isolated from brain inputs.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.10

Topic: E.06. Posture and Gait

Support: NSF REU REPS

Title: Identification of co-contraction muscle synergies involved in the movement of the rat tail using fine wire electromyography

Authors: *L. A. FRAYSER, M. D. PAVLICHENKO, L. E. HARRIS, M. W. CHRISTENSEN, C. L. CLELAND;
Biol., James Madison Univ., Harrisonburg, VA

Abstract: Accurate movement planning by the central nervous system (CNS) is computationally difficult, a problem exacerbated by non-linear muscle properties and muscular redundancies. To decrease computational load, the CNS must use strategies that simplify computations, including muscle synergies. Co-contraction muscle synergies produce simultaneous agonist and antagonist muscle activity around a joint. The rat tail is an ideal model system for studying co-contraction synergies as the tail is hyper-redundant and acted upon by over 300 intrinsic and extrinsic muscles and muscle fascicles. The specific aims of our study were to identify co-contraction synergies and their role in the control of the tail by utilizing electromyographic (EMG) recordings and assessing magnitude of activity in four extrinsic and four intrinsic muscles acting on the rat tail during isometric contraction arising from the nociceptive withdrawal response (NWR). In adult rats (n=22), eight bipolar fine-wire EMG electrodes were inserted percutaneously and bilaterally, four of which targeted the extrinsic muscles in the pelvis, and four of which targeted the dorsal lateral intrinsic muscles in the tail. Placement of electrode location in target muscles was verified by electrical stimulation and postmortem dissection. The NWR was evoked by lateral noxious heat (980 nm infrared laser diode) at five stimulus locations on both sides of the tail. We found that the magnitude of EMG in extrinsic muscles depended on stimulus location, with stimulation of the more distal location showing an approximate 90% decrease in magnitude compared to stimulation of the more proximal location. In contrast, the magnitude of EMG in the intrinsic muscles did not demonstrate dependence on stimulus location, suggesting that intrinsic muscles serve a different function in the movement patterns of the tail. Factor analysis suggested that the activity of EMG throughout the tail is dominated by co-contraction synergies that span extrinsic and intrinsic muscles. Based on our preliminary results, the EMG of agonist and antagonist muscle pairs suggests that movement and braking of the tail is created by both differences in timing and magnitude. The pronounced decrease in extrinsic EMG with distal progression of the stimulus correlates tightly and perhaps functionally with parallel progression of the local bend. Finally, the presence of co-contraction synergies throughout the intrinsic and extrinsic muscles of the tail may contribute to tail stiffening during the NWR.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.11

Topic: E.06. Posture and Gait

Support: NSF REU REPS

Title: Temporal organization of the rat tail nociceptive withdrawal response arises from sequenced muscle EMG activity

Authors: *M. D. PAVLICHENKO, L. A. FRAYSER, L. E. HARRIS, M. W. CHRISTENSEN, C. L. CLELAND;
Biol., James Madison Univ., Harrisonburg, VA

Abstract: The nociceptive withdrawal response (NWR) of the tail is an ideal model system for studying how the CNS simplifies the control of complex muscular systems. The NWR in the rat's tail can be organized temporally into four stages: a local bend matched to stimulus location followed by rotation of the entire tail around the tail base, distal progression of the tail bend and lastly stiffening of the tail. These four features arise from the coordinated contraction of over 300 extrinsic or intrinsic muscles or muscle fascicles. The specific aim of this study is to use fine wire EMG to determine how activity in extrinsic and intrinsic, agonist and antagonist muscle pairs, is sequenced to create the tail NWR. Rats (n=22) were anesthetized with isoflurane and 8 bipolar fine wire electrodes were inserted percutaneously; four were placed bilaterally in the extrinsic SDL muscles with one pair placed rostrally and the second caudally. Electrode placement was tentatively established by weak electrical stimulation to evoke tail bend. The remaining four electrodes were similarly placed in dorsolateral intrinsic tail muscles. To ensure an isometric response, the tail was restrained. Rats were constrained in a stoppered acrylic tube, and the electrodes were connected to 8 amplifiers. Tail NWR was elicited by focal noxious heat stimuli (980 nm laser) delivered to the lateral aspect of the tail at five rostral-caudal locations over a range of 1-3xT. EMG was amplified and filtered. Finally, the rat was euthanized, and a post-mortem was conducted to determine the physical location of the extrinsic electrodes. Our overall results showed sequential responses. Extrinsic agonist EMG occurred first, followed by extrinsic antagonist EMG (Δ12 ms), then both agonist and antagonist rostral intrinsic EMGs (Δ18 ms), and finally both agonist and antagonist caudal intrinsic EMGs (Δ10 ms). While antagonist extrinsic EMG lagged agonist EMG, there was no difference between agonist and antagonist intrinsic EMG pairs. The time course of extrinsic and intrinsic EMG also differed; extrinsic EMG, both agonist and antagonist, was largely pulsatile, while intrinsic EMG activity was persistent. Our results suggest that the temporal organization of tail the NWR arises from sequential tail muscle activity. The initial local bend could be driven by initial extrinsic agonist contraction, and its distal progression may arise from extrinsic rostral-caudal sequencing of muscle activity. Rotation and braking around the tail base could be explained by sequenced

activation of the extrinsic agonist and agonist muscles. Tail stiffening could arise from more persistent muscle activity over time seen in the intrinsic muscles.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.12

Topic: E.06. Posture and Gait

Title: Forelimb cutaneous inputs modulate the locomotor pattern of all four limbs during locomotion in intact cats

Authors: *P. JEHANNIN[1], A. MERLET[1], S. MARI[1], C. LECOMTE[1], J. AUDET[1], J. HARNIE[1], I. A. RYBAK[2], B. I. PRILUTSKY[3], A. FRIGON[1];
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Abstract: Coordinating the four limbs is critical for terrestrial quadrupedal mammalian locomotion. When the foot dorsum hits an obstacle during the swing phase, cutaneous inputs trigger a functional reflex response called the stumbling corrective reaction to rapidly move the leg away from and over the stimulus or obstacle. Although we know that cutaneous inputs from the forelimb in response to a perturbation are distributed to the four limbs during locomotion and phase-modulated, we do not know how they affect the interlimb pattern at different times during the step cycle. The purpose of this study was to characterize the locomotor pattern of all four limbs with cutaneous nerve stimulation of the forepaw at four different phases of the forelimb step cycle during quadrupedal treadmill locomotion. In three cats, we stimulated the superficial radial (SR) nerve with a relatively long train at four different phases (mid-stance, stance-to-swing transition, mid-swing, and swing-to-stance transition) of the forelimb step cycle at speeds of 0.3-0.5 m/s. Functional changes in the locomotor pattern were mainly observed during mid-swing and the stance-to-swing transition of the ipsilateral forelimb. Preliminary results suggest that stimulating forelimb cutaneous afferents at mid-swing and at the stance-to-swing transition prolongs ipsilateral forelimb swing (+18.8%, +19.0%, respectively) and contralateral forelimb stance (+9.2%, +4.0%, respectively). In the hindlimbs, we observed slightly longer homolateral and diagonal (+11.4%, +6.7%, respectively) swing durations with stimulation at the forelimb stance-to-swing transition. Additionally, we observed several changes in muscle activity. For example, with stimulation at mid-swing and at the stance-to-swing transition of the forelimb, we observed increased activity in the ipsilateral elbow flexor biceps brachii, (+27.4%, +20.3%, respectively). With stimulation at mid-swing, we observed in the diagonal hindlimb an increase of hip extensor activity (biceps femoris anterior, +3.8%) and in the homolateral hindlimb an increase of ankle extensor activity (soleus, +3.6%). Our results show that correcting or

preventing stumbling with stimulation of forelimb cutaneous afferents involves functional contributions from all four limbs.

Disclosures: **P. Jehannin:** None. **A. Merlet:** None. **S. Mari:** None. **C. Lecomte:** None. **J. Audet:** None. **J. Harnie:** None. **I.A. Rybak:** None. **B.I. Prilutsky:** None. **A. Frigon:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Topic: E.06. Posture and Gait

Support: NIH

Title: Modulation of interlimb cutaneous reflexes during tied-belt and split-belt locomotion in intact adult cats

Authors: ***S. MARI**[1], C. G. LECOMTE[1], A. N. MERLET[1], J. AUDET[1], J. HARNIE[1], I. A. RYBAK[2], B. I. PRILUTSKY[3], A. FRIGON[1];
[1]Univ. de Sherbrooke, Sherbrooke, QC, Canada; [2]Dept. of Neurobio. and Anat., Drexel Univ., Philadelphia, PA; [3]Biol. Sci., Georgia Inst. Technol., Atlanta, GA

Abstract: During locomotion, cutaneous reflexes play an essential role in rapidly responding to perturbations, for example to prevent a fall when the foot contacts an obstacle. In cats and humans, cutaneous reflexes are distributed to all four limbs, participating in a whole-body response, and are modulated by phase to generate functionally appropriate responses. To date, most studies have evaluated cutaneous reflexes during treadmill locomotion at a single speed. Here, we investigated cutaneous reflexes during quadrupedal locomotion in eight intact cats by electrically stimulating the superficial radial or superficial peroneal nerve and by recording fore- and hindlimb muscle activity during tied-belt (equal left-right speeds at 0.4 and 0.8 m/s) and split-belt (different left-right speeds of 0.4-0.8 and 0.8-0.4 m/s) locomotion. Results showed phase-dependent modulation of homonymous and interlimb (crossed, homolateral and diagonal) responses during tied- and split-belt locomotion. The probability of eliciting responses and the minimal response latencies were similar across locomotor conditions. The depth of reflex modulation, measured as the difference between the smallest and largest responses out of 10 phases, was significantly reduced in the two split-belt conditions compared to the fast tied-belt condition in all four types of responses. Higher temporal and spatial coordination indexes, measures of left-right symmetry and step-by-step variability, revealed that left-right coordination was more asymmetrical and variable. Higher coordination indexes correlated with a decrease in the depth of reflex modulation. These results suggest that signals related to left-right symmetry regulate interlimb cutaneous reflexes (i.e. between homologous limbs and between the fore- and hindlimbs). Our results provide a better understanding of the spinal pathways involved in interlimb coordination during locomotion and their modulation by cutaneous inputs, serving as a

basis to assess changes following incomplete spinal cord injury and determine if interlimb reflexes contribute to locomotor recovery.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Topic: E.06. Posture and Gait

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Stratton VA Medical Center
NYS Spinal Cord Injury Research Board

Title: Prediction of H-reflex size using multiple linear regression in operant conditioning studies

Authors: *J. MCLINDEN[1], D. E. GEMOETS[2], J. A. BRANGACCIO[2], D. HAHN[2], J. CARP[2,3], Y. SHAHRIARI[1], J. R. WOLPAW[2,3], J. NORTON[2,4];
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Abstract: Recent developments in our understanding of the CNS are leading to a new class of clinical methods for movement rehabilitation following neurological disease or injury, such as Hoffmann (H)-reflex operant conditioning, based on targeted neuroplasticity. During H-reflex conditioning, participants are asked to either increase or decrease the size of their H-reflex by completing training over the course of several months. During training, experimenters manually control multiple variables (e.g., M-wave size, background muscle activity, etc.) to ensure consistent training conditions throughout. We use multiple linear regression (MLR) to predict H-reflex size during training to better understand the strategies participants use to change the size of their reflexes and to increase the speed and efficacy of the H-reflex training protocol. We recorded electromyographic (EMG) data from four healthy participants (three men) while H-reflexes were elicited from their right soleus muscle. Participants completed five runs (each 50 trials) of data collection. During each run, subjects/experimenters manipulated one of the measured variables; M-wave size, antagonist EMG, background EMG, or stimulus current in 10-trial steps. A run was also collected without any variable manipulation. For each participant, we trained a MLR model to predict H-reflex size based on the measured variables, and compared the results to those obtained using an intercept-only model. Five fold cross validation was applied to prevent overfitting. The MLR model reduced root mean squared error by 31.2% over the intercept-only model (0.11±0.03 vs 0.16±0.05, respectively). There was a similar reduction of

30.8% between the MLR and intercept-only model in mean absolute deviation (0.09±0.03 vs 0.13±0.04). This effect was consistent across all four participants. We have demonstrated that MLR models can predict the H-reflex. Developing models capable of predicting H-reflex size can also deepen our understanding of the mechanisms and strategies employed by participants during training, and potentially lead to new methods for improving the speed and efficacy of H-reflex operant conditioning for the rehabilitation of movement following neurological disease or injury.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Title: The Heksor and Negotiated Equilibrium concepts can explain otherwise inexplicable experimental results

Authors: *J. WOLPAW;
Natl. Ctr. for Adaptive Neurotechnologies, Albany Stratton VA Med. Ctr. and State Univ. of New York, Albany, NY

Abstract: Recognition that the CNS is plastic through life leads to a new paradigm for understanding acquisition and maintenance of adaptive behaviors. The paradigm's core is a CNS entity now called a *heksor*, from the Greek *hexis*. A heksor is a distributed network of neurons and synapses that changes itself as needed to maintain the key features of a behavior. Through their concurrent changes, the many heksors that share the CNS negotiate the properties of the neurons and synapses they all use. They keep the CNS in a negotiated equilibrium (J Physiol 596:3469-91, 2018) that enables each heksor to maintain its behavior. This new paradigm explains otherwise inexplicable results. When operant conditioning increases or decreases soleus (SOL) H-reflex (HR) in one leg, the other side of the spinal cord also changes (J Neurophys 61:563-72, 1989). This is explicable as the action of heksors underlying bilateral behaviors (e.g., locomotion). When the spinal cord is transected in a cat that has regained locomotion after nerve

transection, treadmill training fails (J Neurophys 77:1979-93, 1997; Neurosci 158:1675-90, 2009). This reflects the changes in brain and spinal components of the locomotion heksor that enabled recovery after nerve transection. When operant conditioning increases or decreases SOL HR, quadriceps HR usually changes in the other direction (J Neurosci 31:11370-5, 2011). This is explicable as part of the response of the locomotion heksor that keeps the altered ankle angle during locomotion due to SOL HR change from tilting the rat to left or right (SOL HR up- or down-conditioning, respectively); it helps maintain key features of locomotion. In a rat with locomotor asymmetry due to incomplete spinal cord injury (iSCI), SOL HR up-conditioning restores symmetry, but down-conditioning does not increase asymmetry, even though the SOL HR decreases (J Neurophys 111:1249-58, 2014). The damaged locomotion heksor and new HR heksor negotiate an equilibrium acceptable to both. When SOL HR is down-conditioned in people without iSCI, brain and spinal components of the HR decrease are equal; when it is down-conditioned in people with iSCI, the spinal component is much larger (J Neurosci 33:2365-75, 2013). In people without iSCI, spinal plasticity disturbs existing heksors (negotiation is adversarial); in people with iSCI, it benefits them (negotiation is synergistic). These five examples show the explanatory power of the new paradigm based on heksors and the negotiated equilibrium they create. The paradigm also offers new answers to extant questions (e.g., generation and function of spontaneous neuronal activity; etiology of muscle synergies; control of homeostatic plasticity).

Disclosures: J. Wolpaw: None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Stratton VA Medical Center;
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The Doscher Neurorehabilitation Research Program (Thompson),

Title: Soleus H-reflex measurements in paretic and non-paretic legs during standing in people with and without hemiparesis due to stroke: with implications for post-stroke motor control of standing

Authors: *J. BRANGACCIO[1], A. M. PHIPPS[2], J. R. WOLPAW[3], A. K. THOMPSON[2];
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Carolina, Charleston, SC; [3]Natl. Ctr. For Adaptive Neurotechnologies, Stratton VA Med. Ctr.,, Albany, NY

Abstract: While spasticity is commonly observed in people after stroke, its role in impaired motor control is not well defined. Neurophysiological studies often focus on examining the paretic side; however, unilateral studies alone do not sufficiently inform us of true post-stroke leg motor control as both legs are inevitably involved in essential daily activities such standing and walking. Thus, in this study, we examined the soleus H-reflex in paretic and non-paretic legs of individuals with spastic hemiparesis due to stroke during standing while monitoring the soleus and its antagonist tibialis anterior (TA) EMG activity bilaterally.

In 11 individuals with spastic hemiparesis due to chronic stroke (63±10 yrs in age) and 13 individuals without stroke (51±14 yrs in age), the tibial nerve was stimulated while the participant maintained a natural standing level of soleus EMG activity (typically 20-25 μV) in the stimulated leg for at least 2 sec. Tibial nerve stimulus intensity was increased from just below H-reflex threshold to an intensity just above that needed to elicit the maximum M-wave (Mmax). Two-to-four responses were obtained at each intensity. Soleus and TA EMG activity was recorded bilaterally.

In people after stroke, peak-to-peak maximum H-reflex (Hmax) in the paretic leg (3.1±1.8mV, 52±21% Mmax) was significantly larger ($p=.04$ by paired t-test) than that in non-paretic leg (2.2±1.0mV, 37±19% Mmax). When the participant was actively maintaining the soleus activity of the paretic leg during its H-reflex measurement, the contralateral non-paretic TA was more active than the paretic TA ($p=.007$); when the participant was maintaining the soleus activity of the non-paretic leg during its H-reflex measurement, the contralateral paretic TA that was quiet during the paretic leg testing was significantly more active (6±4 μV when maintaining the paretic soleus activity vs. 16±11 μV when maintaining the non-paretic soleus activity, $p<0.05$ by paired t-test). Among individuals without stroke, the side-to-side difference in H-reflex size was present (12±11% Mmax); but TA activity was minimal (i.e., < 8μV) in both legs and in both testing conditions.

These results suggest that hemiparetic stroke not only increases the soleus H-reflex excitability on the paretic side but also alters descending drive to the TA from unilateral side-specific drive to less side-specific more bilateral drive during standing. The fact that the paretic TA (resulting in foot drop) can be activated more robustly when maintaining the non-paretic soleus activity in standing may support a possibility of training the non-paretic leg to improve the paretic leg function in post-stroke.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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The Doscher Neurorehabilitation Research Program

Title: An automated algorithm for precisely determining the functional onset and duration of soleus M-wave and H-reflex in humans

Authors: *N. J. HILL[1,2], M. L. MCKINNON[4], I. P. CLEMENTS[4], J. R. WOLPAW[1,2,3], A. K. THOMPSON[5];
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Abstract: The Hoffmann or H- reflex is a valuable tool for examining the excitability of spinal reflex pathways and the plasticity that affects it. In humans and animals, an operant conditioning protocol can gradually increase or decrease H-reflex size; this changes the spinal cord (review: Front Integ Neurosci 8:25, 2014). Appropriate H-reflex conditioning can enhance recovery of locomotion in people with spinal cord injury and probably in other chronic neuromuscular disorders as well (review: Neuroscientist 21:203-215, 2015). Thus, it offers a powerful new noninvasive therapy that could be readily adapted for use in rehabilitation centers, and eventually in the home. The H-reflex conditioning protocol requires initial characterization of a patient's H/M recruitment curve, i.e., the relationship between stimulation intensity and the size of both the H-reflex itself and also the earlier muscle response (M-wave) produced by direct stimulation of the efferent axons. Precise determination of the analysis windows of the H-reflex and M-wave is essential for both the accurate assessment of the reflex pathway's input-output property and the efficacy of a therapy (e.g., reflex operant conditioning) that relies on precise measurement of these response sizes. At present, the onset and duration of the H-reflex and M-wave are determined by visual inspection by individual investigators. This makes the protocol vulnerable to inter-personnel variability and human error. Here we describe a wavelet-based approach for automatic detection and reliable determination of the analysis window and amplitude of the soleus H-reflex and M-wave. We tested the algorithm's ability to make accurate determinations of H-reflex size, given only a single initial recruitment-curve run as training data, in each of 20 subjects. These predictions were evaluated using the resulting bounds to compute H-reflex sizes in a set of repeated measurements at H_{max} (1350 trials per subject across 6 sessions). The algorithm showed good agreement with estimates made by an experienced investigator via visual inspection of each subject's complete data-set (95% LOA: -5.7% to +5.4% H_{max}). With further development and validation, the algorithm should permit objective, repeatable determination of the parameters of soleus H-reflex and M-wave analysis and hence enable fully automated analysis of the corresponding recruitment curves. The algorithm is expected to become an essential part of neuromodulation and neurorehabilitation research, as it can reduce the impact of inter-personnel variability and enhance the validation of newly developed neuromodulation approaches for inducing beneficial neuroplasticity.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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NYS SCIRB C33279GG
Stratton VA Medical Center

Title: Automation of a noninvasive, multielectrode array-based platform for reflex operant conditioning therapy

Authors: *M. MCKINNON[1], J. HILL[2,3], D. HOCHMAN[1], J. R. WOLPAW[2,3,4], A. K. THOMPSON[5], I. CLEMENTS[1];
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Abstract: Spinal cord injury can produce spasticity and impair locomotion; operant conditioning of the soleus H-reflex can reduce spasticity and improve locomotion. At present, reflex conditioning is highly specialized and technically challenging, and inter- and intra-subject variability makes automation of this therapy difficult, hindering widespread dissemination of this powerful therapy. Here we explore how array-based algorithms can reduce sources of extrinsic variability and thereby improve the robustness of automation. These algorithms are a key part of a larger effort to develop a clinically practical system for semi-automated H-reflex operant conditioning by leveraging the capabilities of custom hardware, software, and transcutaneous multielectrode stimulation and recording arrays.
We obtained soleus H-reflex and M-wave data from human subjects of either sex. High density surface EMG (HDsEMG) signals were acquired with a custom 32-channel recording device that

provides high quality, low-noise electrophysiological data. Flexible, adhesive arrays of hydrogel electrodes were used to selectively stimulate the tibial nerve and record HDsEMG of the resulting M- and H-waves in the soleus. Custom software was used for data acquisition. Multielectrode recording arrays cover a larger area of the soleus with higher spatial resolution compared to standard bipolar electrodes, providing a more comprehensive picture of soleus recruitment. An optimization algorithm uses these array-based recordings to automatically account for electrode placement variability to target the soleus motor point on a given person on subsequent days, reducing the burden on clinicians to precisely target the motor point. A wavelet-based approach to automatically detect the H-reflex onset and reliably determine its amplitude was developed and tested on clinical datasets of patients undergoing operant conditioning therapy. This approach was validated on novel datasets and showed similar performance to expertly curated data. A multielectrode array designed for stimulation allows selective targeting of the tibial nerve with stimulation parameters shown to minimize discomfort. Algorithms for automatically selecting the parameters for optimal stimulation are in development.
The combination of multichannel stimulation and multielectrode recording in a single platform will enable clinicians who wish to use operant conditioning therapy to treat patients with SCI. Furthermore, this platform may prove useful for additional therapeutic applications beyond the soleus H-reflex conditioning, and in other patient populations suffering from spasticity.

Disclosures: **M. McKinnon:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **J. Hill:** None. **D. Hochman:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **J.R. Wolpaw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Three patents related to operant conditioning of spinal reflexes (US patent number 8862236, 9138579, and 9545515). **A.K. Thompson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Three patents related to operant conditioning of spinal reflexes (US patent number 8862236, 9138579, and 9545515). **I. Clements:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Stratton VA Medical Center

Title: Development of an Isometric Visuomotor Task for Evaluating Myoelectric Adaptation after Operant Conditioning

Authors: *R. HARDESTY, J. BRANGACCIO, J. R. WOLPAW;
Natl. Ctr. for Adaptive Neurotechnologies / Albany Stratton VA Med. Ctr., Albany, NY

Abstract: Animal and human studies show that spinal reflexes can be operantly conditioned in both healthy and neurologically impaired populations. Moreover, in patients with spinal cord injury (SCI), down-conditioning of the H-reflex has been shown to improve locomotor speed and symmetry; yet similar conditioning in individuals without motor deficits does not alter movement kinematics despite changes in the reflex magnitude. These seemingly contradictory observations prompt the question: How are motor behaviors preserved in the presence of change in the underlying neural substrates? One plausible explanation is that the key features of a behavior are maintained by appropriate changes in the responsible muscle activity. This study sought to develop a 1-D visuomotor task that can examine myoelectric adaptation after operant conditioning paradigms. It asks participants to control the horizontal position of a cursor by isometrically contracting the soleus muscle. The target cursor location is a virtual path that scrolls vertically down the screen at a constant rate. Participants receive continuous visual feedback of cursor location in relation to the path. Activity of four leg muscles (soleus, gastrocnemius, tibialis anterior, rectis femoris) are sampled electromyography (EMG) (1000 Hz). Signals are amplified and filtered (10Hz-1kHz). cursor location was controlled using a moving window average (1000ms) of the soleus EMG. To assess performance, an RMSE error was calculated from the differences between cursor location and the middle of the path for each trial. Preliminary results show participants are capable of acquiring a high degree of cursor control; they demonstrated stereotypical fast and slow learning phases. Performance sharply increased within the first 30-45 sec of practice and increased more slowly thereafter. Future work will assess associated changes in: 1) concurrent activity in muscles not controlling the cursor (agonists (gastrocnemius), antagonists (tibialis anterior), others (rectis femoris)); 2) H-reflexes and other spinal, corticospinal, and spinocortical evoked responses. This very simple behavioral task provides a model for exploring skill acquisition and maintenance in people with or without CNS injury or disease, and may guide design of novel therapies that can enhance functional recovery.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

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Stratton VA Medical Center

Title: The effect of tDCS on operantly conditioning the soleus H-reflex in people

Authors: *L. M. MCCANE[1,2], J. R. WOLPAW[1,3], A. K. THOMPSON[4,1];
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Abstract: Transcranial direct current stimulation (tDCS) can modulate cortical activity and enhance motor skill acquisition and retention (Brain Cogn 102: 1-12). The responsible mechanisms are unclear. Remarkably, little is known about tDCS's impact on the spinal cord. Spinal reflexes participate in our everyday movements, change throughout life, and can be modified by experience and injury (J Physiol 596(16): 3469-3491). People can change the size of a spinal reflex through operant conditioning (J Neurosci 29(18): 5784-5792). Acquisition of this simple motor skill (a larger or smaller spinal reflex) requires supraspinal drive (J Neurophysiol 87: 645-652). Here, tDCS's impact on spinal cord plasticity is examined using H-reflex down-conditioning. We hypothesize that tDCS will enhance inhibitory cortical descending influence on the H-reflex spinal pathway. The study was approved by the MUSC IRB. 14 neurotypical participants (9 W, 7 Active tDCS, Age 22-36) received either Sham or Active tDCS over contralateral leg motor cortex. Positive current entered the brain over leg M1 and left over the opposite forehead. Active (2mA, 0.06mA/cm^2, 30 min) or Sham tDCS (30s ramps) was applied during 4 baseline (BL) and 12 soleus H-reflex down-conditioning sessions (CS) (3/wk). H-reflexes were elicited by tibial nerve stimulation at the popliteal fossa while participants maintained soleus background activity in a defined range during comfortable standing. EMG recording electrodes were placed longitudinally below the gastrocnemii. An H/M recruitment curve and a reflex block (1x10) were collected before tDCS. After tDCS onset, 4 blocks were collected (1x20 & 3x75). During BLs, H-reflexes were simply recorded. During the 12 subsequent CSs, participants practiced reducing the reflex size during the 3 x 75 blocks. Visual feedback of H-reflex size occurred after each conditioning trial. H-reflex change was calculated using the mean reflex sizes of the last 4 CSs as a percentage of the 4 BLs. Conditioned H-reflex size decreased -18.4% (range -40.3, 2.6%) in Active and -0.9% (range -27.5, 30.9%) in Sham participants. The conditioned H-reflex was the sum of within-session (i.e., task-dependent adaptation (TDA)) and across-session (i.e., long-term change (LTC)). In both groups, TDA began after 5 CS sessions and persisted; it averaged -8.5% in Active and -11.2% Sham. The final LTC was -11% in Active and 10% for Sham. This study demonstrated tDCS improved H-reflex down-conditioning in heathy controls over 12 CSs. Further research is needed to determine if tDCS can enhance beneficial plasticity in spinal cord pathways in people with spinal cord injury, stroke, or other chronic disorder.

Disclosures: L.M. McCane: None. **J.R. Wolpaw:** None. **A.K. Thompson:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.21

Topic: E.06. Posture and Gait

Support: NIH NINDS Grant R01NS114279
The Doscher Neurorehabilitation Research Program
NIH NIBIB Grant P41EB018783

Title: Effects of speed and added weight on human locomotion: temporal parameters, joint motion, and muscle activity during walking

Authors: *B. A. P. DAMEWOOD, A. K. THOMPSON;
Med. Univ. of South Carolina, Charleston, SC

Abstract: INTRODUCTION: In humans, locomotion includes different motions (modes) that can occur in different conditions. Within a mode of locomotion (e.g., walking), a typical human adult can adjust to different speeds and different weight-bearing conditions (e.g., carrying a heavy backpack); however how people adapt to different walking conditions is not well understood. Here, to further our understanding of the neural control of locomotion, we investigated the effects of added weight and different speeds on step cycle duration, muscle activity, and joint kinematics during walking. Two walking speeds and three weight-bearing conditions were examined. METHODS: To ensure the robust impact of fixed weight and speed conditions, participants were young women of similar physical size (n=11, 27±6 yr) with no history of neurologic or orthopedic injury. Each walked on the treadmill at 1.0 and 1.5 m/s with or without an added 20.4 kg, worn as a weighted belt and vest set either on the front or back of the body. During the six ≈10-minute walking bouts (2 speeds (1.0 and 1.5 m/s) x 3 weight conditions (none: 0W, front: FW, and back: BW), ankle, knee, and hip joint motion; EMG (soleus, lateral gastrocnemius, tibialis anterior, biceps femoris [BF], rectus femoris, vastus lateralis, vastus medialis, gluteus maximus [GM]); and force signal (for cycle detection) were measured.RESULTS: A 2x3 ANOVA revealed: Cycle duration: At 1.5 m/s, the step cycle duration and stance phase were shorter, and the swing phase was longer than at 1.0 m/s ($p<.001$). Step cycle duration with FW was shorter than that with 0W ($p=.006$). Stance phase was longer and swing phase was shorter with BW and FW than with 0W ($p<.01$ for both). Joint motion: At the ankle, compared to 1.0m/s, 2-4° less dorsiflexion and 5-8° more plantarflexion was observed at 1.5 m/s. Only minor effects of speed and weight (all within 1-4°) were observed at the knee. At the hip, added weight caused more flexion across the entire step cycle (4-5°) and greater peak flexion (7-8°) and extension (4-5°). EMG: Peak EMG amplitudes were 56-132% larger at 1.5 than 1.0 m/s across all muscles but GM. Added weight also increased EMG in all muscles (5-78%), with various positional (i.e., BW vs. FW) effects. Notably, the increase in EMG to added weight was larger at 1.0 than 1.5m/s (except soleus and BF). DISCUSSION: Walking speed affected ankle joint motion, increased EMG amplitude, and shortened the step cycle and stance durations. Added weight impacted hip joint motion, lengthened stance duration, and affected EMG activity at 1.0 m/s. These findings underscore the importance of ankle joint motion in generation of locomotion, especially its role in producing faster walking.

Disclosures: B.A.P. Damewood: None. **A.K. Thompson:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.22

Topic: E.06. Posture and Gait

Support: NINDS NS114279 (Thompson)
Neilsen Foundation Allied Health Professional Research Award of ASIA A22-0037-001 (Dellenbach)
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the Doscher Neurorehabilitation Research Program (Thompson)
NIH/NIBIB P41 EB018783 (Wolpaw).

Title: Effects of MEP Up-Conditioning on Silent Period in the Upper Extremity of People with CNS Injury

Authors: *R. K. COTE, B. S. DELLENBACH, A. M. PHIPPS, A. K. THOMPSON;
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Abstract: After CNS injury, corticospinal excitability diminishes, resulting in weak voluntary activation of muscles below the injury and impaired motor control. However, such deficits are reversible at least partially, and an intervention that increases corticospinal excitability may enhance motor function recovery. Recent studies showed that operant conditioning of the motor evoked potential (MEP) can increase corticospinal excitability for the ankle dorsiflexor and improve locomotion in individuals with incomplete spinal cord injury (SCI) (J Neurophysiol 2018:120:2745-60; 2019:121:853-66). Thus, MEP up-conditioning to increase corticospinal excitability may improve the activation of the targeted muscle and enhance upper extremity (UE) motor function recovery. As the first step in investigating the mechanisms of corticospinal plasticity associated with UE MEP up-conditioning, we are currently examining the silent period (SP) after MEP, which reflects cortical inhibition at least partly, in people with CNS injury.Adults with chronic stable incomplete SCI or stroke are exposed to an MEP up-conditioning or control protocol that consists of 6 baseline and 24 up-conditioning or control sessions over 10 wk. In all sessions, 225 MEPs are elicited at ~10% above active threshold in the Extensor Carpi Radialis (ECR) or Extensor Digitorum (ED) while the participant maintains ~30% maximum voluntary contraction level of ECR or ED background EMG. During baseline sessions, MEPs are simply measured. During conditioning trials of the conditioning sessions, the participant is encouraged to increase MEP size and is given immediate feedback as to whether MEP was larger than a criterion. Along with MEP sizes, the SP is measured as the period from the end of the MEP (i.e., absolute EMG amplitude fell below the prestimulus level) to the recovery of EMG activity to the prestimulus level).To date, 3 individuals with chronic cervical SCI and 1 person after a subcortical ischemic stroke have been studied with the MEP up-

conditioning protocol and 1 person with cervical SCI has been studied with the MEP control protocol (6 baseline + 24 control [=baseline] sessions). In the up-conditioning participants, SP duration decreased over the course of 24 conditioning sessions by 48±16%, whereas in the control protocol participant SP didn't change (95% of the baseline over the final 6 control sessions). Observation in these initial participants suggest that MEP up-conditioning decreases SP duration in UE muscles of individuals with chronic CNS injury. To understand potential link between SP changes and UE function improvements, further studies and analyses are currently underway.

Disclosures: R.K. Cote: None. **B.S. Dellenbach:** None. **A.M. Phipps:** None. **A.K. Thompson:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.23

Topic: E.06. Posture and Gait

Support: NM4R Pilot Project Program, National Institutes of Health National Center of Neuromodulation for Rehabilitation, NIH/NICHD Grant Number P2CHD086844

Title: Short-latency spinal reciprocal inhibition in individuals with post-stroke hemiparesis

Authors: *J. LIANG[1], A. K. THOMPSON[2];
[1]Univ. of Nevada, Las Vegas, Univ. of Nevada, Las Vegas, Las Vegas, NV; [2]Med. Univ. of South Carolina, Med. Univ. of South Carolina, Awendaw, SC

Abstract: The short-latency reciprocal inhibitory pathway has been well examined in the non-impaired nervous system. Electrical stimulation of a mixed muscle nerve induces reciprocal inhibition (RI) of ongoing electromyography (EMG) activity in the antagonist muscle. The magnitude of inhibition increases with increase in intensity of stimulation. Individuals with post-stroke hemiparesis exhibit the inability to adequately activate paretic tibialis anterior (TA) muscle, which is often accompanied by inappropriate antagonist plantarflexor activity that would hinder toe clearance during the swing phase of gait. Thus, the problem of footdrop is attributable to reduced spinal RI between the ankle dorsiflexor and plantarflexor. However, this short-latency spinal RI has not been examined systematically in individuals post-stroke. The purpose of this study was to characterize the spinal RI in paretic and non-paretic legs of the stroke-impaired nervous system.Seven individuals chronically post-stroke and 5 age-similar non-impaired individuals participated in the study. EMG was recorded from TA and soleus (SOL) muscles in each leg. During standing, the common peroneal nerve (CPN) was electrically stimulated from below threshold to the maximal M-wave (M_{max}) level to obtain a TA recruitment curve. Then, 25 pulses were delivered to the CPN at intensities that produced 25%, 50% and 75% of the TA M_{max}. SOL EMG was rectified and RI was measured over a 7ms period (typically 40-50 ms post

stimulus) including the peak suppression. The magnitude of RI was calculated as the difference in EMG amplitude between the 7ms inhibition period and the 50ms prestimulus period, and expressed as % prestimulus EMG. Negative values denote inhibition and positive indicate facilitation. In both legs of controls, magnitude of RI increased linearly with increasing intensity of CPN stimulation (Left = -36.1%, -46.1%, -56.2%; Right= -35.4%, -47.5%, -51.8%, at 25%, 50%, 75% TA M_{max} respectively). In both the paretic and non-paretic legs, on average, magnitude of RI was smaller than controls, and magnitude of RI did not consistently increase linearly with increasing intensity of CPN stimulation, and had greater variability (Paretic = -11.8%, -12.6%, -14.9%; Non-paretic = -25%, -31.6%, -36.1%). We observed facilitation in the SOL EMG under all intensities of CPN stimulation in one paretic leg. Altered RI potentially underlie the leg motor control impairment after stroke, such as foot drop and development of compensatory strategies. Characterizing the stroke-impaired RI allows future development of targeted strategies to restore this neural function, thereby improving post-stroke gait.

Disclosures: J. Liang: None. **A.K. Thompson:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 302.24

Topic: E.06. Posture and Gait

Support: NIH NICHD P2C HD086844-01 (Kautz)
NINDS NS114279
The Doscher Neurorehabilitation Research Program

Title: Effects of latent trigger point dry needling on spinal reflexes

Authors: *G. SEIF[1], A. M. PHIPPS[2], B. DELLENBACH[2], J. DONNELLY[3], C. FERNANDEZ-DE-LAS-PENAS[4], A. K. THOMPSON[2];
[1]Dept. of Rehabil. Sci., [2]Dept. of Hlth. Sci. and Res., Med. Univ. of South Carolina, Charleston, SC; [3]Col. of Rehabilitative Sci., Univ. of St. Augustine for Hlth. Sci., Miami, FL; [4]Dept. of Physical Therapy, Occup. Therapy, Rehabil. and Physical Med., Univ. Rey Juan Carlos, Alcorcón, Madrid, Spain

Abstract: Background: Trigger point (TrP) deep dry needling (DDN) is becoming a common method to treat active and latent TrPs. Latent TrPs are defined as a hyperirritable taut band in a muscle that is associated with tenderness with palpation and can elicit a local twitch response. Latent TrPs can alter functioning of the muscle, agonists, and antagonists. Presence of latent TrPs can lead to muscle cramping, restricted range of motion (ROM), muscle weakness, altered co-contraction of antagonists, and accelerated fatigability. As the therapeutic use of DDN grows, mechanistic understanding of DDN has been increasing, albeit slowly and incompletely. The aim of this study was to investigate the effects latent TrP DDN in the medial gastrocnemius (MG) on

the triceps surae reflexes.
Methods: Seventeen adults 22-57 years old (median 27) with no known neurological conditions with latent TrPs in the MG participated in this study. Before and 0, 90 minutes, and 72 hours after DDN of the MG at TrP the maximum H-reflex (H_{max}), maximum M-wave (M_{max}), and reciprocal inhibition (RI) to antagonist muscle nerve stimulation was measured in the soleus, MG and lateral gastrocnemius (LG). Passive ankle range of motion (ROM) was also measured before and after DDN.
Results: The MG M_{max} was significantly decreased at 0- and 90-minutes post DDN (14 and 18%, respectively) and returned to pre DDN level at 72 hours post. LG and soleus M_{max} did not change. RI of soleus was increased significantly at 0 minutes (15% background EMG, bEMG) and 72 hours (19% bEMG) post DDN. Ankle dorsiflexion ROM was increased significantly by ≈4 deg at 0 minutes and 72 hours post DDN.
Conclusion: In the present study, MG M_{max} was decreased immediately after DDN but not in the soleus or LG, indicating that mechanical effects of DDN was specific to the treated muscle, MG. The increases in soleus RI at 0 minutes and 72 hours post DDN suggest immediate and longer lasting neurophysiological effects of DDN. The changes in RI may also partially explain the changes in ROM noted at 0 minutes and 72 hours post DDN. The present findings indicate a potential window of elevated neural plasticity induced by DDN, which may be harnessed in therapy administration to maximize the impact of motor practice/training or certain therapeutic interventions.

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Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.25

Topic: E.06. Posture and Gait

Support: NIH NICHD P2C HD086844 (Kautz)
NIBIB P41EB018783 (Wolpaw)
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NINDS U44 NS114420 (Clements/Thompson/Wolpaw)
The Doscher Neurorehabilitation Research Program (Thompson)
South Carolina Spinal Cord Injury Research Fund SCIRF #2021 PD-01

Title: Altered short-latency crossed spinal inhibition during standing in people with chronic incomplete spinal cord injury

Authors: *A. K. THOMPSON, M. MELVIN, A. M. PHIPPS;
Dept. of Hlth. Sci. and Res., Med. Univ. of South Carolina, Charleston, SC

Abstract: Introduction: Stimulation of the tibial nerve in the popliteal fossa produces suppression of ongoing EMG activity in the contralateral soleus (Stubbs and Mrachacz-Kersting, J Neurophysiol 2009:102:3596-3605). This short-latency crossed spinal inhibition (CSI) mediated by muscle afferents could be a useful tool for examining interlimb coordination (Stubbs et al., J Neurophysiol, 2011:105:503-511). Here, as the first step in understanding if and how excitation of the ipsilateral extensor muscle afferents affects the contralateral extensor activity in spastic individuals with chronic incomplete spinal cord injury (SCI), we examined the soleus CSI bilaterally in people with and without SCI and compared the amount of soleus CSI between the legs (e.g., less affected vs. more affected/spastic legs).
Methods: Seven adults with spasticity due to chronic incomplete SCI (20-74 yrs in age) and 8 age-matched individuals with no known neurological conditions participated in this study. EMG signal was recorded from the soleus and tibialis anterior (TA) bilaterally. To elicit the H-reflex and M-wave in the ipsilateral soleus and measure CSI in the contralateral soleus, the ipsilateral tibial nerve was stimulated in the popliteal fossa with a 1-ms square pulse when the standing participant had maintained soleus EMG activity within a pre-determined range (i.e., one's natural standing level) for at least 2 s. Stimulus intensity was varied from soleus H-reflex threshold to the maximum H-reflex (H_{max}) to an intensity just above what was needed to elicit the maximum M-wave (M_{max}). CSI, typically observed around +6 ms from the H-reflex onset, was averaged across stimulus intensities that produced $\geq 50\% M_{max}$ in the ipsilateral soleus for each individual's each leg and expressed as %background EMG (bEMG).
Results and Discussion: In both groups, the H_{max}/M_{max} ratio was highly correlated between the legs (r=0.94 for non-SCI, r=0.76 for SCI). In participants without SCI, the amount of CSI did not differ between the leg with higher H_{max}/M_{max} ratio and the leg with lower H_{max}/M_{max} ratio (26% vs. 23% bEMG), and the correlation between the legs was weak (r=0.40). In participants with SCI, CSI of the more affected leg was significantly less than CSI of the less affected leg (20±8% vs. 34±13% bEMG, p=0.002), and the amount of CSI was positively correlated between the legs (r=0.89). Less CSI found in the more affected leg than the less affected leg and stronger interlimb correlation between the legs found in spastic individuals with SCI may suggest altered CSI in chronic SCI. Future studies are needed to further investigate a potential relationship between CSI and spastic movement disorders.

Disclosures: A.K. Thompson: None. **M. Melvin:** None. **A.M. Phipps:** None.

Poster

302. Posture and Gait: Reflexes and Reflex Modulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 302.26

Topic: E.06. Posture and Gait

Support: SCIRF#2019 PD-01 (Thompson)
R01 NS114279 (Thompson)
the Doscher Neurorehabilitation Research Program

Title: Modulation of cutaneous reflexes in lower extremity muscles during walking and running

Authors: *A. M. PHIPPS, A. K. THOMPSON;
Med. Univ. of South Carolina, Med. Univ. of South Carolina, Charleston, SC

Abstract: Cutaneous reflexes (CRs) are modulated between standing and running (Brain Res. 1993;613; 230-238). Currently, the extent of task-dependent CR modulation is not well understood. Thus, to evaluate the effects of mode (walking vs. running) and speed of locomotion, we examined CRs in the lower limb during walking, jogging, and running.
Ten individuals (33.7 ± 12.2 yrs old) with no known neurological conditions participated. CRs were elicited by stimulating the superficial peroneal (SPn), sural (SRn), and distal tibial nerve (DTn) near the ankle with a 5 x 1-ms pulse train (200 Hz) at ≈1.9 x radiating threshold. Stimulation was delivered during walking at 3 or 4 km/h (slow walk: SW), walking at 6 or 7 km/h (fast walk: FW), jogging at 6 or 7 km/h (JOG), and running at 9 or 10 km/h (RUN). For the soleus (SOL), medial (MG) and lateral gastrocnemius (LG), tibialis anterior (TA), biceps femoris (BF), and vastus lateralis (VL) of the stimulated leg, short (50-80 ms post-stimulus, SLR) and medium (80-120 ms, MLR) latency CRs (measured as the difference in EMG between stimulated and non-stimulated steps) were quantified at early stance, late stance, and end swing phase of each locomotion condition. At early stance, the effect of mode on SLR was clear. Suppressive SOL CRs to SPn stimulation was greater in JOG (-30% maximum unstimulated EMG) than in FW (-16%) (p = 0.001). A similar pattern was observed for SRn (-33% JOG vs. -11% FW, p = 0.009) and DTn (-39% JOG vs. -5% FW, p = 0.017) stimulation. In response to SPn and SRn stimulation, TA SLR showed reflex reversal; suppressive CRs with FW (-20 and -24%) to excitatory with JOG (1% and 2%) (p = 0.016 and p = 0.011, respectively). TA CRs to SRn stimulation were different between JOG (2%) and RUN (11%) (p = 0.027). MG SLRs to SPn and DTn stimulation were mode-dependently modulated between JOG and FW (p <0.001 and p = 0.007, respectively). MG SLRs to DTn stimulation differed between JOG (-12%) and RUN (-24%) (p = 0.002). At end of stance, the effect of speed was significant only for the SOL SLR to SRn stimulation (2% JOG vs. 6% RUN, p = 0.014). At the end of swing, the effect of speed (not mode) was significant only for the LG SLR to DTn stimulation (2% JOG vs. -2% RUN, p = 0.021). There were no significant differences found for VL or BF SLRs. Trends in MLRs were similar to those in SLR but were less robust across different muscles.
CRs are modulated speed- (i.e., SW vs. FW or jog vs. run) dependently during locomotion; mode- (FW vs. JOG) dependent modulation was significant at early stance. CR modulation was more robust in plantar and dorsiflexors than in VL and BF. Phase, mode, and speed dependent modulation of CRs may reflect complex and extensive impact of cutaneous input on human locomotion.

Disclosures: **A.M. Phipps:** None. **A.K. Thompson:** None.

Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.01

Topic: E.09. Motor Neurons and Muscle

Title: Motor neuron cell body morphology with age, exercise, and gender

Authors: *A. J. LOPEZ, A. F. CINTRÓN-COLÓN, J. M. VANGYSEGHEM, J. M. SPITSBERGEN;
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Abstract: The number of individuals of old age has continued to increase within the last few years, accompanied by an increase in senescence - an increased risk for diseases and ailments, one of the most common being sarcopenia, or loss of muscle mass. In aged rat models, the neuromuscular junction shows morphological changes such as decreased end plate areas, pointing to possible changes in the muscle's associated motor neuron and proposes an interest in the motor neuron cell bodies in the ventral horn of the spinal cord. Prior studies in our laboratory have shown a 13% decrease in the number of motor neurons in old male rats compared to younger male rats (Cintron-Colon, 2022). Males only make up 50% of the population and have distinctive features from females regarding certain health risks and effects of aging. The focus of this study is to examine the motor neuron cell bodies in aged female rats, including the number, area, and area distribution of cell bodies. Time points of 8 weeks, 12 weeks, 1 year, and 18 months were analyzed to correlate to major developmental markers of the Sprague-Dawley rat model. The lumbar region (L4-5) of the spinal cord was cryoprotected and sectioned at 20 µm, followed by immunohistochemical staining with anti-choline acetyltransferase, a neuronal marker, and DAPI (nuclear stain). Sections were viewed under a Nikon confocal microscope to identify, count, and measure motor neurons in lamina 9 of the ventral horn. A comparison of average cell body sizes showed no significant ($p<0.05$) difference between 52-week vs 78-week females (354.01 ± 28.75 μm^2 and 314.81 μm^2 ± 14.39, respectively). However, there was a significant difference between 12-week (642.14 ± 22.32 μm^2) vs 52-week (354.01 ± 28.76 μm^2) females, and between 12-week vs 78-week (314.81 ± 14.38 μm^2) females. Following histogram analysis, 12-week females showed a higher frequency of cell bodies >1000 μm^2 in size, while 52-week and 78-week females showed a higher frequency of cell bodies between 100 and 500 μm^2 in size. The average number of cell bodies showed a decreasing trend with age, and there was no significant difference in the average number of cell bodies per section between 52-week (17.83 ± 0.877) vs 78-week (16 ± 0.895) females. The decrease in the frequency distribution of cell bodies >1000 μm^2 in size may be attributed to an overall decrease in size of all cell bodies with age or may suggest a loss of fast-type motor neurons with age since larger cell body sizes are observed in lower numbers in aged rats. The next step in this study is to examine the effects of exercise on cell body morphology with age, and the possible neuroprotective effects it exhibits in a comparison between male and female rat models.

Disclosures: A.J. Lopez: None. **A.F. Cintrón-Colón:** None. **J.M. VanGyseghem:** None. **J.M. Spitsbergen:** None.

Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.02

Topic: E.09. Motor Neurons and Muscle

Support: NIH grant 1 R15 AG022908-01A2
NSF grant DBI 0552517

Title: From young to old: the effects of sedentary-aging and exercise interventions on structural plasticity of lumbar motor neurons, end plates and GDNF

Authors: *A. F. CINTRÓN-COLÓN, J. M. SPITSBERGEN;
Western Michigan Univ., Western Michigan Univ., Kalamazoo, MI

Abstract: Altered neuromuscular communication has been proposed to contribute to the loss of muscle mass and strength observed with age, leading to frailty and loss of independence. Exercise provides protective effects for both muscle and nervous tissue. Glial cell line-derived neurotrophic factor (GDNF) is a potent survival factor for somatic motor neurons (MNs).In this study, changes in GDNF content, number of MNs and structural plasticity of MNs and end plates were evaluated. We hypothesized that GDNF content, MN cell number, and MN cell body and end plate size would all decrease with sedentary-aging and that exercise would slow or reverse those changes.Lumbar spinal cord, soleus and plantaris muscles were taken from sedentary and exercised rats between 1 and 24 months of age. Sedentary groups consisted of 1-, 3-, 6-, 12-, 18-, and 24-month-old rats, while exercised groups consisted of 3-, 12-, 18-, and 24-month-old rats. For immunohistochemical analysis of spinal tissue, antibodies against choline acetyltransferase (ChAT), neuronal nuclei (NeuN), and the fluorescent dye DAPI were used to identify MN cell bodies in the ventral horn of the spinal cord. In skeletal muscle, alpha-bungarotoxin was used to identify end plates. GDNF protein content of spinal cord and muscle tissues was measured by ELISA.The results show that in sedentary rats, MN size peaked at 6 months of age (1562 μm^2 +/- 121.1) and declined thereafter (12 months of age - 949.4 μm^2 +/- 92.44, 18 months of age - 476 μm^2 +/- 39.42, 24 months of age - 420.6 μm^2 +/- 31.49). Number of MNs decreased (13%) in sedentary rats from 6 to 24 months of age (6-month-old - 148.5 +/- 2.3 cells vs 24 month-old - 129.3 +/- 4.9 cells). Exercised rats had increased MN area at 18 and 24 months of age (657.7 μm^2 +/- 49.86 and 602.8 μm^2 +/- 42.18, respectively) compared to their sedentary counterparts (476.1 μm^2 +/- 39.42 and 420.6 μm^2 +/- 31.49, respectively); and exercise prevented the decline in MN number seen with age (6-month-old - 148.5 +/- 2.3 cells vs 24-month-old exercised - 140.7 +/- 7.3 cells). Exercise also resulted in an increase in the size of end plates in soleus muscle at 12 and 24 months of age and in plantaris muscle at 3, 12, 18 and 24 months of age. Exercised rats had increased GDNF protein content in soleus and plantaris muscle, but no effect on GDNF protein content in spinal cord.Our findings suggest that starting long-term exercise at different time points may aid in preserving GDNF expression in skeletal muscle and may contribute to neuroprotection by conserving size and number of MNs, and size of end plates. Altogether, exercise acts as an encouraging method to slow-down effects of aging and enhance plasticity.

Disclosures: A.F. Cintrón-Colón: None. **J.M. Spitsbergen:** None.

Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.03

Topic: E.09. Motor Neurons and Muscle

Support: PGC2018-098229-B-100

Title: A forced physical exercise maintenance model to study the role of the dopaminergic system in adolescent rats.

Authors: *D. GARRIGOS[1,3], A. BARREDA[3], M. MARTÍNEZ-MORGA[1,3], A. TOVAL[4], Y. KUTSENKO[1,3], B. RIBEIRO DO-COUTO[2], K.-Y. TSENG[5], J. L. FERRAN[1,3];
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Abstract: When performing exercise, our ability to move is the result of hierarchically arranged central nervous system circuits. These motor plans are originated in the cortex and then modulated and refined through different cortical and subcortical circuits. Dopamine is known to act as one of these modulators through different pathways, although the precise role of dopamine in training motor responses remains unclear. In previous studies, we determined that physical capacity is dependent on D1 striatal and D2 extra-striatal actions in Sprague-Dawley rats, measured as the maximal running time during an incremental test. Here we want to define a model to study the causal mechanisms regulating the maintenance of physical exercise responses. To accomplish that, we first defined a model of physical exercise maintenance that involved conducting three incremental tests 72 hours apart, with active rest in the intervals between tests to preserve performance. To validate the model, in the second test, we administered a D1-like receptors antagonist (SCH23390) in a dose of 0.1 mg/kg intraperitoneally or saline (Sodium Chloride 0.9%). According to our results the habituation protocol and active rests between tests can maintain the physical exercise performance. Sprague Dawley rats administered with D1 SCH23390 (0,1mg/kg) antagonist decreased the performance in the second test compared to the first and third ones (Test 1: 27.99±6.04 min.; Test 2: 5 min. (SCH23390, $p<0.05$) and 26.72±9.49 min. (Saline); Test 3: 24.03±7.09 min.). Rats injected with saline were able to maintain the performance throughout the incremental tests. Rats injected with SCH23390 significantly decreased their performance in the second test but recovered performance in the third test. Finally, our model significantly reduces the number of experimental animals required for the experimental design.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.04

Topic: E.09. Motor Neurons and Muscle

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Title: Minocycline treatment preserves Ia synapse structure but not function following peripheral nerve injury

Authors: *T. M. ROTTERMAN[1], S. N. HOUSLEY[1], P. NARDELLI[2], V. GARCIA[2], T. C. COPE[1];
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Abstract: Peripheral nerve injury (PNI) results in the permanent reorganization of spinal motor circuitry leading to behavioral deficits. One example is the degradation of the proprioceptor Ia afferent synapses on axotomized motoneurons which ultimately results in the absence of the stretch reflex. Our previous work demonstrated that this loss is due to a central microglia-macrophage mediated neuroinflammatory response. Therefore, we hypothesized that suppressing inflammation would result in the preservation of Ia connections and ultimately restore function. To investigate this, we transected and repaired the medial gastrocnemius nerve in adult rats. Rodents were then treated daily with an anti-inflammatory drug (minocycline) or vehicle for two weeks post-injury. Synaptic preservation, functional connectivity, and synaptic efficacy were assessed at 3 and 6+ months post injury using retrograde tracing techniques in combination with immunohistochemistry and *in vivo* intracellular motoneuron recordings. At 3 months post-axotomy motor axons had regenerated and reformed neuromuscular junctions in both treated and untreated animals alike, but sensory axon reinnervation was limited. However, retrograde labeling revealed that motoneurons retained Ia afferent synapses in minocycline treated rats compared to the vehicle treated cohort. The maintenance of Ia synapses persisted even at 6 months post-injury in minocycline treated rats when peripheral regeneration and muscle reinnervation were substantial for both motor and sensory axons. This retention led to increase in the proportion of motoneurons that responded to muscle stretch though the amplitude of the evoked synaptic potentials were significantly smaller than control animals and resembled nerve injured animals receiving vehicle alone. This ultimately prevented the recovery of the stretch reflex in minocycline treated rats. These data suggest that while minocycline treatment did in fact better preserve structural and functional connectivity, synaptic efficacy is not restored with minocycline treatment alone. Future studies will aim to strengthen these now preserved connections with the intent of improving behavior outcomes in response to axotomy.

Disclosures: T.M. Rotterman: None. **S.N. Housley:** None. **P. Nardelli:** None. **V. Garcia:** None. **T.C. Cope:** None.

Poster

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Topic: E.09. Motor Neurons and Muscle

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Title: Sciatic nerve transection and sciatic nerve crush induce different central neuroimmune responses and varying degrees of spinal circuit plasticity

Authors: T. M. ROTTERMAN[1], **Z. HALEY-JOHNSON**[2], W. MCCALLUM[4], T. C. COPE[1], *F. J. ALVAREZ[3];
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Abstract: Peripheral nerve injury results in a central neuroimmune response followed by plasticity of essential spinal motor circuits, the extent of which depends on the severity of the injury. Following nerve transection and immediate repair there is a die-back of ventrally projecting Ia afferent axons and their synaptic boutons are permanently removed from axotomized motoneurons undergoing regeneration. Intriguingly, nerve crush injuries result in temporary displacement of Ia synapses, but these recover to near-normal levels following muscle reinnervation. These differences have functional consequences. After eight to twelve weeks of regeneration time there is no recovery of stretch reflexes after nerve cut but an exaggerated reflex appears following nerve crush. Previously, we showed that microglia activation and a CCR2 mechanism are both necessary for permanent removal of Ia afferent synapses from regenerating motoneurons axotomized by nerve transection. We thus hypothesized that to induce such long-lasting changes on Ia synapses, nerve transection should evoke a more robust neuroimmune response compared to nerve crush. Three to seven days after injury both injury models showed similar microglia proliferation and activation, but while microglia activation was sustained for three weeks following transection, it significantly decreased one week after nerve crush. Furthermore, we used *ccl2-RFP* reporter mice to show that the pro-inflammatory chemokine CCL2 is briefly upregulated in motoneurons in both injury models during the first week post-injury, but in contrast, microglia express CCL2 only after nerve transections but with a delay onset and during the prolongation period of microgliosis in the second week after injury. To test the significance of microglia-derived CCL2 we use a floxed *ccl2* allele to eliminate CCL2 in microglia (CX3CR1-CreERT2) or motoneurons (Chat-IRES-Cre). We found that *ccl2* removal from microglia, but not from motoneurons, significantly rescued Ia synapses. This demonstrates that time-dependent changes in reactive microglia that are specific to nerve transection are involved in Ia synapse permanent elimination. However, this synaptic rescue did not result in recovery of normal stretch reflexes. We are currently investigating possible remaining deficits in Ia synapse mechanisms and/or peripheral Ia-spindle reinnervation to explain lack of functional recovery of the reflex. In conclusion. microglia CCL2 mechanisms are involved in the removal of inefficient Ia synapses after nerve injury.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Topic: E.09. Motor Neurons and Muscle

Support: R15NS099983

Title: Estrogen signaling is required for treadmill exercise mediated effects on synaptic plasticity around axotomized spinal motoneurons

Authors: G. BADER, V. L. KENNEDY, Jr., S. WILSON, ***J. C. WILHELM**;
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Abstract: After peripheral nerve transection, changes in spinal cord circuitry occur including the withdrawal of synaptic inputs from the somata and proximal dendrites of axotomized spinal motoneurons. Moderate daily treadmill exercise after transection injuries has been shown to mitigate the reduction in synaptic coverage. The mechanisms that underlie the effect of exercise are not well understood. Estrogen receptor (ER) signaling has been shown to have an important role in the effects of exercise on regeneration of injured motoneuron. Therefore, in this study we tested the hypothesis that ER signaling also is a part of the mechanism by which treadmill exercise mediates its effects on synaptic inputs. Gonadally intact male and female C57BL/6 mice underwent a unilateral sciatic nerve transection. Injured motoneurons were retrogradely labeled using a fluorescent dye immediately after transection. Mice then were divided into groups and treated for two weeks with 1) treadmill exercise at 10 m/min for 1 hour (continuous training), 2) treadmill exercise of 4 repetitions at 20 m/min for 2 minutes with 5 minute breaks interspersed (interval training), 3) ER antagonist Fulvestrant, 4) continuous treadmill exercise and Fulvestrant, or 5) interval treadmill exercise and Fulvestrant. Lumbar spinal cord tissue was harvested fourteen days after surgical injury. Tissue sections containing injured motoneurons were reacted with antibodies to vesicular glutamate transporter 1 (vGLUT1) and glutamate decarboxylase (GAD67). The average synaptic coverage of glutamatergic and GABAergic inputs onto labeled motoneurons was assessed using Image J. Similar to previous studies, we found no significant reduction in coverage in continuous trained male mice or interval trained female mice. We did find reductions in coverage in interval trained male mice and continuous trained female mice. Fulvestrant treatment during exercise prevented the sustaining effects of the continuous exercise in males and interval exercise in females. Fulvestrant treatment resulted in an increased loss of inputs compared to mice not receiving the ER antagonist. Based on these results we suggest that ER signaling is an important part of the machinery required for the treadmill exercise-mediated effects on synaptic inputs onto axotomized motoneurons after sciatic nerve transection.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Title: Specific preservation of KCC2 expression in axotomized abducens motoneurons and its enhancement by VEGF modifying inhibitory synaptic function

Authors: *P. M. CALVO[1], R. R. DE LA CRUZ[2], A. M. PASTOR[2], F. J. ALVAREZ[1]; [1]Emory Univ., Atlanta, GA; [2]Univ. de Sevilla, Facultad de Biología, Sevilla, Spain

Abstract: The potassium chloride cotransporter 2 (KCC2) is the main Cl^- extruder in neurons. Any alteration in KCC2 levels leads to changes in Cl^- homeostasis and, consequently, in the sign and amplitude of inhibitory synaptic potentials mediated by GABA or glycine. Axotomy downregulates KCC2 in many different motoneurons and it is suspected that interruption of muscle-derived factors maintaining motoneuron KCC2 expression is in part responsible. In here, we demonstrate that KCC2 is expressed in all oculomotor nuclei of cats and rats, but while trochlear and oculomotor motoneurons downregulate KCC2 after axotomy, expression is unaltered in abducens motoneurons. KCC2b is the isoform expressed and regulated on the cell bodies of oculomotor and also spinal motoneurons. Exogenous application of vascular endothelial growth factor (VEGF), a neurotrophic factor expressed in muscle, upregulated KCC2 in axotomized abducens motoneurons above control levels. In parallel, a physiological study using cats chronically implanted with electrodes for recording abducens motoneurons in awake animals, demonstrated that inhibitory inputs related to off-fixations and off-directed saccades in VEGF-treated axotomized abducens motoneurons were significantly higher than in control, but eye-related excitatory signals in the on direction were unchanged. This is the first report of lack of KCC2 regulation in a motoneuron type after injury, to propose a role for VEGF in KCC2 regulation and to demonstrate the link between KCC2 and synaptic inhibition in awake, behaving animals.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Topic: E.09. Motor Neurons and Muscle

Support: R21 NS114839
R01 NS111969

Title: Inhibitory synaptic activity in injured motoneurons promotes axon regeneration after axonal injury.

Authors: *W. MCCALLUM[1], R. L. WOOD[2], K. K. CARTER[2], A. W. ENGLISH[2], F. J. ALVAREZ[2];
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Abstract: Regenerating axons after peripheral nerve injuries may take years to reinnervate muscle. Electrical nerve stimulation and exercise are two techniques that have been shown to enhance neural recovery and axonal regeneration after injury, but the mechanisms that drive this activity in injured motoneurons remains unknown. Following nerve injury, most excitatory synaptic boutons are removed from the cell body of injured motoneurons while most inhibitory synapses are retained. In healthy motoneurons, inhibitory synaptic activity prevents neuronal firing, however after axotomy KCC2 is downregulated and inhibitory synapses become depolarizing, suggesting that GABA and/or glycine neurotransmission could be a possible source of synaptic drive that promotes regeneration. We investigated possible regenerative effects of post-injury inhibitory synaptic activity by blocking release of inhibitory neurotransmitters after injecting tetanus toxin in the left tibialis anterior (TA) muscle that flexes the ankle. Specific tetanization of the target motor pool was confirmed by estimating disinhibition of TA responses to a pressure stimulus applied to the paw, while the response of the ipsilateral lateral gastrocnemius (LG) remained unaltered. Thereafter, we performed a mid-thigh ipsilateral sciatic nerve crush to axotomize the motoneurons. The time course of muscle reinnervation was monitored by EMG recordings of muscle (M) responses and histological confirmation of neuromuscular junction (NMJ) reinnervation by motor axons and motor endplates. Two-month-old male and female C57/BL6 mice received either tetanus toxin (2 uL of a 0.25 ng/uL) or vehicle (saline) injected into the left TA. One week later, half of the mice in the tetanus and saline groups had their left sciatic nerve crushed. Ten-, twenty- and thirty-days post-crush, M responses were recorded from one third of the mice in each group, followed by euthanasia. Mice with crushed nerves that received tetanus toxin exhibited slower reinnervation of the TA estimated by delayed recovery of M response amplitude: 56.4% smaller at 30 days postinjury (0.41mV ±0.07 SD) compared to saline-crushed counterparts (0.94 mV ±0.32) ($P<0.001$, Bonferroni t-test). Mice that received tetanus with no nerve injury yielded M responses similar to uninjured control animals, suggesting no toxin effects at the NMJ (1.17 mV ±0.15 vs 1.15 mV ±0.14). We conclude that inhibitory neurotransmission on the cell bodies of axotomized

motoneurons contributes to the speed of regeneration and muscle reinnervation. We expect that the ongoing analysis of NMJ reinnervation will further confirm this conclusion.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Title: Spinal circuits early dysfunction in the SOD1G93A mouse model of Amyotrophic Lateral Sclerosis

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Abstract: There is increasing evidence that motoneuron death in Amyotrophic Lateral Sclerosis is preceded by a series of events that are not restricted to motoneurons but may involve alterations at the pre-motor circuit level. In particular, alteration in the density and strength of both excitatory and inhibitory synapses onto motoneurons have been reported in the early stages of the disease. Here we compared the strength of identified sensory afferent and motor efferent synaptic pathways in SOD1G93A mice and Wild Type littermates in the P15-P25 age range. While no changes were observed in di-synaptic Ia/Ib inhibition, we found that Ia mono-synaptic excitation was stronger in SOD1G93A mutants. The excitatory component of the recurrent circuitry was not altered, however, Renshaw cell mediated inhibition exhibited a 2-fold reduction in mutant mice, with no concomitant change in the strength of motoneurons to Renshaw cells synapses. A reduction in quantal size, demonstrated by quantal analysis and by asynchronous release experiments, is responsible for the alteration in recurrent inhibition strength. Interestingly, the impairment of recurrent inhibition is preferentially restricted to late firing motoneurons (putative fast), while early firing motoneurons (putative slow) are not affected. In vivo electromyography recordings confirmed the impairment in Renshaw inhibition in juvenile animals, but also showed that at later stages (around P60 and P90) the strength of recurrent inhibition is restored, suggesting the possibility of a compensation effect.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Title: Early reversible structural and functional impairments of excitatory synapses on ALS motoneurons

Authors: M. BACZYK[1], N. OUALI ALAMI[2], K. L. GRYCZ[1], N. DELESTRÉE[3], C. MARTINOT[4], T. LINYUN[2], B. COMMISSO[2], M. MANUEL[5], F. ROSELLI[2], ***D. ZYTNICKI**[4];
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Abstract: Excessive excitation is hypothesized to cause motoneuron (MN) degeneration in amyotrophic lateral sclerosis (ALS), but actual proof of hyperexcitation in vivo is missing: how are synaptic inputs to MN affected by the disease, and are they increased or decreased? We demonstrate, by in vivo intracellular MN electrophysiology, that, contrary to expectations, excitatory post-synaptic potentials evoked by electrical or mechanical stimulation of Ia sensory fibers are reduced in MNs of adult presymptomatic mutSOD1 mice. This synaptic impairment correlates with disrupted postsynaptic clustering of Homer1b, Shank, and GluR4 subunits. Moreover, this impairment has a deep impact on the whole MN biology since mechanically-induced Ia inputs translate in a reduced phosphorylation of the CREB transcription factor in MNs. Interestingly, a similar functional impairment is observed in synapses on MN originating from the brainstem descending medial longitudinal fasciculus, indicating a widespread phenomenon. Restoration of excitatory synapses can be achieved by activation of the cAMP/PKA pathway, by either intracellular injection of cAMP or DREADD-Gs stimulation. Furthermore, we reveal through independent control of signaling and excitability in MN allowed by multiplexed DREADD/PSAM chemogenetics, that PKA-induced restoration of synapses triggers an excitation-dependent decrease in misfolded SOD1 burden and autophagy overload. In turn, increased MN excitability contributes to restoring synaptic structures. Thus, the decrease of

excitation to MN is an early but reversible event in ALS. Failure of the postsynaptic site, rather than hyperexcitation, drives disease pathobiochemistry at this stage of the disease evolution.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Title: The motoneuronal receptorome in ALS reveals adrenergic entry points to modulate motoneurons excitability and firing

Authors: *G. CARON[1], S. ANTONUCCI[2], M. BACZYK[3], D. ZYTNICKI[1], F. ROSELLI[2,4]; [1]Saints Pères Paris Inst. for the Neurosciences, Univ. de Paris Cité, Paris, France; [2]Dept. of Neurol., Ulm Univ., Ulm, Germany; [3]Dept. of Neurobio., Poznan Univ. of Physical Educ., Poznan, Poland; [4]German Ctr. for Neurodegenerative Dis., Ulm, Germany

Abstract: Modulation of motoneuron (MN) excitability and synaptic excitation constitutes an important entry point to affect MN degeneration in several MN diseases. We have previously demonstrated that chemogenetic interventions on excitability and PKA signaling exert beneficial effects on synaptic integrity and disease burden in ALS MN. To achieve a similar upregulation of PKA signaling and MN firing through natural receptor, we explored the PKA-coupled motoneuronal receptorome in ALS. Among the receptors prioritized by screening available databases (Allen Spinal Cord Atlas, GPCR database), adenosinergic, histaminergic, cholinergic and several peptidergic receptors are downregulated in G93A SOD1 mice, whereas beta-1 adrenergic receptor is distinctively upregulated and the expression of dopaminergic D5 and beta-2 and beta-3 adrenergic receptors are preserved. Importantly, in vivo intracellular recordings of MN in G93A SOD1 mice show that acute activation of Dopaminergic D5 and beta2/3 adrenergic receptors by selective agonists increase MN excitability, through a decreased recruitment current for D5 agonist, and through an increased gain of the frequency-current relationship for beta2/3 agonists, indicating a role for D5 and beta2/3 receptors in the regulation of MN excitability in ALS. In addition, intracellular iontophoretic injection of cAMP-sP increases the gain of the frequency-current relationship, suggesting that beta2/3 receptors increase MN excitability

through the PKA pathway. The ALS MN receptorome is nevertheless highly dynamic and all studied receptors are down regulated in advanced stages of the disease. Our data show that MN display extensive entry points for modulation of their electrophysiological properties, which can be accessed with small molecules with translational potential.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Title: Electrical stimulation of rat sensorimotor cortex activates the spinal cord TrkB-ERK signaling pathway and changes glutaminergic terminals on spinal motoneurons

Authors: *Y. WANG[1], B. HERRON[2], Y. CHEN[3], A. VATO[4], J. S. CARP[5], J. R. WOLPAW[6]; [1]Albany Stratton VA Med. Ctr., Albany Stratton VA Ctr., Albany, NY; [2]Univ. At Albany, Sch. of Publ. Hlth., Univ. At Albany, Sch. of Publ. Hlth., Albany, NY; [3]Albany Stratton VA Med. Ctr., Albany Stratton VA Med. Ctr., Albany, NY; [4]Albany VA Med. Ctr., Albany VA Med. Ctr., Albany, NY; [5]Stratton VA Med. Ctr., Natl. Ctr. For Adaptive Neurotechnologies, Albany, NY; [6]Jonathan r Wolpaw, Natl. Ctr. for Adaptive Neurotechnologies, Delmar, NY

Abstract: Weak electrocortical stimulation (ECS) of rat sensorimotor cortex (SMC) increases soleus (SOL) H-reflex (HR) and GABAergic terminals on SOL motoneurons (MNs) (Chen et al 2007; Wang et al 2012). We are exploring ECS effects on spinal cord gene expression, immunohistochemistry, and physiology (Wang et al 2021, 2022; Herron et al 2021). To test the hypothesis that ECS acts through the TrkB-ERK pathway to change terminals on MNs, the present study examined ECS effects on TrkB gene expression, ERK activation-mediated synapsin 1 phosphorylation, and VgluT2-labeled terminals (TER_{VGT2}) on SOL MNs. Rats were implanted with SOL EMG electrodes and a posterior tibial nerve stimulating cuff in the right (R) leg and ECS electrodes over left (L) hindlimb SMC. ECS (25-Hz train of 0.1-ms biphasic pulses for 1 s every 10 s) was delivered 24 hr/d X 30 d; it produced no visible behavioral response. The R SOL HR was recorded throughout. After the 30 days, 8 ECS rats and 8 naïve control (NC) rats were injected with cholera toxin subunit B in SOL muscles and perfused 3 days later. L4-S1 lumbar sections were cut for immunostaining TER_{VGT2} and phosphorylated TrkB or ERK1/2 or

synapsin1 as well as *in situ* hybridization targeting *Trkb*. Blinded analysis of 3-D micrographs quantified TER_{VGT2} and immunoreactivity (IR) and mRNAs on SOL MNs. Data from the ECS group were expressed as % of the NC control values. Intergroup differences were assessed by ANOVA. The SOL HR increased over the 30 days of ECS. In SOL MNs of ECS rats, TrkB-IR, p-TrkB-IR, and *Trkb* mRNA puncta/MN averaged 114(±3SE)%, 125(±6)%, and 116(±4)% of NC values, respectively (p=0.007, 0.036, 0.036 *vs* NC); p-ERK1/2-IR and p-synapsin1-IR averaged 127(±6)% and 120(±4)% respectively (p=003 & 0.004 *vs* NC). ECS also significantly increased TER_{VGT2} on both left and right SOL MNs (116±5 and 117±5% (p=0.015 & 0.010 *vs* NC)). These results suggest that weak stimulation of sensorimotor cortex acts through the TrkB-ERK pathway to increase glutaminergic afferent terminals on SOL MNs. Together with earlier data, they indicate that cortical stimulation has multiple effects on the synaptic coverage of spinal motoneurons.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Title: Aging affects more the Hoffmann-reflex than the M-wave pathway of the flexor carpi radialis

Authors: *M. HENRY, J. DUCHATEAU, S. BAUDRY;
Lab. of Applied Biol. and Res. Unit in Neurophysiol. (LABNeuro), Univ. Libre De Bruxelles, Brussels, Belgium

Abstract: Sensory axons are the target of many peripheral nerve disorders and may be more affected than motor axons by aging [1]. The age-related changes in the sensory and motor axons can partly be documented by the Hoffmann (H) reflex and M wave. Thus, this study compared the responsiveness of the H reflex and M wave evoked with different pulse widths in young and old adults. H-reflex and M-wave recruitment curves were recorded at rest in the flexor carpi radialis of 12 young (YA, 21-36 yrs) and 12 older adults (OA, 62-80 yrs) by stimulating the median nerve with 0.05-ms, 0.2-ms and 1-ms pulse widths. We measured the maximal H-reflex amplitude (Hmax), the associated M wave (M-Hmax), and the H-reflex amplitude for a stimulus intensity evoking an M-wave of 5%Mmax (H-M5%) for each pulse width. Weiss’ formula was used to estimate the strength-duration time constant (SDTC) and response threshold from the charge/stimulus-duration relation for the H reflex and M wave. The SDTC was determined as the

negative intercept of the regression on the duration axis, and the response threshold as the slope of the regression [2]. Regardless of pulse width, OA had a smaller Hmax (YA: 30.9%; OA: 16.6% Mmax; $p = 0.029$) but a greater M-Hmax (YA: 22.2%; OA: 47.6% Mmax; $p < 0.001$) than YA. M-Hmax decreased similarly in both age groups with increased pulse widths ($p < 0.001$). The H-M5% was lesser in older compared with young adults with 1-ms and 0.2-ms ($p < 0.001$) but not with 0.05-ms pulse width ($p = 0.36$). Moreover, the increase in pulse width had a lesser effect on the H-M5% of old compared with young adults ($p = 0.005$). The SDTC was smaller with age for the H reflex (YA: 860 µs; OA: 618 µs; $p = 0.048$) but not the M wave (YA: 555 µs; OA: 436 µs; $p = 0.21$). The response threshold was greater in OA ($p = 0.003$) for the H reflex (YA: 3.1 mA; OA: 7.5 mA) and M wave (YA: 6.0 mA; OA: 12.3 mA). The SDTC for the H reflex was negatively associated with the response threshold ($r^2 = 0.42$; $p < 0.001$). The interaction of pulse width with age for H-M5% but not M-Hmax is consistent with the lesser SDTC for the H reflex but not the M wave in OA compared with YA. This briefer SDTC, associated with a greater response threshold, in OA should mainly reflect a decrease in persistent non-inactivating Na+ inward current for sensory fibers [3]. Overall, these results indicate greater age-related changes in the responsiveness of the H-reflex than the M-wave pathway, and suggest an increased homogeneity of the biophysical properties of the sensory and motor fibers with aging. [1] Mogyoros et al. 1998. Brain, 121, 851-859. [2] Kiernan & Kaji, 2013. Handb Clin Neurol, 115,43-53. [3] Kiernan et al. 2005. Brain, 128,1841-1846.

Disclosures: **M. Henry:** None. **J. Duchateau:** None. **S. Baudry:** None.

Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.14

Topic: E.09. Motor Neurons and Muscle

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NYS SCI Research Trust Fund C32241GG

Title: Electrocortical stimulation induces diverse transcriptional effects in the spinal cord.

Authors: *B. HERRON[1,2], Y. WANG[2], Y. CHEN[2], A. VATO[2,3], X. CHEN[2,4], J. CARP[2], J. R. WOLPAW[2,4];
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Abstract: Electrocortical stimulation (ECS) has local effects, and it also has remote effects on regions to which the stimulated region connects. We are studying its short- and long-term effects on gene expression in the spinal cord. Our experimental paradigm delivers just-threshold ECS to rat sensorimotor cortex and monitors the H-reflex of soleus motoneurons as a biomarker of remote effects. Our previous work has shown that ECS increases the H-reflex, which persists for months after the end of stimulation. This increase is correlated with short-term increases in synaptogenesis of GABAergic interneurons on soleus motoneurons and with decreases in GABA receptors in soleus motoneurons that also persist for months post-stimulation. We are now investigating genome-wide transcriptional effects of ECS in sagittal spinal cord sections from rats shortly after completing 30 days of ECS (25-Hz train of 0.1-ms biphasic pulses for 1 s every 10 s with no visible response to stimulus in rats). Targeted transcriptomic analyses were performed on sagittal lumbar spinal cord sections from ECS treated and control rats 3 days after completion of stimulation using the TempO-Seq™ platform. We uncovered more than 900 significantly differentially expressed genes. Gene function annotation was used to better understand the short-term effects of ECS. We identified gene sets related to GABAergic and glutamatergic neurotransmission, indicating a complex short-term response to ECS. A robust plasticity response was implicated by the modulation of genes involved in axonogenesis, ion channel function, neuronal kinesins, and neurotrophins. Alteration of genes previously implicated in neurodegeneration uncovers biomarkers and targets potential therapeutic application of ECS. Studies of the long-term transcriptional effects of ECS are underway. We predict that long-term ECS transcriptomes will show fewer differences than are in short-term ECS samples and a subset of long-term ECS transcriptome effects will correlate with the long-term H-reflex maintenance in soleus motoneurons. Identification of these genes will be critical to our understanding of the mechanisms of the remote and lasting impact of ECS on the spinal cord.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

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Program #/Poster #: 303.15

Topic: E.09. Motor Neurons and Muscle

Support: National Research, Development and Innovation Fund, Hungary, GINOP 2.3.3-15-2016-00032 and TKP2021-EGA- 35

Title: The effect of body position on the spasticity of quadriceps muscle after spinal cord injury

Authors: P. MAYER[1], N. ZENTAI[1], M. MRAVCSIK[2,1,3], H. BARTOK[4], B. RADELECZKI[5,2], ***J. LACZKO**[2,1,5];
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Locomotor Dis. and Disabilities - Natl. Inst. for Med. Rehabil., Buadpest, Hungary; [4]Semmelweis Univ., Budapest, Hungary; [5]Pazmany Peter Catholic Univ., Budapest, Hungary

Abstract: Spasticity is a consequence of upper motoneuron lesion. Spinal cord injury (SCI) may seriously affect the spasticity of leg muscles. For the objective and quantitative assessment of spasticity of the quadriceps muscle group, the pendulum test has often been applied. We performed the pendulum test with SCI patients in sitting and supine positions. The patient was a) sitting or b) laying on a bed with his thigh on the bed, the knee at the edge of the bed and the knee was fully extended while a physiotherapist held the foot of the patient. Then unexpectedly the foot was released and the shank started the pendulum like movement. This was repeated 3 times for both body orientation conditions for the left and also 3 times for the right leg. Kinematic measurement were made using a VICON motion capture system that recoded marker coordinates with a sampling frequency of 120Hz. Knee angle was computed from marker coordinates. We investigated the change of knee angle after foot release, especially the number of oscillations that occurred until the shank stopped and remained in stable position. We checked the time differences between consecutive local maxima of knee angle time profiles. We present in the case of an SCI patient that the number of oscillations was higher in sitting than in supine position. For the left leg the average number of oscillations was 2.7 and 3.7 in supine and sitting positions respectively. These values for the right leg were 2.7 and 5. Regarding the average time between consecutive maxima of knee angles, it was longer in sitting than in supine position: 0.92s and 0.90s for the left leg and 0.97s and 0.86s for the right leg. Our results confirm that the assessed spasticity is larger in supine than is sitting position. This is in line with other studies that calculated and analyzed the relaxation index derived from pendulum tests. The quadriceps muscle group is more stretched in supine position because in this case the distance between the origin and insertion of the rectus femoris is larger than in sitting position. We present that the number of oscillations and average duration of single oscillations are also useful metrics that are obtained from measurements with high sampling frequency and show the differences in spasticities derived from measurement performed in different body positions. Our work shows that when this test is applied for quantitative assessment of the disruption in central control of muscle stretch reflexes, the body orientation during assessment need to be taken into consideration.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.16

Topic: E.09. Motor Neurons and Muscle

Title: Changes in electromyographic and stabilometric indices during rehabilitation in amateur athletes with back pain

Authors: *E. KOLOSOVA, Z. BONDARENKO, O. LAZARIEVA;
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Abstract: It is known that inadequate exercise stress of deep dorsal spine or limb muscles could lead to functional and structural disorders of athlete neuromuscular system, such as compression of nerve roots or peripheral nerves. As a result, prolonged nerve fibers compression leads to local demyelination and axonal degeneration. In turn, this may be the cause of postural balance deterioration. Therefore, there is a need for early diagnosis of musculoskeletal disorders for timely rehabilitation in order to preserve the health of the athlete. Various rehabilitation effects have been reported, such as increased range of motion. At the same time, influence of rehabilitation activities (RA) on neuromuscular system remains insufficiently studied. The objective of the research was to evaluate changes in muscle strength, electromyographic (EMG) and stabilometric (SM) indices under the influence of RA in amateur athletes experiencing back pain. Twenty four athletes, specializing in functional training (11 men and 13 women, 19-55 years of age, M_{age}=30.2, SD=8.8) took part in the studies before and after RA (myofascial release lasting 40 min). The method of soleus H reflex was performed using neurodiagnostic complex Nicolet Biomedical Viking Select (Viasys Healthcare, USA). The strength of muscle groups of the trunk and lower extremities was measured using the BackCheck complex (Dr Wolff, Germany). Stabilometric study was performed using a stabilometric platform Nintendo Wii Board (Japan). Mean comparisons were made using the paired-sample Student's t-test in SPSS Statistics 17.0. It was found that H- and M-response thresholds were significantly decreased after RA, as well as the stimulation magnitude required to elicit maximum H- and M-responses ($p<0,05$). It was also revealed that muscle strength increased after RA in tests for trunk lateroflexion and hip extension ($p<0,05$). This might be due to some decrease in compression of the spinal nerves, which made it possible to reduce swelling and activate additional motor units for the development of greater muscle effort. It was found that under conditions of upright stance with feet positioned together, eyes closed, which required active involvement of proprioceptive system, statokinesiogram area and Center of Pressure trajectory length decreased under the influence of RA ($p<0,05$). Such data could be considered a sign of improvement in postural balance as a result of rehabilitation. Obtained data demonstrate that changes in EMG and SM indices could be indicators of a positive rehabilitation effect on the neuromuscular system. It is assumed that EMG and SM studies should be carried out to monitor the rehabilitation process.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.17

Topic: E.09. Motor Neurons and Muscle

Title: Targeted stimulation of movement-related ensembles in the motor cortex drives learned movements

Authors: *A. WU[1], T. KOMIYAMA[1,2];
[1]Dept. of Neurobiology, Ctr. for Neural Circuits and Behavior, Dept. of Neurosciences,
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Abstract: The motor cortex is necessary for producing complex movements. Learning of a specific motor skill induces specific activity patterns in the superficial layer (L2/3) of motor cortex, which can reliably decode the movement kinetics. However, it is unclear if this emerged activity pattern causally drives the learned movement. To address this question, we combined *in vivo* 2-photon calcium imaging and holographic optogenetic stimulation to precisely activate ensembles of L2/3 pyramidal neurons in the motor cortex in mice skilled in pushing a lever with the left forelimb. We found that stimulating a small subset of movement-related neurons (~20 cells) in the contralateral but not ipsilateral motor cortex can reliably initiate stereotypical lever pushes. Targeted stimulation of same numbers of cells that are not part of the movement ensemble also drove lever pushes; however, the kinematics of these movements differed from the learned movement. Analysis of the neural activity shows that targeted stimulation of movement ensembles recruited the activity of other, non-targeted movement cells, suggesting preferential connections within the movement population. Evoked population activity patterns predicted the evoked movement kinematics on a trial-by-trial basis. These results support the notion that neural activity patterns of L2/3 of the contralateral motor cortex instruct the kinematics of the learned movement.

Disclosures: A. Wu: None. **T. Komiyama:** None.

Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.18

Topic: E.09. Motor Neurons and Muscle

Support: NSF Grant 2015317

Title: Improving a balance control neural model through numerical optimization

Authors: *J. MCNEAL, A. HUNT;
Portland State Univ., Portland, OR

Abstract: A primary goal of neuromechanical models is to discern the relationships between neural networks and animal movement, including balance control. Previous work, based on a functional subnetwork approach, has generated and tested models that envision human balance control as an inverted pendulum. That work demonstrated that by pairing engineering control theory with trials of human subjects with severe vestibular deficiencies, different aspects of balance control can be decomposed and modeled by a proportional-derivative (PD) controller. Some subsequent work has demonstrated that this controller can be modeled with a relatively simple neural system. The effort used a hand-tuned real-time neural model to drive a single,

torque-controlled motor, however, this previous work still fell short of fully matching the experimental data. It is our hypothesis that the existing neural model can be made to match the human data more accurately through improved parameter setting. We accomplish this using numerical optimization techniques in PyTorch to set connection strengths and neural time constants within the model. Here we present our methods and an assessment of the impact of training by simulations and real-time trials on the inverted pendulum hardware.

Disclosures: **J. McNeal:** None. **A. Hunt:** None.

Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.19

Topic: E.09. Motor Neurons and Muscle

Support: NINDS
VA
Craig H. Neilsen Foundation

Title: Intracortical inhibition in elbow flexor and extensor muscles in intact humans

Authors: *C. L. P. BUTLER[1,2,3], S. SANGARI[1,3], M. OUDEGA[1,4,5], J. P. A. DEWALD[2,3,4], E. J. PERREAULT[1,2,3], M. A. PEREZ[1,3,4,6];
[1]Shirley Ryan AbilityLab, Chicago, IL; [2]Biomed. Engin., Northwestern Univ., Evanston, IL; [3]Dept. of Physical Med. and Rehabil., [4]Physical Therapy and Human Movement Sci., [5]Neurosci., Northwestern Univ., Chicago, IL; [6]Edward Hines Jr., VA Hosp., Hines, IL

Abstract: Differences have been reported in the neural control of elbow flexor and elbow extensor muscles in humans. For example, studies using transcranial magnetic stimulation (TMS) showed decreased amplitude of motor evoked potentials in elbow extensor compared with elbow flexor muscles, as well as inhibition in the elbow extensors elicited through ipsilateral stimulation of the primary motor cortex. The neural mechanisms contributing to this differential control remain poorly understood. Low-intensity TMS inhibits ongoing corticospinal output, likely through the activation of intracortical inhibitory circuits. In this study, we examined the contribution of intracortical circuits by using low-intensity TMS to measure the suppression of electromyographic activity (svEMG) in the biceps and triceps brachii muscles in 12 control subjects during 10% of maximum voluntary output (MVC) into isometric elbow flexion and extension, respectively. We found that the svEMG area (expressed as a % of the area under background EMG activity) was larger in the triceps brachii (23.9±7.3%) compared with the biceps brachii (10.5±8.1%, p=0.0003). All subjects showed larger svEMG area in the triceps compared to the biceps, suggesting more pronounced intracortical inhibition in elbow extensors compared to elbow flexors. The svEMG latency (biceps=22.7±6.7 ms, triceps=25.2±4.2 ms; p=0.18) and svEMG duration (biceps=17.1±8.2 ms, triceps=15.0±4.0 ms; p=0.3) was similar

between muscles. Our results show differences in the cortical control of biceps and triceps brachii muscles in intact humans. Future studies will test this question in people with cervical spinal cord injury who present poor motor recovery in elbow extensor compared with elbow flexor muscles.

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.20

Topic: E.09. Motor Neurons and Muscle

Title: Primary afferent depolarization facilitates, not inhibits sensory transmission in the spinal cord of non-human primates

Authors: *A. A. MAHROUS[1], L. LIANG[2], J. M. BALAGUER[3], J. C. HO[4], E. M. GRIGSBY[5], V. KARAPETYAN[6], J. A. GONZÁLEZ-MARTÍNEZ[6], P. C. GERSZTEN[6], D. J. BENNETT[8], C. HECKMAN[1], E. PIRONDINI[4], M. CAPOGROSSO[7];
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Abstract: Primary afferent depolarization (PAD) is a $GABA_A$-mediated response that occurs at the axo-axonic synapses of sensory afferents in the spinal cord. For decades, PAD was thought to be responsible for pre-synaptic inhibition by suppressing transmission at afferent terminals. However, recent studies in rodents show that $GABA_A$ receptors are expressed at branch points in sensory axons and not at their terminals, and that PAD can prevent action potential failure at branch points. These studies suggest that PAD is facilitatory reversing a long-standing idea of spinal sensory processing. However, this revolution in understanding of sensory gating is founded almost exclusively on experimental data in rodents which lack the sophisticated functional organization of the nervous system for dexterous motor control of primates. To fill this gap, we studied the effects of PAD on sensory transmission in the cervical spinal cord of macaque monkeys. We used an acute experimental setup in anesthetized monkeys to record PAD in sensory afferents and its effect on arm and hand motor output. Following dorsal laminectomy, we isolated dorsal rootlets at the C8 segment, severed them distally and wrapped them around silver hook electrodes to record intracellular PAD signals. In parallel we recorded intra-spinal neural signals from a multi-electrode (32-64 ch) linear probe dorsoventrally at the C5/C6 segments throughout different spinal laminae. Finally, we recorded EMG from forearm and hand muscles. To evoke PAD, we stimulated sensory afferents at the C6-C7 dorsal roots or through nerve cuffs on the radial and median nerves. Electrical stimulation of sensory afferents generated

clear PAD signals in the rootlets and across multiple laminae in the grey mater. Then we assessed the function of PAD via causal perturbations by intrathecal administration of bicuculline, a $GABA_A$ antagonist. We found that PAD was significantly reduced by the drug which confirms that PAD in monkeys is also $GABA_A$-mediated. In addition, monosynaptic responses of sensory-evoked EMG activity were significantly suppressed by bicuculline which, given the inhibitory role of GABA on somatic excitability, can only be explained by a facilitatory action of PAD on Ia-afferents. In summary, we directly measured PAD in monkeys for the first time and showed that PAD is facilitatory, not inhibitory and that suppression of PAD significantly reduces monosynaptic reflexes. These findings contribute to a radical shift of current models of sensory gating and because of the human-relevance of our animal model they can significantly impact our understanding of sensory-motor dysfunctions in patients with spinal cord injury and stroke

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Poster

303. Motor Neurons: Effects of Exercise, Injury, and Disease

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 303.21

Topic: E.09. Motor Neurons and Muscle

Support: NIH - R01NS102870
McDonnell Center for Systems Neuroscience
WUSTL Dept. of Radiology

Title: Light-based motor mapping of multiple limbs using deep learning reveals behaviorally relevant cortical motor representations

Authors: ***N. KHANAL**[1], J. PADAWER-CURRY[2], K. SCHULTE[1], T. VOSS[1], B. KIM[3], A. R. BICE[1], A. Q. BAUER[1];
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Abstract: Recent developments in optogenetics have allowed for quick and minimally invasive methods of studying functional brain organization in animal models. Studies incorporating light-based mapping of forepaw motor movement representations in rodents have reported distinct cortical representations of specific forepaw movements. However, previous mapping techniques have been limited to either tracking one-dimensional motion, bulky electronics that hamper natural movement, or manual labeling of specific features, any of which preclude simultaneous mapping of multiple limbs. DeepLabCut (DLC), a deep neural network toolbox for markerless

pose estimation, offers the ability to quickly track multiple user-defined features in 3-dimensions with human level accuracy. To demonstrate the utility of DLC for mapping cortical representations of limb movements, we performed light-based mapping in 7 transgenic mice expressing channelrhodopsin under a Thy1 promoter. A 473nm laser was used to stimulate 238 cortical sites separated by 300 microns positioned primarily over motor and somatosensory regions of the left hemisphere. Photostimulus induced movements in awake and anesthetized mice were captured by 3 cameras positioned around the animal. A set of representative frames were manually labeled and used to train the neural net for a specified set of iterations and the resultant models were used to automate labeling of videos. Label coordinates output by DLC were imported into MATLAB for analysis. Movement trajectories were baseline subtracted and significant movements were interpreted as displacements larger than 2 standard deviations above baseline fluctuations. Robust, repeatable motor movement representations of the left and right forepaws and hindpaws as well as the mouth were observed in each mouse across multiple mapping sessions. Stimulus evoked awake movements were approximately 4-10x larger than those under awake conditions, consistent with other studies. Photostimulation of overlapping motor movement representations of multiple limbs (e.g. left and right forepaw) produced combined, behaviorally relevant movements of each limb (e.g. grasping). These 3D movement trajectories were repeatable and persisted independent of state. These results suggest that motor circuits for specified motor output are spatially preserved in the cortex. Future studies include characterizing recovery of finer, articulated movements of individual paws (grasping, supination, etc.) after stroke, as well as mapping brain network activity during naturalistic behavior.

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Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.01

Topic: E.09. Motor Neurons and Muscle

Support: BCCN Berlin
Humboldt-Universität zu Berlin
DFG under Germany´s Excellence Strategy – EXC-2049 – 390688087

Title: Elephant facial motor control

Authors: *L. V. KAUFMANN[1,2], U. SCHNEEWEIß[1], E. MAIER[1], T. HILDEBRANDT[3], M. BRECHT[1];
[1]Humboldt Univ. zu Berlin / BCCN, Berlin, Germany; [2]Berlin Sch. of Mind and Brain, Berlin, Germany; [3]Leibnitz Inst. for Zoo and Wildlife Res., Berlin, Germany

Abstract: We studied facial motor control in elephants, animals with muscular dexterous trunks. Facial nucleus neurons (~54000 in Asian, ~63000 in African elephants) outnumbered those of other land living mammals. The large-eared African elephants (3 female, 1 male, 1 sex unknown; 0-41 y.) had more medial facial subnucleus neurons than the Asian elephants (2 female, 2 sex unknown; 0-52 y.), reflecting a more elaborate ear-motor control. Elephant dorsal and lateral facial subnuclei were unusual in elongation, neuron-numerosity and showing a cell-size gradient. We suggest this subnucleus-organization is related to trunk-representation with the huge distal neurons innervating the trunk-tip with long axons. African elephants pinch objects with two trunk-tip-fingers, whereas Asians grasp/wrap objects with larger parts of their trunk. Finger 'motor foveae' and a positional bias of neurons towards the trunk-tip-representation in African elephant facial nuclei reflect their motor strategy. Thus, elephant brains reveal neural adaptations to facial morphology, body size and dexterity.

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Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.02

Topic: E.09. Motor Neurons and Muscle

Title: Characterisation of iPSC-derived motor neurons using an accelerated maturation protocol

Authors: S. PRIME, S. SPRINGE, J. LYNDSAY, ***S. HUMPHREYS**, A. BARNES;
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Abstract: Understanding neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) requires a supply of cells for *in vitro* modelling and drug screening. Motor neurons derived from induced pluripotent stem cells (iPSC) provide an unlimited source of such cells and can potentially facilitate precision medicine approaches to compound testing when derived from somatic cells from patients. Furthermore, in co-culture with iPSC-derived muscle cells, a fully human preclinical screening model of the Neuromuscular Junction is possible. However, iPSC-derived motor neurons traditionally required up to six weeks maturation to exhibit relevant functional phenotypes. We demonstrate use of an *in vivo* environment-mimicking supplement, to produce a more physiological, modified growth medium for maturation, which reduces the time to reach functional maturity from six weeks to 10 days. Cells showed typical morphology with abundant neurites and were positive for expression of the mature motor neuron marker SMI-32 along with HB9, ChAT and Islet-1, as assessed by immunocytochemistry. Recordings using a 48-well multi-electrode array (MEA) demonstrate synchronised spontaneous firing at a similar timepoint. Motor neurons matured using the accelerated protocol formed junctions with muscle cells in a microfluidic co-culture system, as demonstrated by staining with fluorescently labelled alpha-bungarotoxin. The phenotype of motor neurons from an ALS patient was also investigated.

These rapidly matured cells provide an invaluable tool for research into neuromuscular disease and a model allowing a higher throughput for compound screening.

Disclosures: S. Prime: A. Employment/Salary (full or part-time):; Axol Bioscience. **S. Springe:** A. Employment/Salary (full or part-time):; Axol Bioscience. **J. Lyndsay:** A. Employment/Salary (full or part-time):; Axol Bioscience. **S. Humphreys:** A. Employment/Salary (full or part-time):; Axol Bioscience. **A. Barnes:** A. Employment/Salary (full or part-time):; Axol Bioscience.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.03

Topic: E.09. Motor Neurons and Muscle

Title: Dendro-axonal structure pattern correlations in M1 PT neurons

Authors: *Y. OTOR, P. AHIRWAL, J. SCHILLER;
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Abstract: The pyramidal neurons of the motor cortex are composed of cell types that differ in their location, projections, gene expression, and electrophysiology. The layer 5 thick-tufted pyramidal tract (PT) neurons, convey the output of the motor cortex down to motor centers in the brainstem, thalamus, and spinal cord to directly control behavior. These thick-tufted PT neurons of the mouse motor cortex can be divided to at least three neuronal sub-populations based on their molecular markers and axonal projections, with the first subtype preferentially projecting to the medulla, the second subtype to the thalamus and a third projecting to both. Our recent work indicates that thick-tufted PT neurons can be also sub-divided according to their morphological apical tree structure. Early bifurcating PT neurons with long nexus (type-1), and late bifurcating with short nexus (type-2). These two PT tuft morphologies exhibited unique tuft computational properties of motor information. Type-1 PT neurons showed marked tuft functional compartmentalization representing different motor variable combinations within and between their two tuft hemi-trees. In contrast, type-2 PT neurons showed synchronous tuft activation.Retrograde viral tracing of PT neurons projecting to the medulla or cervical spinal cord and full anatomical reconstruction of the apical dendritic structure revealed that M1 cortico-spinal PT neurons yield a high proportion of type-1 dendritic morphology, 90% of reconstructed neurons in 4 mice, were classified as type-1 neurons.

Disclosures: Y. Otor: None. **P. Ahirwal:** None. **J. Schiller:** None.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.04

Topic: E.09. Motor Neurons and Muscle

Support: NIH NINDS 1F31NS120500

Title: Single dose noradrenergic pharmacological probes modulate human motor units and attenuate chronic stroke-induced motor impairments

Authors: *J. A. BEAUCHAMP[1], S. T. JENZ[2], T. A. PLAISIER[3], S. URDAY[4], F. SOROND[4], C. HECKMAN[5], J. P. DEWALD[6];
[1]Northwestern Univ., [3]PTHMS, [4]Neurol., [2]Northwestern Univ., Chicago, IL; [5]Dept. of Physiol., Northwestern Univ., Oak Park, IL; [6]Physical Therapy and Human Movement Sci., Northwestern Univ., Chicago, IL

Abstract: Persistent inward currents (PICs) augment excitatory synaptic input to motoneurons and are facilitated by monoamines, setting a motoneuron's state. This monoaminergic dependence is critical for normal motor function, with motor impairments post neural injury commonly attributed to dysfunction of monoaminergic drive. Indeed, accumulating evidence attributes chronic stroke-induced limitations in upper extremity motor function to an increased monoaminergic drive and a greater reliance on contralesional corticoreticulospinal tracts. To investigate this interplay, we performed a series of experiments using single-blind and single-dose noradrenergic pharmacological probes, high-density surface electromyography (HDsEMG), and novel robotic devices in a neurologically intact and chronic hemiparetic stroke population. In the first set of experiments, a cohort of ten neurologically intact participants performed isometric elbow flexion, shoulder abduction (SABD), and finger abduction contractions before and 1.5 hours after oral administration of tizanidine, yohimbe, atomoxetine, or placebo. Isometric paradigms were designed to highlight PIC behavior, with MUs decomposed from HDsEMG of the biceps brachii, deltoids, and first dorsal interosseus. In a second set of experiments, individuals with chronic hemiparetic stroke were interfaced with a novel robotic device and similarly asked to generate isometric elbow flexion and SABD torque profiles. To highlight independent joint control an additional dynamic task was conducted, involving an isometric elbow flexion task with a downward SABD load. All tasks were performed before and 1.5 hours after oral administration of tizanidine, with MUs decomposed from HDsEMG of the biceps brachii and deltoid muscles. Initial analysis shows noradrenergic probes to modulate MU discharge profiles in both experiments. Preliminary findings from the second set of experiments show a significant decrease in MU excitability post tizanidine, with a 1.29 pps decrease in ΔF (95%CI: [0.8, 2.5]), and a 10.5% decrease in the proportion of involuntary elbow flexion generated during sub-maximal SABD. These findings elucidate the acute effects of noradrenergic perturbations on human MUs in a neurologically intact population. This knowledge is critical for understanding the motor effects of monoaminergic dysfunction post neural injury. Additionally, findings from the second set of experiments highlight the noradrenergic dependence of motor impairments in chronic stroke and show tizanidine to decrease measures of MU excitability and increase independent joint control in this population.

Disclosures: **J.A. Beauchamp:** None. **S.T. Jenz:** None. **T.A. Plaisier:** None. **S. Urday:** None. **F. Sorond:** None. **C. Heckman:** None. **J.P. Dewald:** None.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.05

Topic: E.09. Motor Neurons and Muscle

Title: M-type potassium currents shape recruitment and firing rates of motoneuron subtypes

Authors: *S. A. SHARPLES, G. B. MILES;
Univ. of St Andrews, St Andrews, United Kingdom

Abstract: Orderly recruitment of motor units and subsequent modulation of firing rate serve as two key mechanisms for the gradation of muscle force. The size principle describes the orderly recruitment of motor units and is believed to be governed by passive properties of the constituent motoneurons (MNs). However, MNs are not simply passive integrators and are endowed with voltage-sensitive ion channels that create non-linearities in their input-output functions. Here we describe a role for the M-type potassium current in the control of the MN recruitment and firing rate in mice capable of producing functionally mature locomotor behaviours (age P10-16). Fast and slow MNs were studied with whole cell patch clamp electrophysiology in transverse spinal slices. MN subtypes were identified based on delayed (fast) and immediate (slow) onsets to repetitive firing during long (7 s) depolarizing current steps. Recruitment and rate coding was investigated by measuring rheobase and subsequent repetitive firing of action potentials in response to slow (100 pA/s) depolarizing current ramps.
XE991-sensitive M-currents were detected in both MN subtypes, however they were significantly larger in delayed- compared to immediate firing MNs. Pharmacological blockade of the M-current with XE991 increased the input resistance of both MN subtypes, however this translated to a reduction in rheobase of delayed firing MNs only. Interestingly, XE991 only increased firing rates at the lower end of the frequency-current range and did not change maximal firing rates. Similarly, an M-current activator (ICA73) hyperpolarized the resting potential and decreased the input resistance of both MN subtypes, but only caused a significant increase in rheobase and depolarization of the spike threshold in delayed firing MNs. These effects of ICA73 were reversed by subsequent application of XE991.
These results demonstrate a MN subtype-specific role for the M-type potassium current in the control of MN recruitment and firing rates. More generally, they highlight the importance of features other than passive properties, specifically voltage-sensitive ion channels, in the differential control of MN recruitment and firing rate modulation.

Disclosures: **S.A. Sharples:** None. **G.B. Miles:** None.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

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Program #/Poster #: 304.06

Topic: E.09. Motor Neurons and Muscle

Support: WUSTL ENDURE Research Stipend (NIH)

Title: Effects of Fluoxetine on Serotonin Transmission at the Egg-laying Behavior Circuit of C. elegans

Authors: *E. ARNOLD[1], K. M. COLLINS[2];
[1]Harvard Univ., Cambridge, MA; [2]Biol., Univ. of Miami, Coral Gables, FL

Abstract: Serotonin (5-hydroxytryptamine, or 5-HT) regulates vertebrate and invertebrate behaviors such as feeding, mood, aggression, perception, reproduction, and sleep. Serotonin has been implicated in psychiatric disorders such as depression, and selective serotonin reuptake inhibitors (SSRI), such as fluoxetine (aka Prozac), inhibit the Serotonin Reuptake Transporter (SERT) and potentiates serotonin signaling to improve moods. However, while SSRIs block SERT activity immediately, behavioral changes may take weeks to develop, raising questions on how serotonin signaling contributes to psychiatric disorders and how long-term restoration of serotonin signaling alleviates symptoms. We are addressing how SSRIs like fluoxetine regulate behavior using the *C. elegans* egg-laying circuit as a model system. Egg laying is promoted by serotonin which is released by a pair of command neurons (HSNs) that innervate and regulate the contractility of the vulval muscles. Low levels of fluoxetine promote egg laying, but high levels of fluoxetine can inhibit it. This suggests that small increases in serotonin activate excitatory postsynaptic serotonergic receptors to stimulate vulval muscle contractility, while saturation of serotonin can inhibit behavior. Using behavior assays and Ca^{2+} imaging, we observed from our model that high concentrations of fluoxetine can inhibit HSN activity and egg laying due to increases in serotonin. We predict that inhibition of egg laying by fluoxetine requires inhibitory serotonin receptors MOD-1 and SER-4, thus mutant animals lacking these receptors will be more active. Together, these results would support a model where long-term treatment with fluoxetine ultimately *decreases* serotonin release and signaling by inhibiting the activity of the serotonin releasing neurons.

Disclosures: E. Arnold: None. **K.M. Collins:** None.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.07

Topic: E.09. Motor Neurons and Muscle

Support: BBSRC grant BB/S005943/1
Sir Henry Wellcome Postdoctoral Fellowship 221610/Z/20/Z
Royal Society Newton International Fellowship NIF\R1\192316

Title: The rules of synaptic connectivity between motoneurons

Authors: *M. BEATO, F. NASCIMENTO, J. OJEDA-ALONSO, **G. OZYURT**;
UCL, UCL, London, United Kingdom

Abstract: Anatomical and electrophysiological evidence shows that motoneurons collaterals can form synaptic contacts with other motoneurons in the lumbar spinal cord. We have recently shown that late firing motoneurons (putative fast) receive 10-fold stronger recurrent excitation than their early firing (putative slow) counterpart. This finding raises the question of whether connections between motoneurons are dominantly between fast motoneurons, or whether it is slow motoneurons that preferentially connect to fast ones. We performed paired recordings from motoneurons labelled from either lateral gastrocnemius (ankle extensor) or tibialis anterior (ankle flexor) muscles. Once a pair of connected motoneurons was identified, we characterized their properties and determined the frequency and strength of connections between the two types of motoneurons. First, for synapses within the same motor nuclei, there was a large dominance of connections between fast motoneurons and only rarely we identified a slow motoneuron as the pre- or post-synaptic partner. In preparations in which both nuclei were labelled, we tested whether it was possible to find connections between motoneurons belonging to antagonist nuclei. Surprisingly, the connectivity across antagonist nuclei was similar to that observed within nuclei, and the synaptic strength of individual connections did not differ. The connectivity between antagonist is similarly biased towards fast to fast connections.The function of these recurrent circuits is unclear, but given the preferential fast to fast connectivity, it is possible that recurrent excitatory circuits are recruited during motor tasks that require the activation of a large number of fast motor units, for instance during explosive movements.

Disclosures: M. Beato: None. **F. Nascimento:** None. **J. Ojeda-Alonso:** None. **G. Ozyurt:** None.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

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Program #/Poster #: 304.08

Topic: E.09. Motor Neurons and Muscle

Support: CONACyT Grant 732830 (DAZL)
CONACyT Grant 628536 (CHB)
NIH Grant 1R01DK20307-01

Title: Chronic Wireless Neuromodulation of Perineal Nerves Improves Micturition Behavior and Urodynamic Metrics in Aging Multiparous Rabbits.

Authors: *D. A. ZACAPA[1], C. HERNÁNDEZ-BONILLA[1], D. L. CORONA-QUINTANILLA[1], O. SÁNCHEZ-GARCÍA[2], F. CASTELÁN[3], M. MARTÍNEZ-GÓMEZ[3], M. I. ROMERO-ORTEGA[4];
[1]Ctr. Tlaxcala de Biología de la Conducta, [2]Facultad de Ciencias de la Salud, Univ. Autónoma De Tlaxcala, Tlaxcala, Mexico; [3]Inst. de Investigaciones Biomedicas, UNAM, Tlaxcala, Mexico; [4]Bioengineering and Biomed. Sci., Univ. of Houston, Houston, TX

Abstract: Multiparity and aging are physiological events that are recognized as contributing factors in stress urinary incontinence (SUI). Patients moderate SUI often resource to cutaneous or intravaginal electrical stimulation to contract the pelvic floor muscles. Unfortunately, these alternatives have demonstrated low therapeutic efficacy. The aim of this study was to evaluate the effect of direct neuromodulation of the perineal muscles as an alternative treatment for SUI. To that end, wireless electrical stimulation was applied on the nerves that control the perineal muscles in old multiparous rabbits, an accepted model of SUI, using a small implantable electrode. Five aging Chinchilla rabbits (4-5 years of age) were implanted with a miniature wireless electrode (OM+NClip) on right bulbospongiosus nerve (Bsn) and compared to old multiparous rabbit (n=5) with sham surgery (ShOM). Two weeks after implantation and for 4 weeks, wireless electrical stimulation was performed with an external RF antenna placed at 4 cm from the pelvic area on all groups for 5 min at 30Hz and threshold energies as reported previously. At the end of stimulation' protocol, we recorded cystometrography (CMG) in all animals and calculated voiding volume (Vv), volumetric intermicturition interval (Vii), threshold voiding volume (ThVv), residual volume (Rv) and voiding efficiency (Ve). Results of the CMG variables show that Vv in ShOM was 2.72 ±0.57 ml, while in OM+NClip was 8.23 ± 1.28 ml. An intermicturition interval of 5.06 ± 1.03 min was observe in ShOM, and, and doubled in the for OM+NClip group (11.14 ± 1.99 ml). The rest of the parameters did not show statistic differences between groups. These results suggest that unilateral wireless electrical stimulation of bulbospongiosus muscle through its specific nerve, improved urinary bladder capacity probably by strengthening the perineal muscle contraction supporting its role as secondary urinary sphincter and improving continence. The effect of chronic implantation of this device on the nerve will be evaluated using electron microscopy. This data supports the use of small implantable neural stimulators as an alternative treatment for SUI.

Disclosures: D.A. Zacapa: None. **C. Hernández-Bonilla:** None. **D.L. Corona-Quintanilla:** None. **O. Sánchez-García:** None. **F. Castelán:** None. **M. Martínez-Gómez:** None. **M.I. Romero-Ortega:** Other; Shareholder of Regenerative Bioelectronics Inc. (RBI Medical), a company that has commercial interest in neuromodulation of the PFM.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.09

Topic: E.09. Motor Neurons and Muscle

Support: HLB Grant 5U01HL133360-05

Title: A 3D anatomical and molecular map of cardiac vagal motor neurons

Authors: *E. HORNUNG[1], S. ROBBINS[1], S. ACHANTA[1], A. SRIVASTAVA[1], J. SCHWABER[1], R. VADIGEPALLI[2];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Jefferson, Philadelphia, PA

Abstract: Dorsal Motor Nucleus of the Vagus (DMV) activity is required for cardioprotection from physiological interventions such as remote ischemic pre-conditioning (RIPC). Herein, we begin to explore the transcriptomic phenotypes of DMV neurons, by combining high throughput transcriptomics, 3D anatomical mapping, and neural tracing in 12 week old male and female Sprague Dawley rats. We find that DMV neurons are not solely cholinergic, but simultaneously catecholaminergic, including those that project to the heart. To distinguish potential mechanisms of cardioprotection, we highlight the functional significance of several transcripts in cardiac-projecting DMV neurons and we begin to validate these findings at the protein level. In our sc-RNAseq data we find that *Snca* and the cardioprotective neuromodulator *Adcyap1* (PACAP) are enriched in the left intermediate DMV of females, a region previously shown to be implicated in ventricular contractility. *Adcyap1* and *Cartpt* are also enriched in this region of males in our sc-qPCR data. We have spatially mapped the location and molecular phenotypes of DMV and Nucleus Ambiguus (NA) neurons, and discovered their differentially expressed genes. *Slc18a2* (VMAT2), *Cartpt, Adcyap1, Ddc,* and Th are enriched in cardiac-projecting DMV neurons relative to those of the NA, implicating catecholaminergic processes and these secreted neuropeptides in cardioprotection. At the protein level, we found PACAP and TH enrichment, as well as co-localization of PACAP, TH, and CHAT in cardiac-projecting left intermediate DMV neurons. Consistent with literature suggesting a role for GLP-1 in cardioprotection from DMV, we also observed GLP-1 co-expression with PACAP in the DMV. A unique phenotype, with transcriptomic markers of strong cardioprotective potential, is present at the rostrocaudal position of left DMV previously shown to affect ventricular contractility and we are able to detect this transcriptomic signature from female sc-RNAseq data in male 10X genomics Visium spatial transcriptomics data. There is consistency between our findings from three spatial transcriptomics techniques and across sexes. Based on these results, we propose a 3D anatomical and molecular map of cardiac vagal motoneurons.

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Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.10

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Title: Measuring Motor Neuron Recruitment Patterns in Response to Mechanical Loading during Aplysia californica Feeding

Authors: *S. KUSHMAN, J. P. GILL, H. J. CHIEL;
Case Western Reserve Univ., Cleveland, OH

Abstract: Measuring Motor Neuron Recruitment Patterns in Response to Mechanical Loading during *Aplysia californica* Feeding
Authors
Sophie C. Kushman, Jeffrey P. Gill, Hillel J. Chiel; Biology, Neuroscience, Case Western Reserve University, Cleveland, OH
Abstract
Studying feeding behavior in *Aplysia californica* makes it possible to understand the relationship between neural activity and biomechanics. To replicate the findings from a prior *in vivo* study (Gill and Chiel, 2020) which showed that increasing mechanical load caused longer duration and higher frequency firing in identified neurons (B6/B9) and recruited an additional motor neuron (B3), and to determine whether this activity could be manipulated and monitored in a more accessible preparation, we studied the effects of mechanical loading in a semi-intact preparation: the suspended buccal mass, buccal ganglia, and cerebral ganglion of *Aplysia californica* (McManus et al. 2012). In this preparation, we used extracellular electrodes on nerves to monitor motor neuronal activity, but because the ganglia are exposed, it would be possible to study activity of individual neurons.We investigated the effects of changing mechanical load. We hypothesized that if the load is increased in the *in vitro* preparation, then the firing frequency of B6/B9 will be greater and B3 would be recruited. Swallowing motor patterns recorded on buccal nerves 2 and 3 were classified by the activity of identified motor neurons. Each animal was used as its own control to compare low and high mechanical loads. The results suggest that a stiff food stimulus (tape nori) increased the recruitment of the motor neurons compared to a soft stimulus (regular nori). Peak swallowing forces were shown to strongly correlate with neuron recruitment: B6/B9 recruitment was correlated with peak force ($n=4$, $p<10^{-7}$, $R^2=0.35$). Additionally, peak force was correlated with B3 and B4/B5 recruitment, respectively ($n=3$, $p<10^{-6}$, $R^2=0.35$; $n=3$, $p=0.00162$, $R^2=0.23$). These results support the hypothesis that load increases *in vitro* may cause neural changes similar those observed *in vivo.*

Disclosures: S. Kushman: None. **J.P. Gill:** None. **H.J. Chiel:** None.

Poster

304. Motor Neurons: Identification, Development, and Recruitment

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 304.11

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: R01CA221363
R01CA268125
Northside Hospital Foundation

Title: In vivo dynamic clamp exposes multidimensional conductance space that governs repetitive firing.

Authors: S. N. HOUSLEY, P. NARDELLI, *T. COPE;
Sch. of Biol. Sci., Georgia Inst. of Technol., Atlanta, GA

Abstract: The ability to fire action potentials and sustain repetitive firing are important physiological functions of numerous cells distributed throughout the mammalian nervous system. The voltage- and time-dependent sub- and supra-threshold conductances that cause repetitive firing depend on a diverse combination of ion channels species. The diversity of ionic conductances between and even within cell types suggests that there is likely no unique solution to achieve repetitive firing. Instead, varying proportions of multiple conductances allows normal function to occur within a range. Moreover, this redundancy results in a potential safety factor that affords adaptability and resiliance to disease or injury. While genetic deletion studies shed light on the binary role many conductances play, much less is known about the proportion of conductances in which normal repetitive firing occurs and the boundaries in which diseased firing emerge in motor neurons. We modeled ion channel kinetics of various Na^+ and K^+ conductances known to be expressed in motor neurons. Using these models, we utilize dynamic clamp to directly manipulate the proportions of conductances *in vivo* in anesthetized rats to explore the boundary conditions of normal repetitive firing. To start, we defined the 2D continuous conductance space representing combinations of Nav1.6 and Kv1 that result in normal MN firing. In doing so, we identified the opposing boundary condition underlying hyper- and hypo-excitable repetitive firing, replicating our recent finding for neurologic disorders in motor neurons. This novel approach has the potential to establish the range of conditions supporting healthy repetitive firing in neurons.

Disclosures: S.N. Housley: None. **P. Nardelli:** None. **T. Cope:** None.

Poster

305. Vocal/Social Communication: Non-Avian

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Program #/Poster #: 305.01

Topic: F.01. Neuroethology

Support: NIH Grant 1R15DC020327-01
NSF Grant IOS 1456743
PSC-CUNY Grant 68877-00 46

Title: Synaptic plasticity associated with reproductive state in the inner ear and auditory efferent system of a vocal fish

Authors: J. PERELMUTER[1], H. ALI[2], A. LAWRENCE[2], C. ZERNER[2], Y. IZRAELOV[2], A. ETOUNI[2], ***P. M. FORLANO**[3];
[1]Neurobio. and Behavior, Cornell Univ., Ithaca, NY; [2]Biol., CUNY Brooklyn Col., Brooklyn, NY; [3]Biol., CUNY Brooklyn Col. and Grad. Ctr., Brooklyn, NY

Abstract: Structural plasticity in the nervous system often occurs in conjunction with reproductive and circadian cycles. The plainfin midshipman, *Porichthys notatus*, is a marine fish which has provided decades of insight into neuroendocrine mechanisms of auditory plasticity. Males migrate to intertidal zones to breed during the late spring and summer, producing advertisement calls. Females are strongly attracted to this call, locating males at night for mating. Both males and females undergo an increase in inner ear auditory sensitivity coincident with the breeding season, particularly within the range of the harmonics of the male call. This increase in auditory sensitivity is associated with increased hair cell density, transcriptional changes within hair cells, a reduction of dopaminergic input to the inner ear and an increase in dopaminergic input to the cholinergic hindbrain octavolateralis nucleus (OE, homologous to the olivocochlear nucleus). Here we investigate whether the synaptic architecture of the saccule, the main endorgan of hearing in midshipman, and its associated efferent systems undergo seasonal remodeling associated with reproductive state. Non-reproductive females (n = 6) were collected by otter trawl in the winter from deep waters offshore while reproductive females (n = 6) were collected from summer intertidal nesting sites northern CA. Nervous system tissue was processed using pre-embedding tyrosine hydroxylase (TH) immunohistochemistry combined with transmission electron microscopy (TEM) to visualize dopaminergic cells and processes. Serial sections from the saccule and the OE were sampled random-systematically and imaged by TEM. After tracing image sets with TrakEM2, reconstructed 3D volumes were analyzed to extract changes in ultrastructure as a function of reproductive condition. Reproductive females have more ribbon synapses per hair cell but fewer and smaller dopaminergic varicosities in the saccule compared to non-reproductive females. In contrast, reproductive females had more direct synaptic and non-synapse-forming dopamine terminals within the OE. Increased numbers of ribbon synapses combined with the summer reduction of dopamine innervation likely results in enhanced inner ear sensitivity, while increased terminals in the OE may reduce cholinergic inhibition in the ear, altogether improving the ability of females to find and evaluate potential male mates. Because increased auditory sensitivity can be induced in winter, non-reproductive females with testosterone or estradiol, we hypothesize that the synaptic plasticity we report here is likely hormone-mediated.

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Poster

305. Vocal/Social Communication: Non-Avian

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.02

Topic: F.01. Neuroethology

Support: NSF IOS 1934386

Title: Phylogenetic conservation of the central vocal pattern generator in genus Xenopus

Authors: *A. YAMAGUCHI, M. PELTIER;
Sch. of Biol. Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Courtship behavior of animals encodes information about the species' identity. Accordingly, the exact cellular and synaptic properties of the neural circuitry underlying the courtship behavior are likely to be species-specific. However, the general architecture of the courtship neural pathways may be shared across closely related species. Here, we examined phylogenetic conservation of the central vocal pathways in five species of the genus *Xenopus*. Male Xenopus produce species-specific advertisement calls made of a series of clicks underwater to attract females of the same species. Although the acoustic morphology of the advertisement calls is diverse, the temporal organizations of the calls within the genus can be divided largely into three categories: fast calls containing clicks repeated at >60Hz, slow calls with clicks repeated at <30Hz, and biphasic calls that contain both fast and slow clicks. Previously, we showed that the biphasic advertisement calls of male *Xenopus laevis* are generated by anatomically distinct fast and slow click central pattern generators (CPGs) contained in the brainstem. The fast click CPG spans between the parabrachial nucleus and the nucleus amphiguus, whereas the slow click CPG is contained in the caudal brainstem including the nucleus ambiguus. In this study, we evoked fictive vocalizations from the isolated brains of two fast clickers (*X amieti, X. cliivi*), one slow clicker (*X. tropicalis*), and one biphasic clicker (*X. petersii*) and compared the functional and anatomical organizations of the vocal CPGs to those of male (biphasic clicker), female (slow clicker), and testosterone-treated female X. laevis (biphasic clicker). The results showed that the location and the function of the fast and slow click CPGs are conserved across species, and each species have one or both CPGs. The results suggest that the species-specific vocal neural circuitries are built upon conserved fast and slow click CPGs.

Disclosures: A. Yamaguchi: None. **M. Peltier:** None.

Poster

305. Vocal/Social Communication: Non-Avian

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Topic: F.01. Neuroethology

Support: NIH Grant DP2HD102042
Rita Allen Scholars Award
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L'Oreal FWIS Fellowship

Title: Dopamine regulation of tadpole social behaviors

Authors: *J. BUTLER, L. A. O'CONNELL;
Stanford Univ., Stanford, CA

Abstract: Our earliest interactions are with our caregivers, whom we rely on to satisfy our basic needs, such as food and safety. Rewarding interactions, such as those with caregivers, stimulate dopaminergic reward pathways in the brain. Despite this, there exists a critical gap in our understanding of how dopamine signaling mediates social decision-making in the neonate brain. We examined the neural basis and dopamine regulation of affiliative social behaviors in Mimetic poison frog tadpoles, which beg for food from their parents by vigorously vibrating their body back and forth to elicit feeding from mom. We first used ribosome capture (phosphoTRAP) to quantify transcripts being actively translated during begging and found that dopaminergic signaling pathways were enriched. Next, we used immunohistochemistry for the phosphorylated ribosome marker pS6 on brains from begging and non-begging tadpoles exposed to caregivers and quantified neural activity in 15 different brain regions. We found several brain regions, including regions involved in dopamine signaling such as the posterior tuberculum (Tp; putative homolog of ventral tegmental area), with higher activation in begging compared to non-begging tadpoles. We also found that dopaminergic neurons in the Tp had higher activation in begging compared to non-begging tadpoles. Finally, we used pharmacological manipulation of dopamine signaling in tadpoles exposed to caregivers to functionally test the role of dopamine in begging behaviors. We found that signaling through dopamine D1 receptors, but not D2 receptors, stimulated tadpole begging behavior. Current experiments involve connecting the sensory and dopamine neurons to motor circuits, which will lead to a circuit level understanding of infant communication and bonding.

Disclosures: J. Butler: None. **L.A. O'Connell:** None.

Poster

305. Vocal/Social Communication: Non-Avian

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.04

Topic: F.01. Neuroethology

Support: NIH RO1 DC017466-01
NSF DEB2121058

Title: The mystery of mismatch between auditory temporal tuning and mating call structure in chorus frogs undergoing speciation by reinforcement: retuning or repurposing?

Authors: *A. MUKHOPADHYAY[1], J. M. MCDOWELL[1], R. K. ALLURI[1], E. M. LEMMON[2], G. J. ROSE[1];
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Abstract: Diversification of reproductive communication behaviors can occur within a species when different populations interact with different heterospecifics, generating divergent selection pressures across geography. Consequently, unique male call properties and female mating preferences may evolve within (sympatry) compared to outside of (allopatry) these species contact zones. Chorus frogs, *Pseudacris feriarum*, show a divergence in call traits (pulse number and rate) across geographical locations; for example, advertisement (Adv) calls of frogs from populations in Georgia (sympatric with *P. nigrita)* and South Carolina (sympatric with *P. nigrita* and *P. brimleyi)*, have faster pulse rates (PRs) and/or more pulses, respectively, relative to an allopatric population from Alabama. To identify the neural correlates of divergence in call preference, we made extracellular recordings from auditory midbrain neurons (AMNs) while presenting synthesized acoustic stimuli that varied in their temporal properties, and Adv calls from each population. Studies in other anurans have suggested that a class of AMNs, Interval Counting Neurons (ICNs), play a crucial role in species recognition; they show selectivity to fast PRs and respond only after a threshold number of pulses have occurred. We tested the hypothesis that the temporal tuning of ICNs would be rescaled to values present in the Adv calls of each population. We found that the selectivity of ICNs in Georgia (sympatric) populations was well matched to pulse rates in their calls; however, ICNs in the allopatric group showed tuning to faster PRs than those seen in their Adv calls and were better matched to the PRs of their aggressive calls. These results suggest that allopatric frogs may represent an ancestral condition in which neurons selective for slow PRs, Long-Interval Neurons (LINs), rather than ICNs, are used to recognize Adv calls, ; the divergence in sympatric populations may, therefore, involve repurposing LINs and ICNs.

Disclosures: A. Mukhopadhyay: None. **J.M. McDowell:** None. **R.K. Alluri:** None. **E.M. Lemmon:** None. **G.J. Rose:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.05

Topic: F.01. Neuroethology

Support: NSF CAREER Award 1149446

Title: Dispersers: Overcoming overspecialization and seizure susceptibility in the African naked mole-rat

Authors: *C. PLATE[1], M. ZIONS[2], D. P. MCCLOSKEY[3];
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Abstract: African Naked Mole-Rats (NM-R) appear to have become overspecialized for the extreme conditions in their crowded underground burrows. Exposure of adult NM-R to the conditions they would encounter at the burrow surface unmasks their sensitivity to alkalosis and produces epileptic seizures. However, evidence suggests that some members of the NM-R colony disperse above ground, presumably without seizures, to propagate new NM-R colonies. Therefore, NM-R may harbor endogenous factors to overcome their own seizure susceptibility. The occurrence of a "disperser morphology" in wild and captive NM-R point to systemic metabolic plasticity which drive a fatty pale appearance in some animals. These animals are also noticeably calm when handling or removed from the colony habitat. We hypothesized that disperser NM-R would show a reduced vulnerability to seizures when exposed to surface conditions. Three adult NM-R were selected based on the physical and behavioral characteristics described above, and of these, two were seen to be physically shoved from the colony nest chamber by the queen or other large colony members as they approached the colony nest. All three of the putative dispersers were males between two and three years old. When placed in a chamber with surface conditions (normal air heated to 42 degrees C) all three of these animals demonstrated initial hypoactivity, followed by a period of intense hyperactivity, but no behavioral seizure activity. Based on our observations, NM-R selected for dispersal may be kept away from the colony nest to trigger a physiological switch preparing them for surface exposure. The details of this transformation will provide insight into the behavioral organization of the NM-R as well as endogenous mechanisms to overcome seizure susceptibility.

Disclosures: C. Plate: None. **M. Zions:** None. **D.P. McCloskey:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.06

Topic: F.01. Neuroethology

Support: NSF CAREER Award 1149446

Title: Hippocampal neuropeptide Y is related to social behavior in African naked mole-rats

Authors: *S. L. NARVAEZ[1], C. A. DUNNE-JAFFE[2], M. SANSONE[3], D. P. MCCLOSKEY[4]; [2]Psychology/ Biotech., [1]Col. of Staten Island, Staten Island, NY; [3]Developmental Neurosci., City Univ. of New York, Col. of Staten Isl, Staten Island, NY; [4]Dept of Psychology and Program in Developmental Neurosci., City Univ. of New York Grad. Sch. and Un, Staten Island, NY

Abstract: The African Naked Mole-Rat (NM-R) provides a unique opportunity to study how the mammalian brain coordinates behavior in a cooperative society. Aside from reproductive behavior, which is restricted to the queen and a few breeding males, the colony tasks of foraging, alloparenting, nest-building and colony defense are distributed among the dozens of members in a NM-R colony. Previous observation of behavior in captive NM-R colonies has identified

specialized roles for NM-R colony members which align with the size dimorphism seen in this species. Larger animals (> 45g) participate in colony defense tasks, while smaller animals participate in foraging, alloparenting, and nest building tasks. However, some behaviors in the crowded nest areas of the NM-R environment may be more difficult to observe. In the present study, we used an RFID-based approach to track the movement of animals and objects through the NM-R colony environment, overcoming observational limitations. We found that larger colony members participated in colony defense and other "nest centric" tasks, such as nest building and alloparenting, while smaller animals were recruited more in tasks that were distal to the colony nest and provided opportunities for colony expansion and foraging. Animals from four colonies were analyzed for behavior and selected based on nest-centric or distal behavior patterns. Brains were collected, and the amount of hippocampal neuropeptide Y (NPY) was measured using an ELISA. Animals selected for distal behaviors had higher levels of hippocampal NPY than animals selected for nest-centric behaviors. Confocal imaging of NPY expression in the hippocampus showed a robust axonal expression in area CA1 in animals that had higher NPY levels. NPY expression increase is consistent with foraging colony members in some insect societies, suggesting convergent evolution of NPY in societal specialization. We propose that NPY additionally helps distal NM-R to maintain adequate brain inhibition as they venture away from the calming colony nest.

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Poster

305. Vocal/Social Communication: Non-Avian

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.07

Topic: F.01. Neuroethology

Support: NSF CAREER Award 1149446

Title: Oxytocin-related suppression of hippocampal hyperexcitability in the naked mole-rat

Authors: X. GEOFFROY[1], ***D. MCCLOSKEY**[2,3];
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Abstract: Adult African Naked Mole-Rats (NM-R) have an impoverished GABAergic system, yet do not exhibit spontaneous epileptic seizures in their natural habitat. We have found previously that the high levels of carbon dioxide found in the NM-R colony nest can help to inhibit the brain and prevent seizures. Here, we tested whether oxytocin, which is related to nest huddling behavior in NM-R, can also help to prevent hyperexcitability. Extracellular recordings were made from the pyramidal cell layer of area CA3 in hippocampal slices of adult NM-R. This

region typically shows spontaneous epileptiform burst discharges under routine recording conditions in NM-R. When the selective oxytocin receptor agonist [Thr4,Gly7]-oxytocin (TGOT) was added to bursting slices (200 nM, 5 slices in 2 animals), the burst amplitude decreased, and pre-incubation with TGOT prior to recording (6 slices in 3 animals) prevented burst activity altogether. Addition of the $GABA_A$ agonist isoguvacine (200 μM) to bursting NM-R hippocampal slices typically exacerbates the bursts and leads to spreading depression. However, when isoguvacine was added after TGOT, bursts stopped completely. The NM-R harbors a variant of the KCC2 cotransporter which may limit its neuronal membrane insertion and reduce GABAergic efficacy. Oxytocin enhances membrane insertion of KCC2 and may help to restore GABAergic function when NM-R are socially congregating in the colony nest. We propose that the increased brain inhibition in the colony nest, through carbon dioxide and oxytocin-enhanced GABA, may have reinforcing properties that help to maintain the eusociality of this species.

Disclosures: X. Geoffroy: None. **D. McCloskey:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.08

Topic: F.01. Neuroethology

Title: Traumatic brain injury resistance in the African naked mole rat

Authors: *C. GAINYO[1], A. MEJIA-BAUTISTA[2], J. H. GOODMAN[3], D. S. LING[4], D. P. MCCLOSKEY[5];
[1]CUNY Col. of Staten Island, Staten Island, NY; [2]SUNY Downstate Med. Ctr., Brooklyn, NY; [3]Developmental Neurobio., NYS Inst. For Basic Res., Staten Island, NY; [4]Physiol. and Pharmacol., SUNY Downstate Hlth. Sci. Univ., Brooklyn, NY; [5]Dept of Psychology and Program in Developmental Neurosci., City Univ. of New York Grad. Sch. and Un, Staten Island, NY

Abstract: Traumatic Brain Injury (TBI) can lead to a lifetime of behavioral impairments and, in some cases, the development of posttraumatic epilepsy due to secondary neuronal death. Protection against primary and secondary neuronal injury is a therapeutic strategy to improve TBI outcomes, but a commonly accepted neuroprotective strategy remains elusive. We hypothesized that the African Naked Mole-Rat (NM-R), an animal with an enhanced brain extracellular matrix and resistance to hypoxia/hypercapnia, would have endogenous neuroprotection against primary and secondary injury in TBI. To test this, adult NM-R were subjected to controlled-cortical impact (CCI) surgery (at 1mm depth and 5m/s velocity), and the number of NeuN+ neurons and PU1/SPi1+ microglial cells was estimated in the cortex one week after insult using the isotropic fractionator method. Despite clear signs of injury upon gross inspection, the neuron and microglia numbers in the impacted cortex did not differ significantly

from the contralateral hemisphere, or from sham animals, suggesting native protection against both primary and secondary neuronal injury. While more work remains to be done, the initial results of neuroprotection against TBI in the NM-R offer promise for new approaches in the prevention and treatment of TBI.

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Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.09

Title: WITHDRAWN

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.10

Topic: F.01. Neuroethology

Title: Midbrain circuits for context-dependent control of vocalization in mice

Authors: *P. ZIOBRO, Z. HE, K. A. TSCHIDA;
Cornell Univ., Cornell Univ., Ithaca, NY

Abstract: Vocalizations are an essential means of communication in humans and other mammals. Animals produce different acoustic categories of vocalizations that serve different communicative functions, but the neural circuits that underlie this process remain unknown. To address this question, we study the neural circuits for vocalization in mice, which produce ultrasonic vocalizations (USVs) during social interactions and lower frequency squeaks in painful or aversive contexts. The midbrain periaqueductal gray (PAG) contains neurons essential for vocalization in mammals, and in the mouse, a specialized population of PAG neurons is both necessary and sufficient for the production of USVs (e.g., PAG-USV neurons). In the current study, we ask whether PAG-USV neurons are also required for the production of squeaks, or alternatively, contribute only to USV production.
To answer this question, we ablated PAG-USV neurons in male and female mice by employing an activity-dependent labeling strategy (TRAP2) which permits the expression of viral transgenes (caspase) in recently active neurons. Ten days later, these mice were presented with a social partner to test their ability to produce USVs and then placed in a footshock paradigm to

test their ability to produce squeaks. Similar to previous work in male mice, we find that ablation of PAG-USV neurons abolishes USV production in both males and females without affecting non-vocal social behaviors. In contrast, our preliminary data indicate that ablation of PAG-USV neurons has no effect on the rates or acoustic features of squeaks produced in response to footshock. Conversely, ablation of PAG neurons that are active during footshock appears to reduce the production of squeaks without affecting the production of USVs. These experiments will shed light on the functional organization of the midbrain circuits that allow animals to select and produce the appropriate type of vocalization for a given behavioral context.

Disclosures: P. Ziobro: None. **Z. He:** None. **K.A. Tschida:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.11

Topic: F.01. Neuroethology

Support: NIH Grant DC018691
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Title: Mouse Motor Cortex Can Influence Vocal Musculature

Authors: *C. D. M. VARGAS[1], R. AGRAVAT[1], E. N. WAIDMANN[1], E. D. JARVIS[1,2];
[1]The Lab. of Neurogenetics of Language, The Rockefeller Univ., New York, NY; [2]Howard Hughes Med. Inst., Chevy Chase, MD

Abstract: Vocalization is a dynamic and complex behavior that can be innate or learned. Vocal learning species use top-down input from the forebrain to coordinate sub-cortical networks during and after learning to precisely execute learned vocalizations. Songbirds have been the standard vocal learning model, but they lack the experimental tractability found in other species. Lab mice, Mus musculus, possess rudimentary features of vocal systems found in advanced vocal learners, including: 1) multisyllabic structure that varies with social context; and 2) a sparse, but direct, input to laryngeal motor neurons in the brainstem from a small pool of layer 5 neurons in the primary motor cortex (M1). We termed this posteromedial M1 population the laryngeal motor cortex (LMC). Another region of M1, the orofacial motor cortex (OFC), is known to be involved in controlling jaw muscles important for vocalizing. Here, we used electrophysiology, neuronal tracing, and lesions to investigate the role of LMC and OFC in controlling vocal behavior in mice. We used short 1- and 4-pulse, biphasic intracortical microstimulaiton with paired electromyography recordings (ICMS-EMG) from laryngeal and jaw muscles to test if the identified direct projection in LMC could provide input to vocal motor neurons, similar to what is seen in vocal learners. We found that stimulating the LMC and OFC regions preferentially activate laryngeal and jaw muscles, respectively. However, in both

muscles, the EMG responses from LMC stimulation have a longer latency (10-20ms) than predicted for the direct projection, whereas the responses from OFC stimulations were very short (5-10ms), which suggest a monosynaptic circuit. Using pseudo rabies virus (PRV), we found M1 contains overlapping representations of different muscles, including some individual neurons which represented both muscles. Using AAV1, an anterograde transynaptic tracer, we show that the OFC may have a direct monosynaptic connection with the jaw motor neurons in the trigeminal motor nucleus (Mo5). Bilateral OFC lesions induce changes in the ultrasonic vocalizations of mice when compared to controls. These results demonstrate that the murine motor cortex may provide influence of vocal musculature, although further work is necessary to identify the specific role of the cortex in facilitating the diverse vocal repertoire seen in mice. Overall, these results represent the first time that stimulation of the primary motor cortex in a non-vocal learning species can generate laryngeal muscle contractions, further establishing mice as a potential model to study fundamental neurobiology in speech and vocal communication.

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Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

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Program #/Poster #: 305.12

Topic: F.01. Neuroethology

Title: Neural circuits underlying the effects of acute isolation on vocal and non-vocal social behaviors in female mice

Authors: *X. ZHAO[1], Y. CHAE[1], A. SADANGI[1], T. GRINBERG[1], S. RABINOVICH[1], K. A. TSCHIDA[2];
[2]Dept. of Psychology, [1]Cornell Univ., Ithaca, NY

Abstract: The absence of meaningful social connections is aversive and motivates us to seek out social interaction. However, the brain mechanisms through which social isolation promotes affiliative social behaviors remain poorly understood. In recent work, we found that acute social isolation increases social interaction time and promotes USV production in female mice during subsequent same-sex interactions. To explore the neural circuit basis for the effects of isolation on female social behavior, we compared c-Fos activation in the brains of group-housed and single-housed females following same-sex social interactions to identify isolation-potentiated neurons. Our results revealed that acute (3-day) isolation significantly increased the number of Fos-positive neurons in the preoptic hypothalamus (POA) following female-female interactions. Returning isolated females to group housing for two weeks not only decreased their social interaction time and USV production during a subsequent same-sex interaction but also decreased the number of Fos-positive POA neurons relative to that seen in single-housed females. To test whether increased POA activity is necessary for increased USV production and

social interaction following social isolation, we used activity-dependent labeling (TRAP2) to chemogenetically inhibit the activity of isolation-potentiated POA neurons during female-female interactions. Our preliminary results indicate that chemogenetic inhibition decreases USV production in single-housed females, and we are currently quantifying effects on non-vocal social behavior. To further characterize isolation-potentiated POA neurons, we are also using in situ hybridization to characterize the molecular phenotype of these neurons, as well as viral tracing to characterize their axonal projections. We anticipate that our findings will shed light on the forebrain-to-midbrain circuits that regulate the production of social vocalizations in female mice, as well as how acute isolation acts on these circuits to influence social behavior.

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Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.13

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Examining the developmental trajectory of mouse ultrasonic vocal emission

Authors: *C. J. COLLINS[1,2], Y. MA[1,2], J. P. NEUNUEBEL[1,2];
[1]Psychological & Brain Sci., [2]Interdisciplinary Neurosci. Grad. Program, Univ. of Delaware, Newark, DE

Abstract: Throughout the life of an animal, vocal communication plays an integral role in modulating behavior (Froemke et al., 2021). For example, mice emit ultrasonic vocalizations (USVs) during distress as pups (Liu, 2009) and while engaging in courtship behaviors as adults (Neunuebel et al., 2015). While adult and pup USVs have been compared between mice (Grimsley et al., 2011), it is unclear how the vocal emission and acoustic characteristics of an individual change over time. Here, we recorded isolation vocalizations of individual male mice (n = 11) across postnatal (PN) days 2 through 63. From PN days 2 through 14, an individual mouse pup emitted an average of 19 vocalizations per day. However, from PN days 15 through 34, an individual mouse emitted an average of less than 1 vocalization per day. Subsequently, as the mice became young adults, from PN days 35 through 63, an individual mouse emitted an average of 52 vocalizations per day. In total, 2,529 pup and 16,027 young adult vocalizations were recorded. To compare the acoustic features of an individual across developmental time points, we grouped the vocalizations an individual emitted between PN day 2 and 14 (pup) or PN day 35 and 63 (young adult). Across individuals, pup vocalizations had significantly lower peak frequencies than vocalizations emitted during young adulthood (Wilcoxon rank-sum test, $W = 8$, $p < 0.05$). Similarly, the low frequencies of vocalizations produced as a pup were significantly

lower than those emitted during young adulthood (Wilcoxon rank-sum test, $W = 5$, $p < 0.01$). However, the range in frequency, or bandwidth, of vocalizations was indistinguishable (Wilcoxon rank-sum test, $W = 55$, $p > 0.05$). The inter-vocal interval, or time between vocalizations, was significantly shorter as a pup compared to those emitted during young adulthood (Wilcoxon rank-sum test, $W = 6$, $p < 0.01$). In contrast, vocalization durations were significantly longer as a pup compared to those emitted during young adulthood (Wilcoxon rank-sum test, $W = 66$, $p < 0.001$). Our results suggest that the acoustic characteristics of vocalizations emitted by an individual mouse change throughout development.

Disclosures: C.J. Collins: None. **Y. Ma:** None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.14

Topic: F.01. Neuroethology

Title: Rates but not acoustic features of ultrasonic vocalizations are related to non-vocal behaviors in mouse pups

Authors: *N. PRANIC, K. TSCHIDA, C. KORNBREK;
Cornell Univ., Ithaca, NY

Abstract: Title: Rates but not acoustic features of ultrasonic vocalizations are related to non-vocal behaviors in mouse pups
Mouse pups produce ultrasonic vocalizations in response to isolation from the nest and cold exposure (i.e., isolation USVs). Rates and acoustic features of isolation USVs change dramatically over the first two weeks of life, and there is also substantial variability in the rates and acoustic features of isolation USVs at a given postnatal age, both between different pups and between different recordings from the same pup. The factors that contribute to within-age variability in the rates and acoustic features of isolation USVs remain largely unknown. Here, we explore the extent to which non-vocal behaviors of mouse pups account for the variability in rates and acoustic features of USVs within a given age. We recorded isolation USVs and non-vocal behaviors of C57BL/6J mouse pups at four postnatal ages (postnatal days 5, 10, 15, and 20) and applied a combination of traditional hand-picked features and an unsupervised machine-learning-based vocal analysis method to analyze acoustic features of pup USVs within each age. When categories of non-vocal behavior were considered (i.e., locomotion, wriggling, grooming, or lying still), we found that mice in all postnatal age groups produced higher rates of isolation USVs when engaged in active behaviors than when lying still, but USVs produced during these different categories of non-vocal behaviors did not differ substantially in their acoustic features. To further examine the relationship between non-vocal behavior and vocalization rates, we quantified ‘movement intensity’ in each trial by creating a vector of the pup’s total movement between pairs of consecutive video frames and compared this vector to the pup’s USV rate. This

analysis revealed that rates of isolation USVs are strongly correlated to movement intensity within a given trial, particularly at younger postnatal ages. Our findings suggest that levels of behavioral arousal account for variability in rates of isolation USVs within a given age group, and future work can continue to explore factors that may account for variability in the acoustic features of isolation USVs.

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Poster

305. Vocal/Social Communication: Non-Avian

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Topic: F.01. Neuroethology

Support: NIMH R01MH122752
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Title: Segmentation of mouse social behavior using an unsupervised machine learning approach

Authors: *J. D. TURK[1,2], M. R. WARREN[3,4], J. P. NEUNUEBEL[1,2];
[1]Psychological & Brain Sci., [2]Bioinformatics Data Sci. Grad. Program, Univ. of Delaware, Newark, DE; [3]Dept. of Biol., Emory Univ., Atlanta, GA; [4]Ctr. for Translational Social Neurosci., Emory Natl. Primate Res. Ctr., Atlanta, GA

Abstract: Animals display diverse behavioral repertoires (Mackintosh & Grant, 1963) and identification of these behaviors is critical for deciphering the relationship between vocal communication, social behavior, and the neural circuits that encode this information (Kingsbury et al., 2019). While there are many existing approaches for distinguishing behaviors (Kabra et al., 2013; Segalin et al., 2021), most methods are hindered by user bias and the inability to partition all of the data. To overcome these limitations, we developed an unsupervised machine-learning approach that applies a Self-Organizing Map (SOM; Kohonen, 2001) model to group similar patterns in movement and social interaction, as defined by a set of numeric features (e.g., velocity, relative orientation) for each frame of recorded video using an egocentric framework. Within the model, a term for weighting features from previous frames gives temporal context during training. We applied our computational model to a 5-hour recording of freely socializing adult male (n = 2) and female (n = 2) B6.CAST-Cdh23Ahl+/Kjn mice (Sangiamo et al., 2020). The model projected each frame of video (n = 540,000 frames) into a 400-node map (20 x 20 node grid), with each node representing a similar set of behavioral features that described the egocentric framework of a mouse. To compare the similarity of the behavioral features in a node to other nodes on the map, we calculated average feature distance and physical distance between ~2.6 billion pairs of frames. We found a positive correlation between the average feature distances and physical distances such that frames with similar features are closer together on the map (distance correlation, n = 219, dCor = 0.88, $p < 0.001$). To compare unsupervised and

supervised machine-learning approaches, we segmented social and non-social behaviors using JAABA (Kabra et al., 2013). We created classifiers for twelve behaviors to label about 137,000 frames of behavior for a single male mouse in our recording. The labeled data was contained within 313 out of the 400 nodes on the map. For the nodes on the map with labeled data, 79 contained a single category of labeled data (e.g., only frames from fights). Another 22 nodes had at least 85% of the frames labeled as a single type of behavioral event. This shows that the behavioral patterns extracted by our model are similar to the output of a supervised machine learning approach. Our preliminary results suggest that our unsupervised machine-learning approach may represent a powerful tool for segmenting dynamic multiple-animal social behavior.

Disclosures: **J.D. Turk:** None. **M.R. Warren:** None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

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Program #/Poster #: 305.16

Topic: F.01. Neuroethology

Title: Elucidating communicative functions of female mouse ultrasonic vocalizations during opposite-sex interactions

Authors: *C. A. MALONE, J. W. SOKOL, K. A. TSCHIDA;
Cornell Univ., Ithaca, NY

Abstract: Vocalizations produced by males and females during courtship interactions may influence mate-choice and reproductive success, and in turn, affect an individual's fitness. While both male and female mice produce ultrasonic vocalizations (USVs) during opposite-sex interactions, females tend to produce a small proportion (~15%) of the USVs, and these female USVs are acoustically similar to USVs produced by males. These factors have made it difficult to study the contribution of female USV production to courtship. To overcome these challenges, we used a combination of viral tools and activity-dependent labelling to ablate male midbrain neurons essential for USV production, generating male mice that are 'muted' for USV production but still court females at normal levels. By measuring the vocal behavior of females as they interact with these muted males, we tested the hypothesis that female courtship USVs act as a signal for sexual receptivity. Female mice were recorded during interactions with muted males, allowing us to assign detected USVs to the female, and vaginal cytology was used to measure female estrous state in each trial. We predicted that sexually receptive females would produce higher rates of USVs during interactions with males than non-receptive females. Surprisingly, our preliminary data suggest that females produce few or no USVs during interactions with muted males, regardless of estrous state. In ongoing work, we are recording interactions between females and muted males while playing back male courtship USVs, to test the idea that male USVs are necessary to elicit female USV production during courtship.

Disclosures: **C.A. Malone:** None. **J.W. Sokol:** None. **K.A. Tschida:** None.

Poster

305. Vocal/Social Communication: Non-Avian

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Topic: F.01. Neuroethology

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Title: Evaluating mate-selection preferences of individual mice during courtship

Authors: *D. PANDE[1,2], M. R. WARREN[1,4], R. S. CLEIN[1], J. D. TURK[1,3], Y. MA[1,2], J. P. NEUNUEBEL[1,2];
[1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci. Grad. Program, [3]Bioinformatics Data Sci., Univ. of Delaware, Newark, DE; [4]Biol., Emory Univ., Atlanta, GA

Abstract: Animals modify courtship behaviors by observing or mimicking the behaviors of other animals (Freeberg, 2000). Female mice, for example, exhibit mate-choice copying, whereby one female prefers a male chosen by a different female (Kavaliers et al., 2006). While olfactory cues play a significant role in mate-choice copying (Ferkin, 2018), it is less clear how other social signals influence courtship and mate selection in naturalistic conditions. To address this question, we recorded adult mice (13-21 weeks old; B6.CAST-Cdh23Ahl+/Kjn) interacting in mixed-sex groups (n = 11; two males and two females per group) (Sangiamo et al., 2020). Using a supervised machine-learning approach (Kabra et al., 2013), we extracted distinct courtship behaviors such as approaching (number: 142.6 ± 49.2 (mean ± sd), duration: 90 ± 29.4 seconds), investigating (number: 189.4 ± 77.8, duration: 127.2 ± 68.1), chasing (number: 92.1 ± 43.4, duration: 173.8 ± 102.2), and mounting (number: 3.4 ± 7.2, duration: 18.6 ± 36.8). For each behavior and animal, we calculated a preference index. To determine preference indices, we took the difference in how long the animal engaged with both animals of the opposite sex and then divided the difference by the total duration. Preference indices were also computed based on how often the behaviors occurred. Indices ranged from -1 to +1, where positive values indicated that the animal engaged with the first animal of the opposite sex more than the second animal. We found a significant positive correlation between the preference indices of simultaneously recorded females for the number of times that females approached males (Pearson's correlation, $r = 0.75$, $p < 0.05$) as well as for the total duration that females approached males (Pearson's correlation, $r = 0.77$, $p < 0.05$), suggesting that both females preferred the same male. We observed a significant positive correlation between the preference indices of concurrently recorded males for the number of times that males chased females (Pearson's correlation, $r = 0.62$, $p < 0.05$) as well as for the total duration that males investigated females (Pearson's correlation, $r = 0.68$, $p < 0.05$), suggesting that both males preferred the same female. In 7 recordings, both males attempted to copulate, and in 6 of these recordings, the males pursued the same female. Our results suggest that socially interacting males and females show similar mate

selection preferences during unrestrained, naturalistic behavior. Future analyses using a sound source localization system (Warren et al., 2018) may shed light on the role vocalizations play in mate-selection preferences.

Disclosures: **D. Pande:** None. **M.R. Warren:** None. **R.S. Clein:** None. **J.D. Turk:** None. **Y. Ma:** None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.18

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Examining social vocalizations and movement of freely interacting anosmic mice

Authors: *K. CHEN[1], J. P. NEUNUEBEL[1,2];
[1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci., Univ. of Delaware, Newark, DE

Abstract: During socialization, the actions of others influence the behavior of an individual, and the social cues that mediate the interactions are detected through multiple sensory modalities, including olfaction and audition (Chen & Hong, 2018). During distinct social behaviors, mice emit specific types of ultrasonic vocalizations (USVs), which modulate the behavior of social partners (Sangiamo et al. 2020). Additionally, olfactory signals act as powerful communication signals (Cheal & Sprott, 1971), but it is unclear how olfaction affects the relationship between vocalizations and behaviors. To answer this question, we first sought to render mice anosmic. We performed nasal lavage on adult (PD49) C57BL/6J mice with either 0.7% Triton X-100 (n = 10, male = 6, female = 4) or saline (n = 10, male = 5, female = 5) and used an olfactory avoidance test adapted from Brai and Alberi (2015) to evaluate olfactory function 1 day before and after treatment. Across all recordings, animals spent 44.5 ± 15.1 seconds (mean ± standard deviation) near the aversive odor (2-MB) and 74.6 ± 16.6 seconds near the neutral odor (distilled water). We used an avoidance index (AI) ranging from -1 to 1 to quantify olfactory function, such that smaller values indicate more time near 2-MB and larger values denote more time near distilled water. We found that mice treated with Triton X-100 spent more time near 2-MB after lavage than before (pretreatment AI: 0.26 ± 0.26; posttreatment AI: 0.07 ± 0.36; paired-samples t-test, $t = 1.87$, $p < 0.05$). Moreover, Triton X-100 treated mice spent more time near 2-MB than saline-treated mice (Triton X-100 AI: 0.07 ± 0.36; saline AI: 0.36 ± 0.11; independent samples t-test, $t = 2.29$, $p < 0.05$). Interestingly, both Triton X-100 and saline-treated mice showed a significant reduction in movement compared to pretreatment (paired-samples t-test for both groups, $t > 3.15$, $p < 0.01$), but Triton X-100 treated mice moved less than saline-treated mice (independent samples t-test, $t = 1.79$, $p < 0.05$). These results suggest Triton X-100 treated mice exhibit anosmia in conjunction with a reduction in movement and set the stage to probe how

olfaction mediates the dynamics between vocalization and social behaviors using a sound source localization system (Warren et al., 2018).

Disclosures: K. Chen: None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.19

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Sex differences in the acoustic directionality of mouse ultrasonic vocalizations

Authors: *Y. MA[1,2], M. R. WARREN[1,3], J. P. NEUNUEBEL[1,2];
[1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci., Univ. of Delaware, Newark, DE;
[3]Biol., Emory Univ., Atlanta, GA

Abstract: In vocally communicating animals, bioacoustic directionality defines the focus of the vocalization (Patricelli, Dantzker et al. 2007) and may affect the exchange of information pertinent to social behavior (Yorzinski and Patricelli 2010). Both male and female animals vocalize across the animal kingdom (Saino, Galeotti et al. 2003, Warren, Clein et al. 2020). However, it is unclear if there are potential sex differences in the acoustic directionality of vocalizations. To determine if sex differences in acoustic directionality exist, we quantified and compared male and female ultrasonic vocalizations (USVs) by employing an eight-microphone array. USVs were recorded and assigned to freely interacting animals in two-mouse (male-female dyads) and four-mouse (2 males and 2 females) social contexts. To gain a comprehensive view of mouse USV directionality, we used two methods. First, we examined the radiation pattern for each vocalization (males emitted 22,268 and females emitted 4,091 in the two-mouse context; males emitted 73,488 and females emitted 16,072 in the four-mouse context). Next, we quantified the overall radiation pattern for each mouse in a given context (8 males and 7 females in the two-mouse context; 22 males and 22 females in the four-mouse context). Radiation patterns were evaluated with a directional index bounded between -1 and 1, which represented a highly focused beam posterior and anterior to the vocalizer, respectively. For individual vocalizations, male and female directional indices had medians of 0.13 (IQR = 0.29) and 0.03 (IQR = 0.34), respectively, in the two-mouse context; and medians of 0.19 (IQR = 0.48) and 0.08 (IQR = 0.55), respectively, in the four-mouse context. For the overall radiation pattern, male and female directional indices had medians of 0.14 (IQR = 0.01) and 0.01 (IQR = 0.03), respectively, in the two-mouse context; and medians of 0.10 (IQR = 0.01) and 0.03 (IQR = 0.04), respectively, in the four-mouse context. Regardless of the method, male mice had higher directional indices than females in both social contexts (Wilcoxon Rank Sum Test, for the overall pattern of each mouse: W = 92 for the two-mouse context; W = 726 for the four-mouse context; for the pattern

for each vocalization: $W > 3.0 \times 10^8$ for the two-mouse context; $W > 3.4 \times 10^9$ for the four-mouse context; all p-values < 0.001). Overall, our results demonstrate sex differences in the bioacoustics of mouse USVs such that male mice produced USVs that were more directional than those of females.

Disclosures: Y. Ma: None. **M.R. Warren:** None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.20

Topic: F.01. Neuroethology

Support: NIMH R01MH122752

Title: Characterizing ultrasonic vocalizations of Piezo2-deficient mice during group interaction

Authors: *C. M. KAWAR[1,2], J. D. TURK[1,3], J. P. NEUNUEBEL[1,2];
[1]Psychological & Brain Sci., [2]Interdisciplinary Neurosci. Grad. Program, [3]Bioinformatics Data Sci., Univ. of Delaware, Newark, DE

Abstract: Vocalizations shape animal social behavior by alerting conspecifics to predators or reproductive opportunities-which in turn elicits distinct responses (Seyfarth et al., 1980; Macedonia, 1990; Evans, 1993). Other sensory stimuli, such as tactile stimulation, are required for the successful performance of particular behaviors (Chen & Hong, 2018). While mice produce specific types of ultrasonic vocalizations (USVs) associated with discrete behaviors (Sangiamo et al., 2020), it is unclear how gentle touch, mediated through Piezo2 receptors (Woo et al., 2014), affects the relationship between vocalization and behavior. To investigate the relationship between vocalizations and gentle touch, we recorded audio and video data with a sound-source localization system (Warren et al., 2018) as mixed-sex groups (2 males and 2 females per group) of adult (8-10 weeks old) Piezo2 knockout mice (KO; n = 4 groups) or wildtype controls (WT; 4 groups) interacted for 5 hours. In total, there was a significant difference between the number of vocalizations emitted by the KO (n = 16; mean = 1777.3; SD = 1473.3) and WT (n = 16; mean = 1327.2; SD = 2437.1) animals (Wilcoxon rank-sum test, W = 328, $p < 0.05$). The difference between the numbers of vocalizations produced by KO males (n = 8; mean = 2751; SD = 1519.2) and WT males (n = 8; mean = 2069.6; SD = 3300.8) was not statistically significant (Wilcoxon rank-sum test, W = 84, $p > 0.05$), and neither was that between KO females (n = 8; mean = 803.6; SD = 419.9) and WT females (n = 8; mean = 584.8; SD = 756.8) (Wilcoxon rank-sum test, W = 81, $p > 0.05$). We next compared the acoustic features of vocalizations emitted by individual KO or WT mice, grouped by sex. Neither low frequency nor duration differed between genotype in either sex (Wilcoxon rank-sum test, $W > 54$, $p > 0.05$). However, in both male- and female-emitted vocalizations, peak frequencies were significantly lower for KO mice compared to WT (Wilcoxon rank-sum test, $W > 45$, $p < 0.05$). Both for male-

and for female-emitted vocalizations, the bandwidths (frequency ranges) of vocalizations were shorter in KO mice than in WT (Wilcoxon rank-sum test, $W > 38$, $p < 0.001$). Taken together, our results suggest that, during multi-mouse social interactions, the acoustic features of the vocalizations produced by mice with tactile sensation differed from those produced by mice lacking the ability to sense gentle touch.

Disclosures: **C.M. Kawar:** None. **J.D. Turk:** None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.21

Topic: F.01. Neuroethology

Support: NIH 2P2G0GM103653
NIMH R01MH122752

Title: Characterizing collective behavior of Shank3b mice using a sound source localization system

Authors: *R. S. CLEIN[1], M. R. WARREN[1,3,4], J. P. NEUNUEBEL[1,2];
[1]Psychological and Brain Sci., [2]Interdisciplinary Neurosci., Univ. of Delaware, Newark, DE; [3]Dept. of Biol., Emory Univ., Atlanta, GA; [4]Emory Natl. Primate Res. Ctr., Ctr. for Translational Social Neurosci., Atlanta, GA

Abstract: Autism spectrum disorders (ASDs) are highly heritable neurodevelopmental conditions characterized by deficits in social behavior and communication. While the influence of genetic alterations on ASD-like phenotypes in individuals is well-studied, the impact of ASD symptoms on group dynamics is less clear. Here, we used a preclinical mouse model of Phelan-McDermid syndrome, a monogenic form of ASD resulting from mutations to the SHANK3 gene (Peca et al., 2011), to investigate group social dynamics. We continuously recorded audio and video data using a sound source localization system (Warren et al., 2018) while mixed-sex groups (2 males and 2 females per group) of either Shank3b knockout mice (KO; $n = 6$ groups) or wild-type controls (WT; $n = 7$ groups) freely behaved for five hours. In KO groups, the most prevalent dyadic social interactions were those between females (Kruskal-Wallis Test with Dunn-Sidak post hoc correction; $H(5) = 30.95$, $p < 0.01$). Social interactions in groups of WT animals were not monopolized by a particular dyad (Kruskal-Wallis Test with Dunn-Sidak post hoc correction; $H(5) = 6.57$, $p = 0.09$). WT males had a higher vocal rate during social interactions than WT females (Wilcoxon rank sum test, $W = 264$, $p < 0.01$) but, there were no differences between males and females in KO mice (Wilcoxon rank sum test, $W = 153.5$, $p = 0.86$). To quantify collective behavior, we developed a computational approach that assesses social cohesion over time based on the spatial location of each mouse, relative to the other mice, at every frame of video. We found that KO females contributed significantly more to group

cohesion than KO males, but there were no differences between WT males and females (Kruskal-Wallis with Dunn-Sidak post hoc correction; $H(3) = 14.77$, $p < 0.05$). We next applied a progressive k-means clustering algorithm to the cohesion metric values and identified eight different categories of group behavioral events. Across the entire dataset, the eight types of behavioral events lasted, on average, from 0.6 to 1.7 seconds, occurred 928 to 15,899 times, and had cohesion values ranging from 5.09 (more cohesion) to 36.14 (less cohesion). When comparing the different types of group behavioral events between WT and KO groups, we found notable differences in temporal patterning, number of events, and vocal emission around the onset of events (Wilcoxon rank sum tests, all significant U values > 60, all significant p values < 0.05). Taken together, our results suggest that genetic modifications to the SHANK3 gene differentially affect males and females, and may lead to alterations in group-level dynamics, thus providing further insight into the collective behavior of animals.

Disclosures: R.S. Clein: None. **M.R. Warren:** None. **J.P. Neunuebel:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.22

Topic: F.01. Neuroethology

Title: Changes in breathing rates as source for acoustic variation in rat ultrasonic whistling

Authors: *T. RIEDE;
Midwestern Univ., Midwestern Univ., Glendale, AZ

Abstract: Vocal production is closely associated with respiratory movements. To generate vocal signals, the brainstem generated breathing rhythm must be modulated to allow the conversion of aerodynamic into acoustic energy at the larynx level. Rodents of the murid family use a whistle mechanism to generate high frequency whistles for vocal communication. The sound is generated during an expiration when the airflow interacts with structures inside the larynx generating pressure fluctuations. The expiratory airflow critically influences acoustic features of the ultrasonic whistle. Here I investigated the effect of breathing adjustments on acoustic properties of rat ultrasonic vocalizations. Rats produce 22-kHz calls, one category of ultrasonic whistles, in different situations, for example in response to a mild noxious stimulus, during treadmill exercises, or in a novel environment. To test whether the overall breathing rate during a specific context is associated with vocal breathing patterns embedded in the prevailing breathing rhythm, subglottal pressure was measured in 8 male SD rats at rest in a novel environment, after a mild noxious stimulus and during treadmill running. If laryngeal vocal movements are associated with overall breathing rate, vocal characteristics should change as dictated by the breathing pattern. Average call duration was shorter when overall breathing rate increased, but fundamental frequency did not change with context. The vocal production mechanism was also susceptible to disturbances at high running speeds when breathing rates were highest. For

example, calls were interrupted or completely absent while the underlying vocal breathing pattern was still present. Results lend support to the hypothesis that vocal patterns are entrained to prevailing breathing patterns and that non-vocal breathing movements surrounding a vocal production affect the vocal breath.

Disclosures: T. Riede: None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.23

Title: WITHDRAWN

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.24

Title: WITHDRAWN

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.25

Topic: F.01. Neuroethology

Support: NIH Grant R01 DC012087

Title: Modeling the natural dynamics of marmoset monkey 'conversations'

Authors: *D. M. GRIJSEELS, D. A. FAIRBANK, C. T. MILLER;
Cortical Systems and Behavior Lab., UC San Diego, La Jolla, CA

Abstract: An important aspect of dyadic communication is determining when each conversation partner vocalizes. Various animal species, including humans, employ implicit rules that determine the communication dynamics between partners. Common marmosets (*Callithrix*

jacchus) use long-range phee calls to make contact with conspecifics in the absence of visual access. During these contact calls, marmosets rarely interrupt each other, and show coordination of calling time and duration, suggesting they also follow implicit rules for turn-taking. However, how marmosets determine when to call in these vocal exchanges is still unclear. In this study, we examined the communicative behavior of pairs of marmosets. We included 110 pairings of 40 unique animals. The marmosets were put in test boxes on either side of a sound-proof room for 30 minutes per session, while visual access was obscured with an opaque curtain. Vocalizations were recorded using directional microphones. We determined phee call timing for each monkey based on the loudness or the sound, and the frequency profile. Based on these timing data, we propose a novel stochastic model that better captures the marmoset calling dynamics. We show that marmosets do not, as previously suggested, act as coupled oscillators, as shown by the lack of coordination in their intercall interval. Instead, a marmoset's proclivity to respond to individual calls of their partner monkey is largely independent of that particular monkey's behavior, including response delay and intercall interval. Instead, they have periods of increased overall calling, which we deemed the active state, in which they are more likely to initiate and continue a conversation. Overall, we used natural monkey calling behavior to develop a novel model of marmoset communication dynamics. We plan to use this model in future experiments where we replace one monkey with a computer generated virtual monkey whose respective vocal behavior is determined by machine learning algorithms, allowing for naturalistic interrogation of the underlying processes that govern this communicative behavior, and development of a powerful platform for neurobiological investigations.

Disclosures: D.M. Grijseels: None. **D.A. Fairbank:** None. **C.T. Miller:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

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Program #/Poster #: 305.26

Topic: F.01. Neuroethology

Support: R01 DC012097
R01 NS109294
R01 NS118457
19RT0316
U01 NS116377
U24 MH123423

Title: Cross-modal representations of individual identity in primate hippocampus

Authors: *T. J. TYREE[1], M. J. METKE[3], C. T. MILLER[2];
[1]Physics, UC San Diego, San Diego, CA; [2]Cortical Systems and Behavior Lab., UC San Diego, La Jolla, CA; [3]Neurosciences Grad Program, UCSD, San Diego, CA

Abstract: The ability to recognize the identity of other individuals is integral to social living, as it is necessary for the myriad cognitive processes routinely employed by individuals navigating the complex dynamics of societies, such as memory, decision-making and communication amongst others. While evidence of neural representations for individual identity for a single, sensory modality (e.g. face, voice, odor, etc.) are evident in several species, compelling evidence for cross-modal representations of individual identity have been limited. The most compelling example is 'concept cells' in human hippocampus that selectively respond to the picture, written and spoken name of famous individuals. Here we tested whether cross-modal representations of identity are also evident in the hippocampus of a nonhuman primate: common marmoset. We recorded the activity of ~2400 single neurons while presenting N=4 subjects with faces and voices of a large battery of familiar conspecifics as both unimodal and cross-modal stimuli. During cross-modal stimulus presentations, the face and voice were either from the same individual (Match) or different individuals (Mismatch). Analyses showed several novel findings that lend unique insight into the nature of this fundamental social cognitive process. We show the first evidence of putative concept cells in a nonhuman animal. A population of marmoset hippocampus neurons exhibited similar, invariant, highly selective responses to the face and voice of specific individuals. Furthermore, a population decoder could almost perfectly distinguish between Match and Mismatch trials, even though subjects were presented with ~10 different individuals in each recording session. This decoder was so robust that ~50 randomly selected neurons from the entire population achieved ~80% reliability suggesting that cross-modal representations of identity in primate hippocampus are highly distributed. In fact, removal of the identified putative concept cells did not impact the performance of the decoder, indicating that these neurons may not play a critical role in identity representations in this structure. Finally, additional analyses identified a power law relationship across the neural population, which supports these social representations as hierarchically organized, consistent with what is known about the social structure of nonhuman primates. These compelling findings shed new insight into the nature of identity representations in primate hippocampus by demonstrating that cross-modal representations of identity are not only evident but are a facet of social recognition that supports a robust, distributed coding mechanism.

Disclosures: **T.J. Tyree:** None. **M.J. Metke:** None. **C.T. Miller:** None.

Poster

305. Vocal/Social Communication: Non-Avian

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 305.27

Topic: F.01. Neuroethology

Support: R01 NS113071

Title: Relating vocal behavior to territorial interactions in the singing mouse (S. Teguina)

Authors: *Y. FUJISHIMA, M. A. LONG;
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Abstract: Vocal interactions between Alston's singing mouse (Scotinomys teguina) provide an excellent opportunity to investigate the neural mechanisms enabling socially-relevant sensorimotor interactions. In response to a conspecific's song, singing mice produce an immediate vocal response, and this temporally precise behavior is known as counter-singing. Despite similarities to human conversational exchanges, the ethological relevance of this behavior in singing mice remains poorly understood. To address this issue, we monitored the behavior of singing mice in a 3' by 4' terrarium. In addition to natural bedding material (e.g., wood chips), arenas were outfitted with 10 custom-built shelters that provided visual occlusion. We used a specialized microphone to continuously record vocalizations and a thermal camera to successfully track the behavior of mice, even in cases where they were actively hiding under a shelter. Machine learning behavioral tracking software (SLEAP) was then applied to the thermal images to quantify physical interactions. In preliminary experiments, we found that 'resident' males often favor a single shelter within the terrarium. When 'intruder' males are placed within the arena, their singing often triggered the resident to initiate physical interactions including aggressive behaviors, suggesting that the song may be used in the context of territorial disputes. This study provides a critical ethological context for vocal sensorimotor transformations in the singing mouse and will help to inform further investigations into relevant neural mechanisms.

Disclosures: Y. Fujishima: None. **M.A. Long:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.01

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Hippocampus-synthesized estrogen and androgen rapidly modulate dendritic spines and LTP in non-genomic manner

Authors: *S. KAWATO, M. SOMA, M. OGIUE-IKEDA;
Univ. of Tokyo, Tokyo, Japan

Abstract: We demonstrated (1) hippocampal synthesis of estrogen and androgen, and (2) non-genomic synaptic modulation by these sex-steroids. [Synthesis] We showed expression as well as neuronal/synaptic localization of essential enzymes in the adult male rat hippocampus. Mass-spectrometric analysis demonstrated that hippocampal levels of estradiol (E2), testosterone (T), dihydrotestosterone (DHT) were around 8 nM for all, higher than in plasma. Castration significantly decreased T and DHT in the hippocampus. Castration did not decrease male hippocampal E2, indicating that E2 is synthesized from hippocampal T. Female hippocampal levels of E2 (0.5-4 nM), and T (1 nM) were less than male, but much higher than in plasma.

[Synaptic Modulation] E2-induced rapid non-genomic modulation (1 h) was demonstrated by analysis of dendritic spines and LTP of adult male rat hippocampal 'acute' slices (steroid-depleted slices). Dendritic spine analysis was performed for pyramidal neurons in hippocampal slices. The density of spines and their head diameters were obtained by mathematical software Spiso-3D which identifies spines by calculating geometrical parameters. E2 at 1 nM rapidly increased the density of small-head spines, in CA1. T and DHT increased the density of small-head and large-head spines. Signaling pathways are: synaptic ERalpha or AR→LIMK, MAPK, PKA, PKC, Src →cofilin or cortactin → actin polymerization→ new spines. LTP analysis showed that 1 nM E2 induced full-LTP (E2-LTP) upon sub-threshold stimulation, although without E2 the sub-threshold stimulation did not induce full-LTP. Kinase inhibitors against MAPK, PKA, PKC blocked E2-LTP. Rapid E2 synthesis can also be measured with LTP in hippocampal slices. References: Kimoto et al., 2001 Endocrinology, Hojo et al., 2004 PNAS, Mukai et al., 2007 J. Neurochem, Hojo et al., 2009 Endocrinology, Mukai et al. 2011 Cerebral Cortex, Ooishi et al. 2011 Cerebral Cortex, Okamoto et al., 2012, PNAS, Kato et al., 2013, Frontier Neurosci. Hasegawa et al., 2015 Brain Res., Hatanaka et al., 2015 Brain Res., Murakami et al., 2015 Brain Res., Soma et al., 2018 Frontier Neurosci., Hojo and Kawato 2018 Frontier Neurosci.

Disclosures: S. Kawato: None. **M. Soma:** None. **M. Ogiue-Ikeda:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.02

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Weston Brain Institute
Ontario Veterinary College
Ontario Graduate Scholarship - Queen Elizabeth II Graduate Scholarship in Science and Technology

Title: Effects of Long-Term 5α-androstane-3α,17β-diol Treatment on Cognition and Alzheimer's Disease-Related Neuropathology in the 3xTg-AD Mouse Model of Alzheimer's Disease

Authors: *K. TANG[1], N. R. KUWAHARA[1], S. BHULLAR[1], K. C. NICHOLSON[1], L. K. ISAACS[1], J. COSCHIGNANO[1], B. D. WINTERS[2], N. J. MACLUSKY[1];
[1]Biomed. Sci., [2]Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: Age-related decline in levels of circulating gonadal steroid hormones has been linked to the development of Alzheimer's disease (AD). Clinical observations indicating an association between low levels of testosterone and an increased risk of AD, further support the idea that androgens may play a neuroprotective role in the brain [Hogervorst *et al* 2004 Exp Gerontol, 39:

1633]. Our group has previously found that the neurosteroid metabolite of testosterone, 5α-androstane-3α,17β-diol (3α-diol), may have a protective effect against β-amyloid (Aβ) induced neurotoxicity *in vitro* [Mendell *et al* 2016 Endocrinology, 157: 4570]. Here, we aimed to investigate the effects of long-term 3α-diol administration in 12-month-old 3xTg-AD mice. At 3 months of age, male and female wild-type and 3xTg-AD mice received subcutaneous implants of blank or 3α-diol dipropionate-filled Silastic capsules. The capsules were replaced every 3 months for a total of 9 months of treatment. At 12 months of age, open-field (OF) and ORM testing was conducted to assess anxiety-related behaviours and memory (n=10-17/group). Animals were sacrificed one month after completion of the behavioural experiments. Hippocampi were homogenized to analyze changes in AD-related protein expression by western blotting (n=6-7/group). Brains were also collected to assess hippocampal (HPC) dendritic morphology using Golgi-Cox staining. For OF behaviours, 3α-diol-treated females had greater locomotor activity compared to vehicle-treated females, whereas 3α-diol-treated males had less locomotor activity compared to vehicle-treated males. In the ORM task, vehicle-treated 3xTg-AD males had significantly impaired ORM at both short-and long-term retention delays, whereas 3α-diol-treated 3xTg-AD males had intact short- and long-term ORM. HPC Aβ expression was greater in 3xTg-AD females than in 3xTg-AD males, but no significant 3α-diol treatment effect was observed. Preliminary dendritic morphology results revealed significant truncation of CA1 apical dendritic length and branching in vehicle-treated 3xTg-AD males, which was less pronounced after 3α-diol treatment. These findings suggest that long-term 3α-diol treatment may play a neuroprotective role against cognitive and morphological AD-related deficits, in a sex-specific manner.

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Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.03

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Administration of progesterone, but not 17-beta-estradiol, tends to improve diazepam-induced place recognition memory deficit in female rats

Authors: *T. HATAKEYAMA[1,2], A. HAYASHI[2], K. TOKURA[2], A. ISHII[3], S. KOBAYASHI[1,2], G. WATANABE[1,2,4], M. KAWAGUCHI[2,3];
[1]Organization for the Strategic Coordination of Res. and Intellectual Property, [2]Lab. of Animal Behavior and Envrn. Science, Sch. of Agr., [3]Lab. of Animal Behavior and Envrn. Science, Grad. Sch. of Agr., Meiji Univ., 1-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa, Japan; [4]Lab. of Vet. Physiol., Tokyo Univ. of Agr. and Technol., 3-5-8 Saiwaicho, Fuchu, Tokyo, Japan

Abstract: Benzodiazepines, including diazepam (DZP), are used in a variety of applications such as anxiolytics, sedatives, and hypnotics. They cause recognition memory impairment as a side effect, which is known to bind to a unique site of the gamma-aminobutyric acid type-A ($GABA_A$) receptor. Its side effect has been reported to change with fluctuations of ovarian hormones across menstrual cycles. However, despite more women use benzodiazepines than men, little is known about the effects of DZP or the other benzodiazepines on place recognition memory of female rats as an animal model. Here, we used female rats to investigate the influence of fluctuations in estrus cycle, progesterone (P4), or 17-beta-estradiol (E2) on DZP-induced place recognition memory deficit by using a spontaneous place recognition (SPR) test. This test utilizes the natural tendency of the rat to explore a novel object location more than the previously experienced one.
All female Wistar-Imamichi rats experienced an SPR test at the age of 11-week-old as follows. Thirty minutes after all animals were administered intraperitoneally vehicle (saline plus tween80) or DZP, they experienced the SPR test that consisted of a sample phase (10 min), in which rats were allowed to explore freely two identical objects in an arena, a delay of 10 min, and a test phase (5 min), in which one object was moved to a new position in the arena. Preference was assessed by comparing the time spent exploring the familiar versus novel object location.
In experiment 1 using 57 intact female rats, we observed the smear in morning for consecutive 10 days, and then decided the estrus cycle in each animal. In the SPR test, the rats during proestrus, but not the other phases (metestrus/diestrus and estrus phases), even with the treatment of DZP explored significantly a novel object location more than the familiar one.
In experiment 2 using 58 female rats, they underwent ovariectomy and the transplantation surgery of the P4 silastic capsules, which were containing P4 powder or empty capsules as a control, or the E2 silastic capsules, which were containing E2 dissolved in oil. At least a week after the surgery, the SPR tests were performed. As a result, rats with the chronic treatment of P4, but not E2, tended to prefer a novel object location to the familiar one even when they were treated with DZP.
In conclusion, the influences of proestrus phase and the higher level of P4 in female rats tended to improve the DZP-induced SPR deficit. This finding suggests that P4 or the P4 metabolites, but not E2, affect $GABA_A$ receptors involved in the ability to remember spatial information.

Disclosures: T. Hatakeyama: None. **A. Hayashi:** None. **K. Tokura:** None. **A. Ishii:** None. **S. Kobayashi:** None. **G. Watanabe:** None. **M. Kawaguchi:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.04

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Appetitive social learning and social competition in rats

Authors: M. Y. C. CHEN[1], ***R. I. WOOD**[2];
[1]Integrative Anatom. Sci., Keck Sch. of Med. of USC, Los Angeles, CA; [2]Keck Sch. Med. USC, USC, Los Angeles, CA

Abstract: Social learning reduces potential risks of trial-and-error learning, and is an efficient way of acquiring information. Rats use social threat learning to minimize risk, as in fear contagion. However, few studies have explored instrumental social reward learning in this species. We determined if male rats acquire a visual cue task (VCT: pressing the lever under an illuminated stimulus light to receive a food reward) faster when they observe a trained demonstrator from behind a mesh screen. Observer rats (n=8/group) were initially trained to respond on both levers to receive 45 mg sugar pellets. Afterwards, they were tested for VCT in 90 trials/day. Before each day of testing, they observed a trained demonstrator perform VCT for 30 min. Controls had no demonstrator. Observing a demonstrator did not enhance VCT acquisition. In a subsequent study, observers were trained separately to operate either the right or left nose-poke to receive pellets from a trough on the opposite wall. They were tested together without barriers to evaluate social learning and social competition. When tested as pairs, rats made 72.2±4.4% of their responses on the nose-poke for which they were trained. When tested alone, they made 91.9±2.7% of responses on the trained nose-poke. Together, these results suggest that rats do not make substantial use of instrumental social reward learning. When tested as pairs, rats compete for sugar pellets. Typically, 1 rat responded, and his partner obtained the pellet. To avoid losing the pellet reward to the partner, omissions increased to 30.9±1.4 per 50 trials, vs 2.7±0.7 when tested alone. In cagemate pairs, the dominant rat made twice as many responses as the subordinate (13.0±2.1 vs 6.1±1.2 trials, $p<0.05$), but the subordinate received more pellets (11.7±1.7 vs 4.0±0.8, $p<0.05$). Both rats guarded the pellet trough when their partner responded on a nose-poke (88.8±5.7% of trials), and the responding partner attempted to displace the recipient 47.5±6.5% of the time. Pretreatment with 0.1 mg/kg oxytocin had no effect, but the dopamine D1-like receptor agonist SKF81297 reduced guarding and displacement. These results demonstrate that rats alter their behavior in response to social competition, and that dopamine D1-like receptors may be involved.

Disclosures: M.Y.C. Chen: None. **R.I. Wood:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.05

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: RF1-AG-041374 from the National Institute on Aging (to J.M.D.)

Title: Rapid and local estrogen synthesis supports long term potentiation of hippocampal Schaffer collateral in ovariectomized mice.

Authors: *M. J. MAROTEAUX[1,3], J. M. DANIEL[1,3], L. A. SCHRADER[2,3];
[1]Psychology, [2]Cell & Mol. Biol., Tulane Univ., New Orleans, LA; [3]Tulane Brain Inst., New Orleans, LA

Abstract: In older humans and in rodent models, higher levels of estrogen activity in the brain are associated with enhanced brain and cognitive aging even in the absence of circulating estrogens (E2). We have previously shown that the growth factor IGF-1 (insulin-like-1 growth factor) can activate or phosphorylate estrogen receptor α in the hippocampus. This activation requires concomitant activity of brain-derived estrogen also known as neuroestrogens (nE2) synthetized by the enzyme aromatase (ARO). Neuroestrogen supports hippocampal excitatory neurotransmission and long-term potentiation (LTP), a critical process for memory formation. This study aims at understanding whether nE2 and IGF-1 support LTP in absence of ovarian estrogen in the hippocampus. We hypothesize that IGF-1 regulates the activity of aromatase promoting the synthesis of nE2 and ultimately improved cognitive functions.In a first set of experiments, we investigated the impact of acute inhibition of ARO on LTP. Sixty to 90 days old female C57BL6 mice were ovariectomized and 10 days later their brains were collected and sliced for electrophysiological recordings. Field excitatory post synaptic potentials (fEPSPs) were evoked in CA3 and recorded in CA1, and long-term potentiation was induced by theta-burst stimulation (TBS) to Schaffer collaterals after 20 minutes of stable baseline. The aromatase inhibitor, letrozole (LET, 100 nM) and/or 17-β-estradiol (10 nM) were applied for at least 30 min prior to LTP induction. The development of LTP was monitored for at least 60 minutes. Blocking nE2 synthesis with LET prior or during, but not after TBS, prevented the development of the LTP, supporting an activity-dependent role of nE2 during the induction but not the maintenance of LTP. Exogenous E2 alone did not affect the amplitude of the LTP but potentiated the fEPSPs at baseline.
A second set of ongoing experiments will assess whether IGF-1 can regulate the ARO activity after TBS. In the same conditions as the previous experiment, des-1,3 IGF-1 (10.8 nM), a synthetic form of IGF-1, will be applied in presence or absence of LET. We expect des-IGF to potentiate LTP when applied alone but have no effect when applied alongside LET. These results contribute to the understanding of the regulation of local neuroestrogens synthesis their role in hippocampal synaptic plasticity suggesting an important role for learning and memory.

Disclosures: M.J. Maroteaux: None. **J.M. Daniel:** None. **L.A. Schrader:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.06

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Williams College

Title: Emotion regulation strategies and latent trait cortisol: The moderating role of internalizing symptoms

Authors: *C. J. SU[1], C. B. STROUD[2];
[1]Psychology, Williams Col., Williamstown, MA; [2]Psychology, Williams Col., WILLIAMSTOWN, MA

Abstract: Stress confers risk for internalizing psychopathology in part by altering hypothalamic pituitary adrenal (HPA) axis activity. Yet, no prior study has tested if the ways individuals modulate their emotional responses to stress (i.e., emotion regulation [ER] strategies) prospectively predict trait cortisol, leaving questions about the physiological effects of such strategies over longer time periods. Further, few studies have tested whether the impact of ER strategies on HPA axis regulation differs for those with current and past internalizing psychopathology. To address these gaps, we tested whether various ER strategies predicted latent trait cortisol (LTC), and whether internalizing symptoms moderated these effects. Emerging adults (n=90; Mage = 19.36 years) completed self-report measures of ER strategies and diagnostic interviews assessing lifetime and current internalizing symptoms. Participants provided saliva samples 4 times per day over three 3-day sampling waves spanning 13 weeks (sampling compliance monitored objectively). An across-wave LTC factor was identified using the first 2 samples from each of the 9 sampling days (CFI=.93, RMSEA=.05). Structural equation models showed that two aspects of emotional reactivity (intensity: β= -.25; p =.013; sensitivity: β= -.23; p =.027) and co-rumination (β= -.36; p=.002) were each related to lower across-wave LTC, regardless of level of internalizing symptoms. However, the effect of excessive reassurance-seeking (ERS) on the across-wave LTC was moderated by internalizing symptoms (β= .33; p =.042), such that greater ERS predicted lower across-wave LTC only for those with lower levels of symptoms (b = -.03, p=.005). All results held when adjusting for sampling compliance, and health and behavioral covariates. Together, findings indicate that the tendencies to easily experience intense emotions, as well as excessively discuss problems and negative emotions in close relationships, predict lower LTC, a pathway to later psychopathology. Further, results suggest that excessive reassurance seeking may have different physiological effects for those without internalizing symptoms. Such findings highlight specific ER strategies that can be targeted in efforts to promote adaptive HPA axis regulation and prevent the later development of internalizing psychopathology.

Disclosures: C.J. Su: None. **C.B. Stroud:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.07

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC Grant
Ontario Graduate Scholarship
Ontario Veterinary College Doctoral Scholarship

Title: Sexually differentiated effects of bisphenols on stress- and sex steroid-sensitive hippocampal signalling cascades and cognition in mice

Authors: *K. C. NICHOLSON[1], J. WOODMAN[1], D. AL-NAJAFI[1], E. CHOLERIS[2], N. MACLUSKY[1];
[1]Biomed. Sci., [2]Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: The ovarian steroid 17β-estradiol (E2) activates rapid non-genomic signalling mechanisms that may mediate E2's neuroprotective and synaptogenic effects in the mammalian hippocampus, thereby enhancing learning and memory. These mechanisms may be impaired by exposure to either endocrine disrupting chemicals (EDCs) or stressful stimuli. Bisphenol A (BPA) is a potent EDC that disrupts the rapid signalling and synaptogenic effects of E2. However, many of these E2-sensitive mechanisms are also sensitive to glucocorticoids and their disruption has been associated with increased anxiety. Therefore, many manufacturing processes have replaced BPA with structural analogues including Bisphenol S (BPS), although its relative safety as a BPA substitute is unclear. To compare the effects of BPA and BPS in the mammalian hippocampus, 2-3-month-old male and female CD-1 mice were administered vehicle, BPA, or BPS-containing peanut butter at the U.S. tolerable daily intake (TDI) dose of 50 μg/kg/day for 10 consecutive days. On day 10, hippocampi were collected 4 hours after treatment and protein lysates were analyzed for E2- and glucocorticoid-sensitive signalling proteins including ERK, JNK, Akt, mkp3, GFAP, IBA-1, and PSD-95 (n=7-9/group). Females treated with 50 μg/kg/day BPA and BPS exhibited significantly reduced hippocampal mkp3 expression (a phosphatase implicated in female-specific stress and depression). Subsequent dose-response studies examined the effects of multiple bisphenol doses on hippocampus-dependent learning, memory, and anxiety behaviours. Male and female 2-3-month-old mice were administered vehicle, BPA, or BPS-containing peanut butter at 5 μg/kg/day, 25 μg/kg/day, or 50 μg/kg/day for 10 consecutive days (n=10-12/group). On day 9, mice were tested for object placement behaviour 4 hours following treatment to assess spatial memory. Males treated with 25 μg/kg/day BPA, 25 μg/kg/day BPS, or 50 μg/kg/day BPS and females treated with 5 μg/kg/day BPA, 25 μg/kg/day BPA, or 50 μg/kg/day BPS exhibited impaired spatial memory. Anxiety behaviour was tested using the light-dark paradigm, and results indicated that light duration was significantly reduced in males treated with 50 μg/kg/day BPS, and females treated with 25 μg/kg/day BPA. On day 10 of treatment, all mice were sacrificed for biochemical, morphological, and immunohistochemical analyses of hippocampal tissue and ongoing studies aim to finalize these endpoints. These findings suggest that both BPA and BPS may exert sexually differentiated effects within the mammalian hippocampus that resemble those induced by physiological stress.

Disclosures: K.C. Nicholson: None. **J. Woodman:** None. **D. Al-Najafi:** None. **E. Choleris:** None. **N. MacLusky:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.08

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Natural Sciences and Engineering Research Council of Canada (NSERC)

Title: Estrogen replacement impairs the learning of environmentally conditioned disgust behaviour (anticipatory nausea) in ovariectomized female rats

Authors: *V. MATIC, I. R. BISHNOI, K.-P. OSSENKOPP, M. KAVALIERS;
Univ. of Western Ontario, London, ON, Canada

Abstract: Anticipatory Nausea (AN) is a form of classical conditioning in which the emetic effects of a nausea-inducing toxin, such as lithium chloride (LiCl), become paired with environmental stimuli. In rats, AN can be modeled through conditioned disgust with the occurrence of conditioned gaping behaviour (gaping), a large opening of the mouth with bottom incisors protruding, displayed upon re-exposure to a LiCl-paired context. A sex difference has been observed in that female rats display significantly more gaping than male rats upon LiCl-free context re-exposure. Further, female rats in the proestrus phase of the rodent estrous cycle (high estrogen) during conditioning and re-exposure display more gaping than those in the diestrus phase (low estrogen), suggesting that estrogen is involved in the learning of conditioned disgust. The present study uses an ovariectomy and estrogen replacement paradigm, removing the influence of other gonadal hormones and isolating the role of estrogen in conditioned disgust. It was hypothesized that rats treated with daily estradiol and conditioned with LiCl in a novel context would display significantly more gaping than controls upon re-exposure. Thirty-two adult female ovariectomized rats were split into four groups of n = 8, receiving daily estradiol (E, 10 µg, 100 µl) or oil (O, 100 µl) for 10 days, and context-conditioned with lithium chloride (LiCl, 128 mg/kg, 20 ml/kg) or saline (NaCl, 0.9%, 20 ml/kg) for 30 min on days 2, 4, 6, and 8. Contrary to the hypothesis, the E-LiCl group displayed significantly less gaping behaviour than the O-LiCl group upon LiCl-free re-exposure to the conditioning context on day 10, suggesting that estradiol impairs the learning of conditioned disgust. These findings suggest that sex differences in conditioned disgust may not be attributable to estrogen.

Disclosures: V. Matic: None. **I.R. Bishnoi:** None. **K. Ossenkopp:** None. **M. Kavaliers:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.09

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Oscar Stern Award/Eisenberg Family Depression Center University of Michigan to NCT

Title: Regulation of stress and anhedonia in a mouse model of hormonal contraceptives

Authors: *K. M. SCHUH[1], T. DAVIS[2], J. AHMED[1], C. XU[1], S. KWAK[1], N. C. TRONSON[1]; [1]Univ. of Michigan, Ann Arbor, MI; [2]Columbia Univ., New York, NY

Abstract: Hormonal contraceptives (HCs) are a critical part of healthcare, with broad health benefits beyond reproductive control, and economic benefits for individuals and families. HCs affect mood and cognition in ways that are not fully understood. For many individuals, these effects are beneficial, with decreased premenstrual mood changes and overall better mood. Yet for ~10% of people, HCs trigger adverse mood effects and increased risk for depression. Research on HC users shows that side-effects of HCs on the brain may be due to (a) direct agonistic effects of the synthetic hormones on estradiol and progesterone receptors; (b) indirect effects of HCs on decreased endogenous hormone levels; or (c) off-target effects of HCs, including androgenicity of progestin components, and modulation of the stress response. Here, we used a mouse model of oral contraceptive hormone exposure, newly developed in our laboratory, to identify how different HC formulations regulate stress responses and contribute to increased or decreased vulnerability to stress-induced behavioral changes. Female C57Bl6 and Balb/c mice (n=8-12) were given ethinyl estradiol (EE, 0.02μg) and a progestin - either the androgenic levonorgestrel (LVNG, 0.75μg) or the anti-androgenic drospirenone (DRSP, 3.75μg) - daily in 0.25mL 10% sucrose; control animals received 0.25mL of 10% sucrose. At these doses, contraceptive hormones have no gross effects on cognition or locomotor activity, and do not increase behavioral despair. They, however, decrease sucrose preference, suggesting specific anhedonic-like effects. In parallel with findings from people using HCs, mice treated with EE+LVNG, but not DRSP, had significantly blunted stress-induced corticosterone. We investigated the interactions of HCs and stress using RNAscope and immunohistochemistry to identify HC-induced changes in glucocorticoid and mineralocorticoid receptors, and stress-induced signaling pathways. Together these findings demonstrate that the interaction of HCs with HPA axis and stress-related signaling are key mechanisms for vulnerability and resilience to stress-induced depression and suggests that identifying individual differences in stress responsivity may help predict which individuals will benefit from which HC formulations.

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Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.10

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant 5 F31 MH126511-02
Rackham Merit Fellowship
Eisenberg Depression Center STAR Award

Title: Effects of ventral subicular activation on open field exploration and hypothalamic-pituitary-adrenal axis reactivity in male and female mice

Authors: *E. S. GEWARGES[1], C. JOHNSTON[2], C. N. SNYDER[1], S. E. YANG[1], J. L. SPENCER-SEGAL[3];
[1]Neurosci. Grad. Program, Univ. of Michigan, Ann Arbor, MI; [2]Michigan Neurosci. Institite, Ann Arbor, MI; [3]Michigan Neurosci. Inst., Univ. of Michigan Med. Sch., Ann Arbor, MI

Abstract: In the face of a potential threat, individual differences in risk-taking behavior are paralleled by changes in hypothalamic-pituitary-adrenal (HPA) axis activation. Previous studies implicated the ventral subiculum (vSUB) in the shared control of the behavioral and endocrine response to a potential threat since vSUB-lesioned rats showed lower risk-taking and higher stress-induced corticosterone (CORT). Therefore, we hypothesized that vSUB activity drives risk-taking behavior and HPA axis inhibition in the face of a potential threat. To test this hypothesis, we chemogenetically activated the vSUB before open field (OF) exploration. In 64 (half female) 12-15 week old C57Bl/6J mice, we injected AAV-hSyn-hM3D(Gq)-mCherry or AAV-hSyn-mCherry in the vSUB. Three weeks later, we intraperitoneally injected mice with either clozapine-N-oxide (CNO) or Vehicle 20 minutes before a 10-minute OF test and collected blood immediately and 25 minutes after testing. Chemogenetic vSUB activation did not clearly alter risk-taking behavior. There was no difference between groups in total distance traveled (no main effect of drug ($p = 0.1717$) or virus ($p = 0.7299$), and no interaction ($p = 0.4631$). DREADD mice spent less time in the center than mCherry mice (main effect of virus $p = 0.0178$), there were no differences between CNO and Vehicle groups (no main effect of drug ($p = 0.1276$), and no interaction ($p = 0.3149$). There were no changes in CORT levels at either time point, but a trend showed that DREADD CNO mice had higher CORT than other groups at the second time point (Virus x drug interaction, $p = 0.1174$). The findings suggest that the presence of DREADD altered risk-taking behavior independently of CNO, though the reason for this is unclear. The lack of effect could be due to insufficient DREADD activation and the specific anatomic subset of vSUB neurons that were activated. Overall, these results show that chemogenetic activation of vSUB neurons in this manner did not change risk-taking behavior or HPA axis reactivity.

Disclosures: E.S. Gewarges: None. **C. Johnston:** None. **C.N. Snyder:** None. **S.E. Yang:** None. **J.L. Spencer-Segal:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.11

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: R15 MH116345
8G12MD007579-279
GM082406

Title: Resiliency to develop a PTSD-like phenotype is associated with low FKBP5 expression in the ventral hippocampus

Authors: *N. IRIZARRY-MÉNDEZ[1], A. HERNANDEZ[2], M. COLON[1], J. T. PORTER[1]; [1]Neurosci., Ponce Hlth. Sci. Univ., Ponce, PR; [2]Neurosci., Ponce Hlth. Sci. Univ., PONCE, PR

Abstract: Fear is an adaptive response to harmful circumstances that includes a physiological stress response to aid learning. Failure to adjust to these responses can lead to the development of neuropsychiatric disorders such as post-traumatic stress disorder (PTSD). PTSD is a condition in which fear acquired during a traumatic experience becomes persistent and difficult to overcome. Animal studies suggest that abnormal glucocorticoid signaling in the ventral hippocampus (VH) caused by stress may play a role in the development of PTSD. Stress-induced excessive glucocorticoid signaling is modulated by FKBP5. FKBP5 is a cochaperone in the glucocorticoid receptor complex that reduces glucocorticoid signaling and could influence conditioned fear responses. However, it is unclear if altered FKBP5 expression contributes to the development of PTSD. In this study, we combined molecular and behavioral approaches to examine whether FKBP5 expression in the ventral hippocampus (VH) is sufficient to affect fear-related behaviors. We hypothesized that reducing FKBP5 protein expression in the VH would increase glucocorticoid signaling to enhance stress-coping behavior and decrease fear acquisition. Adult male rats were injected with either a control virus or an AAV5 vector expressing four distinct shRNAs targeting FKBP5 and the fluorescent marker mCherry into the VH, to reduce FKBP5 levels. After infusing rats, we confirmed that shRNA against FKBP5 reduced FKBP5 levels. Infusion of FKBP5-shRNA in VH decreased fear acquisition and facilitated extinction memory. Reducing VH FKBP5 did not affect anxiety-like behavior in either the open field test or the elevated zero maze test. However, the reduction of FKBP5 increased passive behavior during the forced swim test, suggesting enhanced stress-coping behavior during an acute stressor. Our findings suggest that lower expression of hippocampal FKBP5 may increase resiliency to the development of PTSD-related behaviors and passive coping behavior after trauma exposure.

Disclosures: N. Irizarry-Méndez: None. **A. Hernandez:** None. **M. Colon:** None. **J.T. Porter:** None.

Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.12

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC Discover Grant

Title: The Effects of Hormonal Contraceptives on Spatial Navigation in Intact Female Rats

Authors: *E. L. GOMEZ-PERALES[1], I. FERRAZ[2], L. C. DE MELO RODRIGUES[2], W. G. BRAKE[1];
[1]Ctr. for Studies in Behav Neuro, Concordia Univ., Montréal, QC, Canada; [2]Univ. Federal do Espírito Santo, Vitória, Brazil

Abstract: Rats can use different strategies to navigate their environment such as response memory, place memory, or the memory of a single cue. The strategy a female rat uses to solve a maze changes depending on circulating levels of estrogens and progesterone. Administration of ethinyl estradiol (EE) and levonorgestrel (LNG), which are synthetic hormones found in the most commonly used hormonal contraceptives, disrupts the estrus cycle. Here we investigated how these hormones affect memory bias. Gonadally-intact Long-Evans female rats were assigned to 6 groups. There were three cycling groups; Proestrus, Estrus, Diestrus - and three hormone treatment groups; EE (10 μg/kg), LNG (20 μg/kg) EE+LNG (10+20 μg/kg). They received daily subcutaneous injections of either sesame oil (for the cycling groups), or EE, LNG, or EE+LNG for 21 days and the phase of their estrus cycle was tracked daily using vaginal cytology. Rats were then tested on two separate Morris Water Maze tasks designed to differentiate the use of response memory, place memory, or the use of a single cue to find the hidden platform. In both tasks, the start position and the position of the hidden platform remained fixed across the 8 learning trials. In the first task, rats were trained to seek the escape platform located directly under a suspended cue. On the probe trial, the position of the cue was changed but the start position remained the same. In the second task, rats were trained to seek the escape platform located in a specific quadrant. On the probe trial, the start position of the rat was changed to the opposite side of the pool in order to distinguish the use of place memory or response memory. While EE and LNG, alone or in combination, prevented cycling in all female rats, they biased a different memory system to solve the maze. Both female rats receiving EE+LNG and those in estrus were biased to response memory, and did not attend to the cue in the cue task. Female rats receiving LNG alone and those in proestrus were biased to place memory in the place versus response task while female rats receiving EE alone and those in diestrus used both place and response memory equally. These data demonstrate that hormonal contraceptives affect spatial memory in rats and their effect depends upon the formulation of the hormonal contraceptive.

Disclosures: E.L. Gomez-Perales: None. **I. Ferraz:** None. **L.C. de Melo Rodrigues:** None. **W.G. Brake:** None.

Poster

306. Hormones and Cognition

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Program #/Poster #: 306.13

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Research Institute of the McGill University Health Centre (RI-MUHC) and Merck, Sharp & Dohme Corp. (TVN)
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McGill University Graduate Excellence Fellowship in Mental Health Research (CC)
Ferring Fellowship in Reproductive Health (SLJ)
Canadian Institutes of Health Research (3D Study; CRI 88413)

Title: Prenatal paternal anxiety predicts child's neuroendocrine function and internalizing symptoms during adrenarche

Authors: *S. JONES[1,2,7], V. DE BRAGA[2,7], C. CACCESE[3,7], J. LEW[3,7], G. ELGBEILI[7,8], N. CASTELLANOS-RYAN[9], S. PARENT[9], G. MUCKLE[11], C. M. HERBA[12,13], W. D. FRASER[14], J. TRASLER[7,4], J. R. SÉGUIN[10,13], T. MONTREUIL[5], T.-V. NGUYEN[6];
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Abstract: INTRODUCTION. The aim of this study was to examine whether paternal pre- and post-natal anxious and depressive symptoms predict child neuroendocrine structures and function, as well as child cognitive and behavioral outcomes during middle childhood. Middle childhood coincides with major neuroendocrine changes including increased secretion of dehydroepiandrosterone (DHEA) and its sulfated metabolite (DHEA-S), as the child enters the first stage of puberty called adrenarche. These hormones are triggered by hypothalamic-pituitary-adrenal axis activation, and are implicated in corticolimbic brain development.
METHOD. Participants were recruited from a subsample of the 3D Study, a large prospective birth cohort study. We conducted a follow-up study to assess the role of paternal prenatal and postnatal factors on child neuroendocrine and behavioral development at 6-8 years old (N=61, 36 boys, 25 girls). Parental anxious and depressed symptoms were collected via validated questionnaires. Prenatal measures assessed at a single point during pregnancy include: an anxiety questionnaire (STR), the Perceived Stress Scale (PSS) and the Center for Epidemiologic Studies Depression (CES-D), and at the 6-8 year follow-up: the Beck Anxiety and Beck Depression Inventories. Children provided salivary hormone samples and structural magnetic resonance imaging (MRI) of the brain. Pituitary gland volume was measured by manual segmentation of the MRI scans. Child behaviors were measured by the Strengths and Difficulties Questionnaire, and cognitive outcomes were measured by a qualified psychologist using the WISC-V. Multiple regression was used to test whether paternal pre- and postnatal mental health predicts child neuroendocrine outcomes, adjusting for the equivalent maternal mental health measure and sex

of the child. Significant regressions were followed up to assess potential cognitive and behavioral repercussions using mediation models.
RESULTS. 1) Father's prenatal anxiety (STR) predicted lower DHEA levels in the child, but not pituitary volume. Sex was not significant. 2) Higher paternal prenatal anxiety predicted more child internalizing symptoms mediated by lower levels of concurrent DHEA. The postnatal model was not statistically significant.
CONCLUSION. These results highlight the often-overlooked role of paternal influence on child development, suggesting that paternal prenatal anxiety can influence child neuroendocrine function and internalizing symptoms during middle childhood. This period is important as children acquire a host of new cognitive and behavioral skills with the transition from kindergarten to elementary school.

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Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.14

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NFSC Grant 32130045
NFSC Grant 31522028

Title: Stress hormone rhythmicity proactively promotes brain network states and dynamic transitions during multi-task processing

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Abstract: Stress hormone, one of human’s major glucocorticoid caused by hypothalamus-pituitary-adrenal (HPA) axis activity, is vital for proactively modulating human’s cognition and emotion to meet the upcoming demands. Recent study identified a proactive role of CAR, the phenomenon of stress hormone’s bursts that occurred upon awakening from night sleep, for adaptively modulating executive control resources through core neural circuit during working memory. However, whether such burst of stress hormone also efficiently regulate large-scale network dynamics to meet both workload and emotional demands and even their interactions remain elusive. Here, we implemented a dataset of functional magnetic resonance imaging (fMRI) together with pharmacological manipulation of the CAR to investigate whether CAR-induced stress hormone bursts serve as a general catalyst to proactively modulate dynamical communicability among brain networks supporting efficient cognitive process. In day 1, two

group participants orally received either a dose of 0.5mg Dexamethasone (DXM group) or an equal amount of Vitamin C (placebo group) and participate in successive Resting, N-back and Emotional matching tasks while under fMRI scanning in day 2. We took the advantage of Hidden Markov Model (HMM) to quantitatively measure time-resolved large-scale brain configuration (states) and estimate state dynamics of two groups across three tasks. We first found that different cognitive tasks are featured by their task-specific brain state dynamics in both DXM group and placebo group. Specifically, we find DXM group and placebo group differed in several specific state during rest and n-back task, featured by decreased activity in lateral executive control network (ECN) and default mode network (DMN), paralleling with previous study. And a dominate state during emotion matching has significantly higher occupancy rate in DXM group compared with placebo group, featured by high-level visual networks. More interestingly, we found significantly more frequent transitions among states during n-back and emotion matching in DXM group while no transition frequency difference during rest between two groups, suggesting a higher state dynamic volatility phenomenon in DXM group during cognitive and emotion tasks, Together, our results extended previous research of cortisol awakening response and find a crucial role of stress hormone in proactively promoting efficient brain state reconfiguration that vital for multi-task processing in human.

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Poster

306. Hormones and Cognition

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Support: Foundation for Science and Technology (FCT) - project UIDB/50026/2020
Foundation for Science and Technology (FCT) - project UIDP/50026/2020
NORTE-01-0145-FEDER-000039

Title: Iodineminho study: impact of iodine supplementation in pregnant women

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Abstract: INTRODUCTION Iodine is required for the synthesis of thyroid hormones, which are essential for the proper development of the central nervous system. At present, pregnant women

are moderately iodine deficient worldwide, including in several European countries. In the Minho region of Portugal, we showed in 2009 that the median urinary iodine concentration (UIC) was below the desired values in women before, throughout and after pregnancy, and in their newborns and infants. Consequently, the Portuguese Health Authorities (DGS) have recommended a daily supplementation with 150-200 µg iodine in preconception, pregnancy, and lactation. OBJECTIVES The IodineMinho study intends to study the impact of this iodine supplementation recommendation on the prevalence of iodine deficiency in pregnant women from the Minho region of Portugal, and whether the timing when the supplementation starts influences maternal and children health. MATERIAL AND METHODS The recruitment of women in preconception or already pregnant is ongoing from 10 representative Family Health Units of Grouping of Health Centers (ACES-Cávado I). Physician's approach and prescriptions, sociodemographic, nutrition and clinical information is obtained at baseline. To evaluate endocrine function and urinary iodine levels, blood and urine samples are collected. Maternal thyroid volume is evaluated by ultrasound scan at recruitment. RESULTS AND CONCLUSION Eighty-two women are currently actively enrolled (from a total of 110 recruitments, 15 were excluded and 13 had a 1st trimester abortion), 28 (34%) were recruited in preconception and 54 (66%) in the first trimester of pregnancy. Mean age was 31.8 ±4.7 years and they belong to class I (61%), II (30%) and III (9%) of Graffar scale. Iodine supplementation was started in 60 (73%) women, 18 (30%) in preconception, 36 (60%) in the 1st trimester and 6 (10%) in the 2nd trimester of gestation. Regarding the type of iodine supplementation, 37 (62%) started supplementation with isolated potassium iodide. Regarding parity, 49 (60%) were nulliparous, 30 (37%) had one birth and 3 (3%) had 2 births; 5 (6%) of the recruited women had thyroid pathology. Ongoing measurements of endocrine parameters will allow understanding its association with the type and the time of iodine supplementation.

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Poster

306. Hormones and Cognition

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.16

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Iowa Osteopathic Education and Research Foundation

Title: Controlling voluntary exercise behavior through interactions between sex hormone and neural activity

Authors: *V. MATHIS, L. POINTS, M. MOHAMED, N. VENKATESWARAN, C.-M. LEE, L. WINTER, S. CLAYTON, L.-L. YUAN;
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Abstract: Exercise exerts beneficial effects on mental and physical health. A sedentary lifestyle, on the other hand, is a major risk factor for chronic disease. However, humans display heterogenous levels of participation in regular exercise and the mechanisms which regulate exercise behavior itself are not clear. Using a rat voluntary wheel running (VWR) paradigm, we identified intriguing sex differences suggesting female sex hormones exert an additional, strong regulation of running behavior which offers a unique opportunity to examine the mechanisms responsible for driving exercise behaviors. Compared to males, female rats exhibited significantly higher levels of average running; individual females also exhibited a repetitive peak-valley pattern of running activity, with peaks coinciding with the proestrus stage (highest estrogen level) in the rat estrous cycle. Ovariectomy (OVX) drastically lowered their running activity and completely eliminated the running peaks. Estrogen replacement in an OVX background restored the running level and reproduced the running peaks observed in naïve females. These results suggest a causal link between estrogen and running activity. However, if rats had no exposure to wheel running prior to OVX, estrogen replacement was unable to restore the running activity of the OVX rats. This surprising discovery suggests both female sex hormone (estrogen) and running induced neural activation are required simultaneously to maintain running activity in females. We are currently investigating what molecular events may underlie the neural activation induced by wheel running.

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Poster

306. Hormones and Cognition

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 306.17

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Assessing whether testosterone enhances place and response learning in male rats through an androgen-dependent pathway

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Abstract: Testosterone has complex dose-dependent effects on spatial working memory in male rats, with a low physiological dose enhancing response learning and a high physiological dose enhancing place learning. Testosterone can influence the brain through androgen-dependent and estrogen-dependent molecular pathways. In a series of experiments, we tested whether the effects of testosterone on place and response learning are androgen dependent. Adult male rats (3-8/group) were bilaterally castrated and given daily injections of various drugs starting 7 days prior to maze habituation and continuing through habituation and testing (12 days total). A plus-maze was used to test rats on either a place task (learning the position of a goal arm relative to

distal spatial cues) or a response task (learning to turn a particular direction). All rats were given 100 trials on the maze, and criterion was reached when a rat made 9 out of 10 correct choices. Our first experiment involved testing the effects of the non-aromatizable androgen 5α-dihydrotestosterone (DHT) at three does (0.5, 1.0, and 2.0 mg/kg) along with a negative control group (sesame oil) and positive control groups (0.5 or 2.0 mg/kg testosterone). We replicated previous results, with low and high doses of testosterone improving performance on the response and place tasks, respectively. On the place task, a high dose of DHT (2.0 mg/kg) significantly improved performance relative to the oil-injected control group. On the response task, both low (0.5 mg/kg) and intermediate (1.0 mg/kg) doses of DHT improved performance. In our second experiment, rats were given testosterone doses that were previously shown to enhance place or response learning along with an aromatase inhibitor (1 mg/kg letrozole) to reduce testosterone's conversion to estradiol. A high dose of testosterone (2.0 mg/kg) was again found to enhance place learning, whereas letrozole had no effect on place learning. In contrast, neither letrozole nor a low dose of testosterone were found to influence response learning, but this experiment needs further replication (n = 3-6/group). Both experiments indicated that testosterone enhances place learning through an androgen-dependent pathway. The results for response learning are less clear but suggest that androgens are only partially responsible for testosterone's effects on response learning. Additional analyses are underway to assess whether hormone manipulations influenced levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and striatum. Overall, our results provide evidence that androgens enhance spatial learning through dose-dependent effects upon place and response learning.

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Poster

306. Hormones and Cognition

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Topic: F.02. Neuroendrocrine Processes and Behavior

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VEGA 1/0635/20, National Scholarship Programme of the Slovak Republic, SAIA, n.o., Comenius University grant UK/14/2022 and Tatra bank foundation (NTB) grant to MP.

Title: The memory-enhancing effects of 17B-estradiol depend on astrocyte-to-neuron glycogenolisis in the dorsal hippocampus of ovariectomized female mice

Authors: *G. BARROSO MACHADO[1], M. PILLEROVA[2], L. TAXIER[1], T. BRANYAN[3], F. SOHRABJI[4], K. M. FRICK[1];

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Abstract: Rationale: Estrogens play a pivotal role in memory consolidation, and the infusion of 17β-estradiol (E_2) into the dorsal hippocampus (DH) enhances memory consolidation in ovariectomized female rodents in multiple hippocampal-dependent memory tasks such as object recognition (OR) and object placement (OP). Although glycogenolysis and subsequent astrocyte-to-neuron lactate transport in DH astrocytes are critical for memory consolidation, little is known about their contributions to the memory-enhancing effects of E2. **Objective:** Our goal was to determine the extent to which E2-mediated memory enhancement depends on astrocytic glycogenolysis in the DH. **Methods:** Young C57BL/6 female mice (7-12/group) were underwent bilateral ovariectomy and triple cannula implantation (targeting the DH and dorsal third ventricle) and recovered for 14 days. Immediately after training in OR and OP, mice were infused into the DH with vehicle or the glycogenolysis inhibitor 1,4-dideoxy-1,4-imino-d-arabinitol (DAB, 1 pmol), and into the dorsal third ventricle with vehicle or E_2 (10 µg). Time spent with the new or moved object was evaluated 24 and 48 h after training for OP and OR, respectively. **Results:** Post-training DH infusion of DAB significantly blocked the E2-induced enhancement of memory consolidation in both OR and OP. Because the dose of DAB used does not impair memory on its own, these data suggest that DAB specifically reduces the beneficial effects of E2. **Conclusions and future directions:** Our data suggest that glycogenolysis and subsequent astrocyte-to-neuron lactate transport in the DH are essential for E_2 to enhance multiple forms of hippocampus-dependent memory in ovariectomized mice. Ongoing studies are assessing treatment effects on lactate activity and cell signaling (CREB, cofilin, and Arc) in DH astrocytes.

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Poster

306. Hormones and Cognition

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Program #/Poster #: 306.19

Topic: F.06. Autonomic Regulation

Support: Canadian Institutes of Health Research (FDN-148413)

Title: Exploring neurotensin receptor type 1 biased agonism : how to induce hypothermia over hypotension ?

Authors: *F. BÉLAIR[1], J. DE NEVE[2], E. VANGELOVEN[2], M. VIVANCOS[1], É. EISELT[1], J. CÔTÉ[1], J.-M. LONGPRÉ[1], S. BALLET[2], P. SARRET[1];

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Abstract: Therapeutic hypothermia represents a strategy for protecting the brain for a variety of emergency situations, including ischemic stroke and trauma. Indeed, mild hypothermia (32-35°C) has shown significant benefits against various acute brain insults, including neuroinflammation and excitotoxicity. To date, targeted temperature management is only possible through internal or external cooling interventions, which have serious drawbacks such as unwanted shivering, vasoconstriction reactions, and poor applicability in a prehospital setting. The pharmacological management of hypothermia with drugs capable of controlling the hypothalamic set point thus represents a promising avenue for regulating body temperature in critically ill patients. Among the pharmacological options, there is compelling evidence demonstrating that neurotensin (NT) analogs targeting the NT receptor type 1 (NTS1) produce a sustained, dose-dependent decrease in body temperature. Importantly, recent studies have pointed to the therapeutic benefits of NTS1 agonists in inducing mild hypothermia in clinically relevant conditions, such as intracerebral hemorrhage, ischemic stroke, or traumatic brain injury. However, NTS1 activation also induces robust hypotension, which may seriously compromise the neuroprotection action of NT analog-mediated hypothermia against acute brain injury. In this context, the concept of ligand-biased signaling represents a very promising way to distinguish desired physiological effects from undesirable ones. We, therefore, characterized the signaling signatures of a series of NTS1 analogs and finely monitored their physiological responsiveness. To this end, we selected from the literature NT analogs with different physiological efficacy on hypotension and hypothermia and with key substitutions with natural and unnatural amino acids at the Arg^{8}, Arg^{9}, Tyr^{11} and Ile^{12} positions. In addition to their binding affinities, BRET-based biosensors were used to determine the potency and efficacy of each compound to activate $G_{\alpha q}$, $G_{\alpha oA}$, $G_{\alpha i1}$, $G_{\alpha 13}$ and recruit β-arrestin 2.Moreover, production of the second messengers IP1 and cAMP was assessed by TR-FRET, and bias factors calculated using the Black-Leff operational model. Finally, each compound was tested on SD rats to determine its physiological effectiveness in lowering body temperature and blood pressure. Together, our preliminary results suggest that certain downstream signaling pathways might be responsible for hypothermia over hypotension.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.01

Topic: F.03. Stress and the Brain

Support: NIAAA R01-AA025844
NIAAA R01-AA013892

Title: Altered neural and behavioral responses to stress and alcohol cues in individuals with early trauma

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Abstract: Introduction: Early trauma (ET) is associated with greater risk of negative health outcomes, including alcohol use disorder (AUD). Prior neuroimaging studies with ET individuals have focused on stress-related neural circuits, and effects on risk of AUD are unclear. To elucidate neural correlates of ET and associated AUD risk, the current study investigated behavioral and neural responses to alcohol and stress cues in social drinkers with and without ET using functional magnetic resonance imaging (fMRI). Methods: Fifty-four social drinkers (mean age = 31.4, 26 females) were recruited, consisting of demographically matched (age, gender, Race) individuals who had experienced ET (N = 26) or no ET (NT; N = 28) and were similar in baseline alcohol use. ET was determined by the Childhood Trauma Questionnaire. During fMRI, participants were administered a well-validated emotion provocation task, where they viewed stress cue, alcohol cue, and neutral-relaxing pictures in a randomized block design and rated their levels of emotional valence, stress, and alcohol craving. Results: Behavioral results indicated group differences in baseline and task responses; at baseline, ET showed greater stress ($p = 0.030$) and more negative valence ($p = 0.013$) compared to NT. During the task, ET showed increased stress ($p = 0.012$) during alcohol cue, while they showed increased craving during stress compared to NT ($p = 0.014$). fMRI results using whole brain analyses at $p<.001$ (cluster corr at $p=.05$) indicated significant group differences in the prefrontal-striato-limbic regions. During stress vs neutral cues, compared to NT, the ET group showed hyperactivity in limbic regions (amygdala, hippocampus, parahippocampal gyrus), dorsal striatum, and dorsal anterior cingulate cortex (dACC), but hypoactivity in the prefrontal cortex (PFC) including ventromedial PFC (vmPFC) and lateral PFC (lPFC). For alcohol vs neutral cues, the ET group showed hyperactivity in the dorsal striatum and dACC, and hypoactivity in medial PFC and dorsolateral PFC compared to the NT group. Discussion: Our findings indicate altered behavioral and neural responses to alcohol cues in addition to stress in ET individuals. Altered neural responses are characterized by hypoactivity in prefrontal regulatory regions, but hyperactive limbic-striatal response to stress, and striatal response to alcohol cue, suggesting compromised prefrontal control over subcortical stress and reward processing regions. These dysregulated neural patterns may signal ET individuals' susceptibility to dysregulation of reward-seeking behaviors, increasing their vulnerability to stress- and to alcohol-related disorders.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.02

Topic: F.03. Stress and the Brain

Support: CIC-UMSNH-BFN-2021-2022

Title: Comparison of chronic- and acute- stress effects on the activities of oxidative stress enzymes in the hippocampus of male and female rat pups

Authors: *L. TORNER[1], D. I. TALAVERA MONDRAGÓN[2], B. FENTON-NAVARRO[2]; [1]Mexican institute of social security (medical) IMSS, Morelia, Mexico; [2]Lab. Glicobiología y Farmacognosia, Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez" UMSNH, Morelia, Mexico

Abstract: Early life stress (ELS) increases the risk of developing psychiatric disorders later in life such as major depression. Maternal separation in rodents reproduces many of the physiological alterations induced by ELS. Stress is known to increase the production of reactive oxygen species (ROS) in the adult brain. However, it has not been fully addressed in the developing brain. Our aim was to analyze the activity of several antioxidant enzymes and parameters of oxidative stress in male and female rat pups under basal, acute stress or chronic stress. Methodology: We used 4 groups of Sprague Dawley rat pups (males and females, n = 7-9 each). Groups were: Control-basal (CB), control-stress (CS), maternal separation-basal (MSB), maternal separation-stress (MSS). In control groups, pups were kept always with the dams in their nests. MS consisted in isolating each pup from its mother and siblings for 3h from postnatal day (PN) 1 to PN14. Acute stress procedures consisted in isolating the pups from their mother and siblings 3h before euthanasia. On PN15, all pups received euthanasia and their hippocampi were isolated and homogenized. International guidelines for animal research were followed. Activities of Superoxide dismutase (SOD), Catalase (CAT), Glutation peroxidase (GPX), reduced Glutation (GR), Glutation transferase (GST), ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA) production were determined with colorimetrical methods. Results: Comparisons of CB and MSB groups showed significant differences in CAT, FRAP, and MDA activities in both sexes; GSH and GST were different only in females. A comparison of basal versus stress conditions (CB, MSB vs CS, MSS) showed differences in CAT and MDA activities in both sexes, GSH, GST, and FRAP in females and GPX only in males. Differences were observed between CS and MSS in GPX, GSH, and GST in males, and MDA in both sexes. Conclusions: the activities of antioxidant enzymes are reduced due to chronic stress (MS) which is insufficient to protect the hippocampus from oxidative damage and results in an increased oxidative stress state in the brain.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.03

Topic: F.03. Stress and the Brain

Title: Hydrogen sulfide improves vascular dysfunction induced by chronic stress restraint in rats

Authors: *J. H. BELTRAN ORNELAS, D. L. SILVA VELASCO, J. TAPIA MARTÍNEZ, A. SÁNCHEZ LÓPEZ, D. CENTURIÓN PACHECO;
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Abstract: Several lines of evidence have shown that chronic stress decreases the levels of hydrogen sulfide (H_2S) in the hypothalamus. Furthermore, chronic stress induces cardiovascular alterations such as vascular dysfunction, and H_2S is implicated in regulating the cardiovascular system. H_2S has a potential pharmacological effect on vascular dysfunction induced by several pathologies. However, up to date, the effect of NaHS (sodium hydrosulfide; H_2S donor) on chronic stress restraint-induced vascular dysfunction has not been evaluated. Thus, this study aimed to determine the effect of NaHS on chronic stress restraint-induced vascular dysfunction in the aorta and mesenteric artery. For this purpose, 24 male Wistar rats were divided into four groups. The first group (n=6) was the control group without chronic stress restraint. The other three groups (n=18) were subjected to restraint stress for 2 h per day for eight weeks in a transparent acrylic tube. At week four of the stress protocol, the animals were divided into three groups that received daily i.p. administration for four weeks with: (1) nothing; (2) phosphate buffer saline (PBS; 1 ml/kg); and (3) NaHS (5.6 mg/kg). Aortas and mesenteric arteries were cut into rings and horizontally mounted with nichrome wire in an organ bath. Then, cumulative concentration-response curves of carbachol (CCh, $1x10^{-9}$ M - $1x10^{-5}$ M), sodium nitroprusside (SNP, $1x10^{-9}$ M - $1x10^{-5}$ M), or noradrenaline (NA, $1x10^{-9}$ M - $1x10^{-4}$ M) were determined and the vascular response was recorded. Chronic stress restraint: (1) increased the vasoconstriction induced by NA, (2) decreased the vasorelaxation induced by CCh, and (3) failed to modify the vasorelaxation induced by SNP in the aorta and mesenteric artery. Remarkably, the treatment with NaHS improved the vasorelaxation induced by CCh and decreased vasoconstriction induced by NA in the aorta. On the other hand, NaHS improved the vasorelaxation induced by CCh and SNP in the mesenteric artery. These findings suggest that NaHS reverted the chronic stress restraint-induced vascular dysfunction and may be used as a therapeutic option for the treatment of the cardiovascular alterations induced by chronic stress.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

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Program #/Poster #: 307.04

Topic: F.03. Stress and the Brain

Support: R01 MH127850-01

Title: Early life adversity alters development of corticolimbic innervation and acoustic startle response

Authors: *C. CODY, J. LARDIZABAL, L. GRANATA, A. PARAKOYI, H. BRENHOUSE; Psychology Dept., Northeastern Univ., Boston, MA

Abstract: Threat evaluation and responsivity are crucial aspects of survival for all animals, and in particular prey animals such as the rodent. Typical development of threat evaluation can be disrupted by early life adversity (ELA), due to the rich postnatal development of the involved circuitry. Prior work from our lab has shown hyper-innervation of gluatamatergic basolateral amygdala (BLA) projections in the prefrontal cortex (PFC) of ELA exposed adolescent rodents. This pathway has been implicated in anxiety related startle potentiation, which is blunted in both ELA exposed human and rodent adults. It remains unknown how these ELA-induced connectivity changes alter the typical potentiation of startle under threatening conditions, and whether the BLA-PFC pathway is directly responsible for the altered startle response. In order to test these questions, we exposed rat pups to an ELA model of maternal separation from post natal day (PND) 2-20. Rats then received an inhibitory DREADD injection in the BLA on PND 26 in order to silence the excitatory projections during the adolescent time period of PND 33-39. Finally, rats were tested in an acoustic startle paradigm under threat in early adulthood to assess the effects of reduced BLA-PFC activity on the previously observed ELA-induced blunted startle response. The acoustic startle paradigm is a two day behavioral assay that first assesses baseline startle response to white noise bursts. Following this initial assessment, rats are tested the following day on the same task, but are first exposed to playback of a 22kHz ultrasonic vocalization (USV). This particular USV signals a potentially threatening environment, and results in an increased startle response when compared to baseline levels in control rats. Here we show that ELA results in a blunted response on the second day of testing, and investigate whether inhibition of this activity rescues this behavior. Density and intensity of BLA axonal boutons in the PFC were then assessed to elucidate how BLA inhibition during adolescence affects innervation later in life. These results shed light on how altered early life environments may affect the development of startle related circuitry within the BLA-PFC pathway, and anxiety responses later in life.

Disclosures: C. Cody: None. **J. Lardizabal:** None. **L. Granata:** None. **A. Parakoyi:** None. **H. Brenhouse:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.05

Topic: F.03. Stress and the Brain

Support: 1R01MD015391
R21MH117987

Title: Olfactory Mechanisms of Trauma Vulnerability and Resilience

Authors: *E. NWULIA;
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Abstract: Experiences of social adversities can lead to negative long-term health outcomes. Minority populations, such as African Americans (AA), suffer a greater lifelong burden of experiencing social adversities. Evidence has found that the olfactory bulb (OB) may also be involved in the mechanisms of traumatic stress through the olfactory system and stress circuitries in the brain. The molecular mechanisms through which social adversities alter brain structure and functions in humans remain poorly understood, because of the inaccessibility of central nervous tissue for prospective sampling. This study aimed to explore these contexts through non-invasive sampled olfactory neurons to demonstrate the molecular and cellular mechanisms of traumatic exposures. This study included 38 female and male adults between the 18-55- years old. The sample was divided into those that experienced trauma and had PTSD, those that experienced trauma and did not have PTSD, and a control group that had not experienced trauma nor qualified for PTSD. This study included one-time measurements of behavioral, social, and psychological constructs through multiple questionnaires focused on past and current experiences. Several clinical tasks were also completed such as salivary cortisol sampling, skin-conductance response sampling, Magnetic Resource Imaging (MRI), and olfactory cell nasal sampling. The nasal exfoliates collected were used for intraneuronal RNA-based epigenomic-transcriptomic analysis. Our study found that olfactory bulb (OB) morphometry is directly associated with PTSD among populations with severe childhood adversities. Also, reduced OB volume is not associated with experiencing traumatic events, but it is predictive of the severity of traumatic distress. Lastly, greater OB volume is associated with an increased score on the Connor-Davidson Resilience Scale. From the intraneuronal RNA analysis, unique and overlapping expression patterns between the three groups are obtained. We identified a novel marker for PTSD vulnerability, SLC7A11-AS1, which corresponds with neuroprotective ability.

Disclosures: E. Nwulia: None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.06

Topic: F.03. Stress and the Brain

Support: NIH Grant R25 NS 80686

Title: Chronic social defeat affects development of sensorimotor gating (PPI) in African Cichlid fish Astatotilapia burtoni

Authors: *O. AGDALI[1], S. KNAPCZYK[1], M. HERNANDEZ[1], Z. CHOUDHRY[1], H. NEUMEISTER[1], T. PREUSS[2];

[1]Hunter Col., New York, NY; [2]Psychology, City Univ. of New York, Hunter Col., New York, NY

Abstract: Prepulse inhibition (PPI) is a neural gating phenomenon which temporally (20-500ms) reduces information flow to diminish disruptive sensory inputs. Deficits in PPI are associated with various information processing disorders, notably schizophrenia. PPI can be studied across species (e.g., humans, rodents, fish) as a modification of the startle response by a preceding sensory stimulus (prepulse). We showed that social stressors (social defeat) reduce PPI in subordinate *A burtoni* males living in dynamic social communities. Interestingly, if the same subordinates become dominant, PPI increases and vice versa, decreasing PPI in previously dominant fish that become subordinate. Here, we ask if PPI remains modifiable in fish that never experience dominance. Accordingly, we paired individual hatchlings with a slightly larger juvenile (Pairs N=7) in arenas for a 6-week period. During this time, juveniles displayed frequent dominant/aggressive behaviors toward the hatchlings. Hatchlings without a juvenile served as controls. Startle behavior and PPI was assessed in all arenas twice a week. Acoustic stimulation consisted of startle stimulus (pulse) only trials and prepulse/pulse trials with either a 50ms or 200ms prepulse/pulse interstimulus interval (ISI). After 6 weeks the larger fish (i.e.,the social stressor) was removed and PPI was tested for an additional 4 weeks. Preliminary results showed a reduced overall PPI effect (p= 0.041; unpaired t-Test; DF=13) in the paired hatchlings (mean: 35.4% ± 5.2 SEM N=7) vs. hatchling controls (mean 49.6% ± 3.7 SEM N=8) for ISI 50. Moreover, we found that removal of the juveniles increased PPI in the previously paired hatchlings (mean increase: 21.7% ± 7.8 SEM; p=0.024; paired t-Test; N=7).These results suggest that PPI in hatchling remains modifiable during development despite chronic social defeat. As such the results are consistent with the changes in PPI seen in adult fish undergoing social transitions. Together, this implies a resilient plasticity of PPI in *A.burtoni* which may serve an adaptive role.

Disclosures: O. Agdali: None. **S. Knapczyk:** None. **M. Hernandez:** None. **Z. Choudhry:** None. **H. Neumeister:** None. **T. Preuss:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.07

Topic: F.03. Stress and the Brain

Support: CIHR
Social Exposome Cluster

Title: Maternal high-sucrose diet alters androgens and aldosterone in the fetal brain and placenta of rats

Authors: *D. R. SEIB, M. M. JUNG, H. W. CHEN, K. K. SOMA;
Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Consumption of sucrose (table sugar) is high around the world. The effects of maternal sucrose intake on the placenta and fetal brain remain unknown. In rats, maternal consumption of sucrose at a human-relevant level alters the mother's physiology and steroids, as well as the adult offspring's brain and behavior. Effects in mothers are impaired glucose tolerance, increased liver lipids, increased adipose macrophages, and decreased corticosterone levels in the blood but not in the brain. In contrast, in adult female offspring, maternal sucrose intake increases corticosterone levels in the blood and the brain. In adult male offspring, maternal sucrose intake increases preference for high-sucrose and high-fat diets as well as motivation for sugar rewards in a progressive ratio task. In this study, we investigated the underlying mechanisms of the observed behavioral and endocrine effects in the adult offspring. We fed rat dams either a high-sucrose diet (26% of kCal) or an isocaloric, matched, control diet (1% sucrose) 10 weeks prior to and during gestation. At embryonic day 19 (E19), we collected maternal serum, placenta, amniotic fluid, fetal blood, and fetal brain. We microdissected the placenta and fetal brain. Next, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure multiple steroids (e.g. corticosterone, testosterone, estradiol, allopregnanolone). We examined multiple regions of the fetal brain (e.g. prefrontal cortex, nucleus accumbens, hypothalamus, hippocampus). A high-sucrose diet increased anti-inflammatory steroids (11-dehydrocorticosterone and aldosterone) in the rat dam and in the amniotic fluid at E19. Sucrose intake also increased aldosterone levels in fetal blood and brain. In contrast, sucrose consumption decreased androgens in the placenta and decidua as well as in specific brain regions such as the nucleus accumbens. Testosterone and androstenedione levels were significantly higher in the amniotic fluid, fetal blood, fetal brain, and placenta of male fetuses compared to female fetuses. However, both androgens were comparable between males and females in the decidua, which is the part of the placenta that is formed by maternal cells. Currently, we are examining cell proliferation, microglia, and tyrosine hydroxylase immunoreactivity in the fetal brain. Moreover, we are characterising the maternal microbiome and using untargeted analyses to examine the metabolome and lipidome. In summary, a maternal high-sucrose diet might be a stressor and alters multiple steroids in the fetal brain.

Disclosures: D.R. Seib: None. **M.M. Jung:** None. **H.W. Chen:** None. **K.K. Soma:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.08

Topic: F.03. Stress and the Brain

Support: National Institute of Mental Health R01MH098348 (D.C.K. and S.M.)

Title: Discrimination, White Matter Microstructure, and Psychological Distress

Authors: *D. K. GREY[1], J. B. PURCELL[1], S. MRUG[1], M. A. SCHUSTER[2], M. N. ELLIOTT[3], S. TORTOLERO EMERY[4], D. C. KNIGHT[1];
[1]Psychology, Univ. of Alabama at Birmingham, Birmingham, AL; [2]Kaiser Permanente Bernard J. Tyson Sch. of Med., Pasadena, CA; [3]RAND Corp., Santa Monica, CA; [4]Sch. of Publ. Hlth., Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Exposure to discrimination has been linked to the dysfunction of emotion regulation processes associated with psychological distress. These processes are mediated by brain regions that include the prefrontal cortex (PFC), hippocampus, amygdala, and hypothalamus. These regions are connected by white matter (WM) tracts that include the cingulum bundle, uncinate fasciculus, stria terminalis, and fornix. We hypothesized that exposure to discrimination would be negatively associated with the microstructure (indexed via quantitative anisotropy (QA)) of these WM tracts. Differences in these WM tracts may affect stress reactivity and mental health. In this study, adolescent exposure to discrimination was assessed at four time points between 11 and 19 years of age. Psychological distress (i.e., depression, anxiety, posttraumatic stress) was assessed and diffusion weighted imaging (DWI) was completed when participants were young adults (Age: 20.13±0.08 years). DWI was acquired in 60 directions in 300 participants (TE=79ms, TR=4600ms, FOV=24cm, b-value=1000s/mm^2, voxel dimensions=2.5mm×2.5mm×2.5mm). Participants who reported discrimination also reported higher levels of depression, anxiety, and posttraumatic stress. Deterministic fiber tracking was used to complete tractography of WM tracts using DSI studio. QA values were averaged across left and right hemispheres resulting in three QA values, one for each tract (i.e., cingulum bundle, uncinate fasciculus, and combined stria terminalis/fornix). Regression models were then used to predict the QA of each WM tract based on discrimination exposure as a dichotomous variable (i.e., any vs. no discrimination) and continuous variable (i.e., sum of discrimination exposures). Lower QA values were observed within the cingulum bundle and stria terminalis/fornix in participants exposed to any discrimination. Discrimination exposure as a continuous variable, was also a significant predictor of lower QA in the cingulum bundle and stria terminalis/fornix. Additional analyses were completed to account for potential confounds (e.g., sex, race) associated with discrimination. Discrimination did not predict QA in models adjusted for sex and race. Given that sex and race are strongly associated with discrimination, further research is needed to distinguish the relationships WM has with sex and race from those WM has with discrimination. However, the present findings suggest that adolescent exposure to discrimination may impact the development of WM tracts linked to emotion processes. Further, these findings may have long-term implications for the mental health of those exposed to discrimination.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.09

Topic: F.03. Stress and the Brain

Support: Dgapa-papiit IN208722

Title: Effects of unpredictable chronic stress on the pancreas in experimental type 1 diabetes mellitus

Authors: *N. A. GARCIA GALICIA[1], L. OCHOA DE LA PAZ[2], D. B. PAZ TREJO[3], H. SÁNCHEZ CASTILLO[3];
[1]Lab. de Neuropsicofarmacología, Facultad de Psicología, [2]Lab. de Neurobiología Mol. y Celular de la Glía, Torre de Investigación, Facultad de Med., Univ. Nacional Autónoma De México, Mexico, Mexico; [3]Lab. de Neuropsicofarmacología, Facultad de Psicología, Univ. Nacional Autónoma de México, Mexico, Mexico

Abstract: Many diabetic patients shown different comorbidities, in accord with the different diseases that could be added in the course of their lives. In this matter, depression and anxiety are two of the main diseases that are enhanced by the stress and they are involved in the course of the development of diabetes. However, there is no much research looking for the effect of stress over the pancreas in subjects suffering from diabetes. The pancreas, can injured when the subjects are exposed to high levels of stress. This experiment aims to evaluate the morphological change of the pancreas in rodents induced to an experimental model of type 1 diabetes and exposed to a model of unpredictable chronic stress (CUSB). The obtained results showed the the weight of the Control, Diabetic, Stressed (CUSB), and Stressed Diabetic (DCUSB) subjects got changes. We observe a decrease in weight in the Diabetic and CUSB subjects, although for these groups the curves show a recovery without reaching the weight of the control group, however, a greater change was observed in the DCUSB subjects. To evaluate the morphological change of the pancreas, hematoxylin and eosin staining was performed. We observe that the Control subjects maintained the islets of the pancreas in optimal conditions, with nuclear forms and without cell reduction, while the CUSB subjects maintained morphological chances in most of the islets. In the diabetic groups, it was found that the damage to the pancreas was significative. These results can lead us to find different treatment targets for patients suffering from diabetes and which are in stressful situations and reduce the damage that can occur in organs of the peripheral system.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

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Program #/Poster #: 307.10

Topic: F.03. Stress and the Brain

Support: R01MH128235
P50MH122379

Title: Early life stress drives divergent network and behavioral states in adulthood

Authors: *G. SCARPA, G. WEISS, J. L. MAGUIRE;
Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Adverse childhood experiences (ACEs) are major risk factors for psychiatric illnesses, including anxiety and depression; however the precise neural mechanisms mediating this vulnerability remain unclear. We propose that ACEs disrupt the conventional development of the amygdala and prefrontal cortex, two reciprocally-connected brain regions that are involved in stress reactivity and valence processing. Network communication within and between the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA) has been shown to govern affective behavioral states. Research from our laboratory has established a critical role for parvalbumin (PV) interneurons in orchestrating BLA network and behavioral states. The overarching objective of this project is to investigate the impact of early life stress (ELS) on mPFC-BLA network states and determine whether deficits in PV interneuron function may lead to different behavioral outcomes following ELS. Here, we present the first evidence to our knowledge that ELS drives divergent mesoscale network state dynamics, especially with regard to state transitions associated with stress. Our results demonstrate that exposure to a predator odor triggers attenuation of theta and potentiation of gamma oscillations in the basolateral amygdala (BLA) of adult C57BL/6J mice, however the network returns to baseline conditions across both frequencies more rapidly in mice subjected to ELS. Furthermore, these divergent network responses appear to be driven by atypical development and function of fast-spiking interneurons which express parvalbumin (PV). These findings suggest a novel mechanism by which ACEs may increase risk for neuropsychiatric disorders.

Disclosures: G. Scarpa: None. **G. Weiss:** None. **J.L. Maguire:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

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Topic: F.03. Stress and the Brain

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Title: Altered brain cortical development and socioemotional behavior in female macaques subject to social subordination and obesogenic diets postnatally.

Authors: *A. GOPAKUMAR[1], Z. KOVACS-BALINT[1], M. KYLE[1], K. SHABBIR[1], J. GODFREY[1], L. LI[1], M. A. STYNER[2], K. ETHUN[1], M. E. WILSON[1], M. SANCHEZ[1]; [1]Emory Univ., Atlanta, GA; [2]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC

Abstract: Childhood obesity is at epidemic levels and is often comorbid with chronic stress. This study investigated the potential synergistic effects of postnatal exposure to psychosocial stress and an obesogenic diet on neocortical development in a translational nonhuman primate model of social subordination. We studied longitudinally forty-one female rhesus macaques from different social ranks (n=21 dominant; n=20 subordinate) with half in each rank assigned to a low-calorie diet (LCD) only (10 LCD/subordinate; 11 LCD/dominant) or to a Choice condition with access to an LCD and obesogenic, high-calorie diet (10 Choice/dominant; 10 Choice/subordinate) at birth. Brain structural MRI scans (sMRI; T1-/T2-weighted) were collected during infancy (2 weeks, 6 months) and the juvenile, pre-pubertal period (16 months), to examine effects of chronic social subordination and diet on the development of cortical structures regulating executive, motor, sensory, socioemotional and motivational functioning, as well as interoception and homeostasis. Behavioral measures of socioemotional development, physiological measures of chronic stress activation (hair cortisol accumulation), inflammation (C-reactive protein; CRP) and body weight were also collected longitudinally. Subordinate animals had higher inflammation (CRP) levels, and Choice subjects consumed more total Kcals than LCD subjects, despite no group weight differences. Choice subjects had larger overall brain size (intracranial volume -ICV-: ($F_{(1, 31)} = 5.037$, $p = 0.032$), parietal cortex ($F_{(1, 32)} = 5.972$, $p = 0.020$), and insula ($F_{(1,32)}=11.376$, $p=0.002$) volumes than those on the LCD diet. Furthermore, subordinate animals had larger temporal visual ($F_{(1, 32)} = 9.954$, $p = 0.003$) and temporal limbic ($F_{(1, 32)} = 10.811$, $p = 0.002$) volumes compared to dominants. After correcting for ICV, social rank and diet region-specific effects only persisted in the insula. Thus, diet and social subordination had region-specific, non-synergistic effects on primate neocortical development, although some were confounded by global effects on whole brain size. We are currently examining effects of social rank and diet on socioemotional behavior. Our findings suggest that social status and postnatal consumption of obesogenic diets have robust effects on homeostatic systems that influence the trajectory of neurodevelopment and behavior in primates.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

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Program #/Poster #: 307.12

Topic: F.03. Stress and the Brain

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core support from IDDRC grant P30HD002528

Title: Sex-dependent effects of voluntary wheel running on hindpaw hypersensitivity, depression-like behaviors, and hippocampal neurogenesis in mice exposed to early life stress

Authors: *B. LEVASSEUR, O. ELLER, J. FRICK, R. FORIGHT, T. MCQUILLAN, J. CHRISTIANSON;
Univ. of Kansas Med. Ctr., Kansas City, KS

Abstract: Exposure to early life stress (ELS) results in suppressed hippocampal neurogenesis and the development of chronic pain and mood disorders across the lifespan. Conversely, voluntary exercise increases hippocampal neurogenesis, expression of Brain-derived neurotrophic factor (BDNF), and improves symptoms of pain and depression. Previous studies have reported sex-dependent effects of both ELS and exercise on hippocampal integrity, therefore here we are using a neonatal maternal separation (NMS) model in mice to determine the impact of voluntary wheel running (VWR) on pain- and depression-like behaviors, hippocampal neurogenesis, and BDNF levels in males and females.
NMS was performed by separating whole litters from the dam for 3h/day from postnatal day 1 (P1) to P21. Mice remained in sedentary caging (-Sed) or were given running wheels (-Ex) at 4 weeks of age. Mice underwent monthly hindpaw von Frey and Hargreaves testing for 4 months, and then tested for sucrose preference and nest building. Ethynyldeoxyuridine (EdU) was used to quantify hippocampal neurogenesis and an ELISA was used to measure bilateral BDNF levels in the hippocampus at 5 months of age.
A significant impact of VWR was observed on mechanical sensitivity in both female and male mice over time, such that NMS-Ex mice had higher thresholds compared to NMS-Sed mice. Male mice also showed a significant NMS/VWR interaction on mechanical sensitivity, with NMS-Ex mice having significantly higher thresholds than both naïve-Ex and NMS-Sed mice. Interestingly, VWR increased thermal sensitivity in both female and male mice over time. VWR increased sucrose consumption in females, whereas VWR increased water consumption only in male NMS-Ex mice. VWR significantly increased nest construction quality in female mice. Unlike our previous study that reported a significant decrease in neurogenesis in 8-week-old NMS-Sed male mice that was increased by 4 weeks of VWR, no significant impacts of NMS or VWR were observed on EdU-positive neurons in the hippocampus of 5-month-old mice. However, there were significantly more EdU-positive cells in females, compared to males. This was mirrored by a significant increase in BDNF levels in female hippocampus, compared to male, with lateralized distinctions.
Taken together, our results suggest that VWR can mitigate the development of ELS-associated outcomes, particularly in female mice, including hypersensitivity and depression-like behaviors. Although hippocampal neurogenesis was not significantly impacted at this later age, stronger effects of VWR in female mice may indicate a more robust response to this highly-effective, non-pharmacological intervention.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

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Program #/Poster #: 307.13

Topic: F.03. Stress and the Brain

Support: internal funding

Title: Maternal oral antibiotics during lactation alter catecholamine homeostasis and peripheral responses to stress in prepubescent offspring mice in a gender-specific manner

Authors: *B. B. NANKOVA[1], F. HU[2], E. F. LA GAMMA[3];
[1]Pediatrics, Biochem. and Mol. Biol., New York Med. Col., Valhalla, NY; [2]Pediatrics, Cell Biol., NYMC, Valhalla, NY; [3]Pediatrics - Div. of Newborn Medicine, Biochem & Molec Bio, NYMC and Westchester Med. Ctr., Valhalla, NY

Abstract: Antibiotic use in the perinatal period in both, mother or infant disrupts initial neonatal gut colonization and has been linked to a wide range of diseases later in life, from gastrointestinal to metabolic, atopic, and neurobehavioral impairments. Recently, using a well-characterized neuronal reflex arc as a model, we discovered that adrenal catecholamine responses to hypoglycemia are attenuated in germ free mice or in young adult colonized animals with an antibiotic-induced gut microbiome depletion. How delayed/perturbed postnatal gut colonization will affect the sympathoadrenal system and the ability of newborns to adapt to adverse conditions in the extrauterine environment is not known. In the current study C57Bl6 mothers were given regular water (control) or a cocktail of non-absorbable broad-spectrum antibiotics (Abx) in the drinking water from postnatal day one. After 4 weeks the pups from each experimental group were injected with insulin or saline and sacrificed 90 min later. Body weights, and plasma levels of corticosterone (marker of the HPA stress axis activation) were not significantly different between groups and between genders. Administration of the antibiotic mixture in mother's drinking water resulted in markedly enlarged ceca in the offspring (macroscopic hallmark for gut microbiome depletion). By-products of bacterial fermentation (sp. SCFA) were not detectable in the male Abx group. Residual acetate was detected in female Abx group, indicating gender-specific shifts in microbial composition, as confirmed by whole genome shotgun sequence profiling of individual stool samples. No differences in cecal SCFA levels were observed between male and female controls. Control female mice also had significantly higher basal urinary epinephrine levels then males. Gut dysbiosis resulted in higher basal epinephrine levels in both, male and female pups (compared to their respective controls) in contrast to our previous findings in adult Abx mice. The effect was more pronounced in female pups. The epinephrine responses to hypoglycemia were significantly reduced only in Abx male group. In female Abx group the individual responses to stress varied in a wide range and were not significantly different from their respective controls. Together, our results revealed that maternal Abx administration dictate neonatal microbiome depletion in the offspring and affects peripheral catecholaminergic pathways and responses to stress in a sexually dimorphic manner.

The implications of these findings portend pragmatic consequences for patient care regarding use of antibiotics, repopulation of gut flora and maintenance of successful stress adaptations.

Disclosures: B.B. Nankova: None. **F. Hu:** None. **E.F. La Gamma:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

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Program #/Poster #: 307.14

Topic: F.03. Stress and the Brain

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CNPq Grant

Title: Maternal childhood adversity and anxiety impact offspring salivary cortisol levels

Authors: *A. CAMARGO RAMOS[1], S. VANBRONKHORST[2], C. ARAÚJO[1], V. SANTANA[1], M. VALVASSORI[1], S. CORRÊA[1], A. CRUZ[1], A. C. MILANI[1], C. PARENTE[3], S. BELANGERO[1], I. SILVA[3], C. DUARTE[2], J. POSNER[4], A. P. JACKOWSKI[1];
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Abstract: Adverse childhood experiences (ACEs) include child abuse and neglect as well as family dysfunction (e.g. parental separation, mental illness, and incarceration). ACEs are risk factors for the development of psychopathologies, not only for the individuals directly affected, but also for their offspring. Exposure to ACEs can also result in alterations of the hypothalamic-pituitary-adrenal axis (HPA Axis) as evidenced by cortisol levels. The present study aims to examine whether maternal ACEs and anxiety impact maternal and infant salivary cortisol during the first month postpartum.Participants are from the Healthy Mother-Infant Neurodevelopment (Healthy MINDs) study, a longitudinal study of pregnant women and their offspring in São Paulo, Brazil. Salivary cortisol was measured in mothers and their infants (30.10 ±8.49 days). Maternal ACEs were assessed using the CDC-Kaiser assessment and anxiety was assessed using the COVEX scale. A univariate generalized linear model (GLM) was utilized to examine the relationship between the exposures (maternal ACEs and anxiety) and the outcomes (salivary cortisol levels of mothers and their offspring), and to investigate whether the interaction between maternal ACEs and anxiety (conditional model) would impact salivary cortisol levels. The model adjusted for offspring age at the time of saliva collection. For multiple comparison correction, we used Bonferroni correction and the significance level was set as $p<0.05$. The sample included 105 mother-infant dyads. We found that maternal ACEs were associated with higher levels of anxiety ($r=0.294$; $p=0.002$). GLM showed a main effect of ACEs ($F=3.385$; $p=0.024$; $\eta 2=0.151$)

and anxiety (F=1.985; p=0.026; η2=0.385) in offspring salivary cortisol levels. Infants born from mothers with higher number of ACEs showed decreased levels of cortisol (ACEs 0-1: 9.475±1.386 ng/mL; 2-4: 9.775±1.061 ng/mL; 5-7: 6.306±1.129 ng/mL; 8+: 5.630±2.706 ng/mL). No effect of ACEs, anxiety, or the ACES x anxiety interaction on maternal cortisol levels (p>0.05) was observed.Maternal ACEs were related to increasing anxiety levels throughout mothers' life. Maternal ACEs and anxiety were related to the levels of cortisol in infants, but not in mothers. Higher maternal ACEs were related to lower infants' salivary cortisol levels. These results point to the possible effects of intergenerational experience of adversities, affecting the functioning of HPA axis of infants, and suggest that ACEs were related to increasing anxiety in mothers, that in turn affect cortisol in their infants.

Disclosures: A. Camargo Ramos: None. **S. VanBronkhorst:** None. **C. Araújo:** None. **V. Santana:** None. **M. Valvassori:** None. **S. Corrêa:** None. **A. Cruz:** None. **A.C. Milani:** None. **C. Parente:** None. **S. Belangero:** None. **I. Silva:** None. **C. Duarte:** None. **J. Posner:** None. **A.P. Jackowski:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.15

Topic: F.03. Stress and the Brain

Title: Early Stress Impacts Food Hoarding and Conditioned Place Preference in Adolescent Male Hamsters

Authors: *K. M. MORAN[1], Y. DELVILLE[2];
[1]Psychology, Univ. of Texas at Austin, Austin, TX; [2]Dept. Psychol, Univ. of Texas at Austin Dept. of Psychology, Austin, TX

Abstract: Early life stress is a consistent predictor of later-life obesity. In hamsters, a two-week exposure to chronic social stress in adolescence causes a consistent 10% increase in weight gain, food intake, and body fat. To examine possible differences in food-related motivation, we tested stressed hamsters with a food hoarding and food conditioned place preference (CPP) task. Male Golden Hamsters (n=24/group) were exposed to a resident-intruder or clean cage control condition for 20 minutes per day from postnatal day 28 to postnatal day 42 (early- to mid-adolescence). In the last five days of the stress period, subjects were allowed to habituate to a V-shaped arena with a blank start area and two distinctly textured wings for 10 minutes at least one hour after stress. After two habituation days, a bowl with banana-flavored pellets was placed in one wing of the arena with subjects allowed to collect as much as desired. On the fifth day (CPP), the food bowl was absent. On the food hoarding day, stressed and non-stressed subjects both spent more time in the food wing. Although animals from both groups spent equal time in the food wing, stressed subjects collected twice as much food as controls. However, on the CPP day, stressed hamsters spent significantly less time in the food-associated wing and more time in

the opposite wing. Food hoarding differences likely exemplify that stressed individuals value food more or require more food to reach similar feelings of reward compared to controls. However, CPP differences are likely associated with a form of frustration having to do with lack of expected rewarding stimuli. Indeed, adult hamsters exposed to unexpected long delays in previously rewarding behaviors rapidly cease those behaviors and spend less time in areas previously associated with rewards. Together, altered motivation for food and frustration in response to delayed rewards are contributing factors to later-life increased body weight.

Disclosures: K.M. Moran: None. **Y. Delville:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.16

Topic: F.03. Stress and the Brain

Support: NIH Grant 1R01HD087509-01

Title: Stress and the epigenome: A role for behavioral interventions

Authors: *T. CAMPBELL, K. DONOGHUE, J. SMITH, U. GHOSH, C. NELSON, S. FERN, T. L. ROTH;
Univ. of Delaware, Newark, DE

Abstract: Aversive caregiving in early life is a risk factor for aberrant brain and behavioral development. This outcome is related to epigenetic dysregulation of the *Bdnf* gene. The *Bdnf* gene encodes for brain-derived neurotrophic factor (BDNF), a neurotrophin involved in early brain development, neural plasticity, learning, and memory. Disruptions in caregiver-infant interactions lead to increased methylation and decreased expression of the *Bdnf* gene. One way to mitigate this effect is hypothesized to be through aerobic exercise. Exercise increases BDNF at the protein and gene expression levels, making it an exciting target for therapeutic interventions. To our knowledge, exercise has never been studied as a therapeutic intervention in preclinical rodent models of caregiver maltreatment. To that end, the current study investigated the effect of an adult voluntary wheel running intervention on *Bdnf* aberrant methylation and expression in the prefrontal cortex and hippocampus of rats who experienced aversive caregiving in infancy. We employed a rodent model (Long Evans rats) wherein rat pups experienced intermittent caregiver-induced stress from postnatal days 1-7 and were given voluntary access to a running wheel (except in the control condition) from postnatal days 70-90 as a young adulthood treatment intervention. Current results indicate that maltreatment and exercise affect *Bdnf* gene methylation in an exon, CG site (loci where methyl groups attach), and sex specific manner. Data collection is ongoing, further demonstrating the dynamic nature of DNA methylation. This work is funded by the National Institutes of Health: Eunice Kennedy Shriver National Institute of Child Health & Human Development (1R01HD087509-01).

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.17

Topic: F.03. Stress and the Brain

Support: NIH Grant P50 MH096889
NIH Grant P50 DA044118
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NIH Grant T32 MH119049
NIH Grant T32 GM008620
TRDRP Grant T31IR1767
Hewitt Foundation for Biomedical Research

Title: A novel role for delta opioid receptor in developmental adversity-induced opioid addiction in female rats

Authors: *Y. XIE[1], S. C. LEVIS[1], M. BIRNIE[2], N. KAMEI[3], T. BARAM[2], S. V. MAHLER[1]; [1]Dept. of Neurobio. and Behavior, [2]Dept. of Pediatrics, [3]Dept. of Anat. & Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: Background: Early life adversity (ELA) is a risk factor for psychiatric disorders that involve changes in reward processing, including opioid use disorders. Previously, we found that female rats are at higher risk for developing opioid addiction-like behaviors after early life adversity than males, suggesting that ELA may lead to a sex-specific derangement of reward circuit maturation. However, the brain mechanisms underlying this phenomenon are still poorly understood. We employ the limited bedding and nesting (LBN) model of ELA to investigate the molecular causes of ELA-induced excessive opioid drug seeking in adult female rats. **Methods**: We analyzed the mRNA expression of opioid receptor genes and their ligands in nucleus accumbens and amygdala of opioid-naïve and opioid-experienced adult female ELA-exposed and control rats. Following up on receptor subtype-specific changes, we then pharmacologically manipulated endogenous delta opioid receptor signaling in both groups during an opioid self-administration task measuring behavioral economic demand for the fentanyl derivative remifentanil. **Results**: ELA enhanced motivation for remifentanil in female rats, as we have previously shown (Levis et al 2021, Mol Psychiatry). We observed a specific reduction in delta opioid receptor expression in reward circuit nodes of ELA females. Pharmacological manipulations during opioid self-administration suggest a possible role of delta opioid receptors in mediating the effects of ELA on motivation for opioid drugs. **Conclusions**: In female rats, ELA causes a sex-specific pro-addiction phenotype, which might be mediated by a disruption in endogenous opioid signaling at the delta opioid receptor, leading to excessive, addiction-like

opioid drug seeking. These findings may have implications for future treatment strategies for opioid addiction in women who have experienced ELA.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.18

Topic: F.03. Stress and the Brain

Support: Southern Illinois University School of Medicine
FI2 GM119962

Title: Opposing effects of NMDA receptor antagonists on early life stress-induced aggression in mice

Authors: *C. J. BARTSCH[1], S. L. SKINNER[1], J. C. NORDMAN[2], Z. LI[3];
[1]Physiol., Southern Illinois University-Carbondale, Carbondale, IL; [2]Southern Illinois Univ. Sch. of Med., Southern Illinois Univ., Carbondale, IL; [3]Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Early life stress is predictive of aggression in adulthood. Unfortunately, there are few clinical options to treat excessive and recurring violent aggression associated with early life stress, and existing options such as atypical antipsychotics and benzodiazepines are replete with side effects. One promising target is the glutamate binding N-methyl-D-aspartate-receptor (NMDAR), which has both immediate and long-term effects on aggressive behavior. Clinically available NMDAR antagonists, such as memantine and ketamine, have successfully reduced violent aggression associated with many psychiatric diseases. In this study, we explore the effects of three non-competitive NMDAR antagonists on aggression brought on by early life stress, MK-801, memantine, and ketamine. We find that a combination of social isolation in early adolescence followed by acute traumatic stress in the form of non-contingent foot shocks in late adolescence leads to long-lasting aggression in mice when measured one week later. We refer to this phenomenon as early life stress-induced aggression. Intraperitoneal injections of MK-801 and memantine 30 minutes before foot shock attenuates early life stress-induced aggression. Interestingly, intraperitoneal injections of ketamine 30 minutes before foot shock significantly enhanced the aggression. These findings indicate that NMDAR antagonists have distinct and opposing effects on early life stress-induced aggression, suggesting these drugs may be mechanistically distinct. We hypothesize this is due to opposite effects on synaptic plasticity, consistent with our previous studies. This study highlights memantine as a promising treatment for aggression brought on by early life stress, while demonstrating the need for greater care when using NMDARs to treat aggression.

Disclosures: C.J. Bartsch: None. **S.L. Skinner:** None. **J.C. Nordman:** None. **Z. Li:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.19

Topic: F.03. Stress and the Brain

Support: NIH Grant 1R01MH127850-01

Title: A neuroendocrine mechanism underlying accelerated pubertal development after early life adversity and its effects on adolescent threat responsivity

Authors: *L. GRANATA[1], A. PARAKOYI[2], P. THAKUR[1], H. C. BRENHOUSE[2];
[2]Psychology, [1]Northeastern Univ., Boston, MA

Abstract: Early life adversity (ELA) increases the risk of developing anxiety disorders later in life, and accelerated pubertal timing mediates the association between ELA and anxiety in women. Using the maternal separation paradigm in rats, ELA-reared females display a vaginal pinhole earlier than control-reared females. However, the neuroendocrine mechanisms underlying female-specific pubertal acceleration and its relationship to threat responsivity are unknown. Stimulatory and inhibitory hypothalamic genes are involved in generating the pulsatile gonadotropin-releasing hormone (GnRH) release necessary for pubertal onset. RF-amide related peptide-3 (*Rfrp*) and Kisspeptin (*Kiss1*) are sensitive to stress and are regulated by corticotrophin-releasing factor (*Crf*). The current study characterizes the development of *Rfrp, Kiss1*, *Crf,* and *GnRH* gene expression in the hypothalamus using qPCR in control and ELA-reared males and females on postnatal days (P)10, P15, and P20 in order to elucidate the underpinnings of pubertal development before external markers of puberty are displayed. ELA and control-reared adolescent (P35) rats underwent the acoustic startle test to determine whether ELA and pubertal timing affected threat responsivity at baseline and following an aversive social cue (22kHz ultrasonic vocalization). Finally, to test whether estrogen-mediated activity is necessary to heighten the startle response, we silenced ERα in the basolateral amygdala, a region involved in threat response circuitry, using a short hairpin RNA on P15. qPCR results show that after ELA, females exhibited increased hypothalamic *Rfrp, Kiss1*, and *GnRH* expression at P20. Results also show a negative correlation between the day of pubertal onset and startle magnitude in females and decreased startle after ERα silencing, demonstrating a neuroendocrine mechanism underlying heightened threat responsivity after ELA.

Disclosures: L. Granata: None. **A. Parakoyi:** None. **P. Thakur:** None. **H.C. Brenhouse:** None.

Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.20

Topic: F.03. Stress and the Brain

Support: Colorado College Research and Development Grant

Title: Maternal separation effects on gut microbiota and behavior, and impact of short-chain fatty acid supplementation

Authors: *L. L. DRISCOLL, E. O'LEARY, T. VIERLING, T. SUI, E. GRANT;
Colorado Col., Colorado Col., Colorado Spgs, CO

Abstract: Early life adversity (ELA), such as the stress experienced by maternal separation (MS), can disrupt gastrointestinal function, induce gut dysbiosis, and impact mental health and cognition. We explored the effects of MS, followed by one week of oral supplementation of a short-chain fatty acid (SCFA) cocktail, on the gut microbiota and behavior of weanling rats. MS pups were isolated from their dams for three hours per day from postnatal days (PNDs) 1-15; non-separated (NS) pups remained with their dams. From weaning on PND21 until the end of the study, half of MS and half of NS pups were supplemented with the SCFAs butyrate, propionate, and acetate in their drinking water. Caecal samples at PNDs 21 and 28 were subjected to deep amplicon sequencing of the 16S ribosomal RNA gene to survey bacterial population dynamics. Beginning at PND28, rats were administered open field, object recognition, Morris water maze, and forced swim tests. MS rats demonstrated lower abundances of caecal genera *Lachnospiracae* and higher abundances of *Bacteroidales* than did NS rats. MS rats continued to show depleted *Lachnospiracae* at PND28, but now had an overabundance of *Bifidobacteria* instead of *Bacteroidales* compared to NS rats. SCFA supplementation induced complex changes in beta diversity but did not mitigate the changes in beta diversity associated with MS. MS rats demonstrated only subtle changes in anxiety, depressive behavior, and short-term memory, but were significantly impaired in the object recognition and Morris water maze tasks at the longest retention periods. The SCFA cocktail attenuated some, but not all, of the long-term memory deficits. These results provide new information about the population-level dynamics of the gut microbiome in response to MS, and suggest that dietary intervention with SCFAs, while beneficial for treating the behavioral deficits associated with MS, does not correct MS-induced gut dysbiosis.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.21

Topic: F.03. Stress and the Brain

Support: KIST program 2E31700
KIST program 2E31522

Title: Particulate matter exposure leads to an ADHD-like phenotype and neurotransmitter changes in infant mice.

Authors: *N. BOESEN, K. KIM, S. SONG, M. OH, S. LIM, G. LEE, B. YU, Y. KIM;
KIST, Seoul, Korea, Republic of

Abstract: Particulate matter (PM) has been recognized as a risk factor for health, necessitating research into its specific effect and reach. Conventionally, larger sized particles (PM_{10} or $PM_{2.5}$) have been shown to negatively influence pulmonary and cardiovascular health. Smaller sized particles (PM_1) can even pass through the blood vessels, enter the circulatory system and penetrate into the brain. An association has been made between an increased prevalence for neurodegenerative disorders and PM exposure during life, but early life exposure has mostly been investigated through epidemiological studies in humans. Early brain development is an important but vulnerable stage and may be adversely impacted by environmental toxicants or stressors. Therefore, it is important to investigate the effect of PM on offspring during pregnancy through maternal exposure and early after birth to determine the potential immediate and long-term consequences. To mimic the natural way of exposure to PM, we made use of a pyrolysis-based soot generating system and exposure chambers. This system has the advantages of whole-body exposure replicating the way we inhale PM and generates an emission of soot which size is measured by a particle analyzer. We used wild-type mice in combination with our exposure system to expose 2 age groups (predominantly in utero from E10-P14 and fully after birth at P6-P28) to high concentrations of PM_1 (175$\mu g/m^3$ in 24h). First, we found an alteration in behavior between exposed and non-exposed offspring, with both age groups showing a significant increase in anxiety as measured in the Open-Field Test (OFT). Further, the group exposed fully after birth (P6-P28) showed a significant increase in hyperactive-like jumping behavior. Second, the brain showed an increase in levels of inflammatory markers as well as a significant increase in dopamine and decrease in serotonin levels in the group exposed after birth (P6-P28). In contrast, the group predominantly exposed *in utero* (E10-P14) did not show any significant changes in brain inflammatory markers or neurotransmitter levels. This atypical hyperactive behavior, generalized anxiety and change in neurotransmitters led us to speculate an ADHD-like phenotype that occurred after early life PM exposure. These results show the early life influence of particulate matter and support epidemiological findings of the far-reaching consequences of increased world-wide pollution, especially in the vulnerable early stages right after birth. It also suggests the possibility of maternal protection leading the offspring to be less affected by detrimental environmental factors while *in utero*.

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Poster

307. Neural, Neurochemical, and Physiological Effects of Early-Life Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 307.22

Topic: F.03. Stress and the Brain

Title: Automated analysis of γ-aminobutyric acid (GABA) and glutamate in microdialysate samples using pre-column OPA-sulfite reagent, UHPLC separation and electrochemical detection

Authors: *M. EYSBERG[1], H.-J. BROUWER[1], C. J. WRIGHT[2], K. M. RENTSCHLER[2], L. M. VAN HEERWAARDEN[1], A. POCIVAVSEK[2], N. J. REINHOUD[1];
[1]Antec Scientific, Alphen a/d Rijn, Netherlands; [2]Dept. of Pharmacology, Physiol. and Neurosci., Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: The quantification of γ-Aminobutyric Acid (GABA) and glutamate (Glu) in freely moving test animals requires the use of microdialysis probes for sampling. This sets three challenges to the analytical method: 1) a very small microdialysis sample is available (5 - 20 µL), 2) GABA levels are very low, and 3) Glu and other amino acid levels are very high. The analytical method of choice that can handle all these requirements at the same time was microbore ultra-high performance liquid chromatography (UHPLC) with automated pre-column OPA-sulfite reagent, an automated post-run column flush and electrochemical detection, on the ALEXYS Neurotransmitter Analyzer. Under optimized separation conditions, GABA and Glu were well resolved from a number of amino acid peaks, and the total runtime was kept as short as possible by applying a fourfold increase of the flow rate. The detection limit for GABA was about 10 nmole/L, which was low enough to measure the low basal levels in microdialysis samples as well as accommodate the measurement of a decrease in those levels. This method was optimized and used to evaluate a new model for the study of prenatal insults. The poster shows a few example chromatograms from this study.
Prenatal insults such as stress or infection are known to increase susceptibility to psychotic disorders like schizophrenia and bipolar disorder in adult life. One of the pathways that is involved is the kynurenine pathway, through the increase of the kynurenine acid (KYNA) levels. KYNA is considered a neuromodulator that decreases the extracellular levels of the neurotransmitters glutamate (Glu) and gamma-aminobutyric acid (GABA) in the brain. Hypofunction of glutamatergic signaling is causally linked to neurodevelopmental disorders. For the study of prenatal insults, pregnant Wistar dams were fed chow laced with kynurenine to stimulate fetal brain KYNA elevation from embryonic day 15 to embryonic day 22. Control dams (ECon) were fed unlaced chow. In separate animals, *in vivo* microdialysis was conducted in the dorsal hippocampus to assess GABA and Glu levels. Using the analytical method, decreases in extracellular glutamate levels were found in the dorsal hippocampus of EKyn male and female offspring, while decreased GABA levels were present only in males during the dark phase.
The automated UHPLC-ECD method with pre-column derivatization was shown to be linear, reproducible, sensitive and fit for purpose for analysis of GABA and Glu in small microdialysate samples of rats.

Disclosures: **M. Eysberg:** None. **H. Brouwer:** None. **C.J. Wright:** None. **K.M. Rentschler:** None. **L.M. van Heerwaarden:** None. **A. Pocivavsek:** None. **N.J. Reinhoud:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.01

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NINDS U19 NS123717
R35 NS097265

Title: Microscopic mapping of extra- and intra-cellular cortical glucose in awake mice

Authors: *P. MAECHLER[1], J. S. MARVIN[3], R. LIU[1], T. BROGGINI[4], L. LOOGER[5], D. KLEINFELD[2];
[1]UCSD, [2]UCSD, La Jolla, CA; [3]Janelia Farms Res. Ctr., Ashburn, VA; [4]Dept. of Physics, UC San Diego, La Jolla, CA; [5]Howard Hughes Med. Inst., Ashburn, VA

Abstract: Blood glucose and oxygen are the main energetic substrates that fuel the generation of ATP, via glycolysis and subsequent oxidative phosphorylation in neurons and glia. With the dye Oxyphor2P in cerebral cortex we found a partial pressure of oxygen gradient around penetrating arterioles of 19.3 +/- 4.5 mmHg (mean +/- SD, n=30 in 6 mice). It is not known if glucose gradients exist in brain tissue. Here we present new data on the in vivo expression and functionality of the membrane bound sensor iGLucoSnFR2 that acts as an indicator for either intra- and extra-cellular glucose. iGLucoSnFR2 is based on the glucose sensor iGLucoSnFR1 (*Keller et al., Cell Rep. 2021*). The dissociation constant K_D = 1.7 mM for *D*-glucose in primary mixed cortical neuronal/glia cultures render iGLucoSnFR2 suitable for in vivo glucose sensing. We injected AAV2/1 encoding for iGLucoSnFR2 under control of the CAG, gfaABC1D, or hSynapsin1 promoter, in black6 mice. The co-expression of the fluorophore mRuby2 with iGLucoSnFR2 allowed for partial ratiometric correction of motion artefacts and vascular artefacts from hemodilution during i.v. injections. Cell-type specific expression was achieved after 2 weeks. Data was obtained up to 500 µm deep using two-photon laser scanning microscopy with adaptive optics implemented to correct for system aberrations (*Liu et al, Nat. Meth. 2019*) and an extended focus (*Abrahamsson et al., Proc SPIE BiOS, 2006; Chen et al., Biomed Opt Exp, 2020*) implemented to reduce motion artifacts. The blood plasma was labeled with Cy5.5 conjugated to 2 MDa dextran. We illuminated at 925 nm and collected green light (465-555 nm), red light (590-650 nm), and far-red light (670-745 nm). The signal-to-noise was sufficient to enable up to 10 Hz mapping of glucose in the somatosensory cortex of awake mice. The dependence of the iGLucoSnFR2 signal on blood glucose levels was demonstrated with consecutive intravenous infusions of fast acting insulin (1 IU/kg) and *D*-glucose (1 mg/kg). We correlated blood plasma glucose, measured via femoral vein coulometry (AlphaTRAK2, Zoetis) with the brain tissue iGLucoSnFR2 signals: a blood glucose modulation from 50 to 550 mg/dL resulted in a $\Delta F/F_0$ change of 0.6 for the cytoplasmic sensors and 0.2 for the membrane bound sensors. Our set-up enables spatial mapping of temporal intra- and extra-cellular dynamics of glucose upon transients in blood glucose levels, with slow kinetics reflecting transport across the

blood brain barrier. iGLucoSnFR2 imaging in awake mice will allow us to further investigate glucose transport across different types of cortical brain vessels and brain states, which is relevant for clinical FDG-PET imaging.

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Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.02

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Texas Woman's University Research Enhancement Program Award (Na, E.S, Brower, C.S)
Texas Woman's University Startup Funds (Na, E.S)
NINDS of the NIH Grant R15 NS 095317-02 CS Brower

Title: Loss in ATE1 may affect energy metabolism through leptin

Authors: *P. FRAYRE[1], M. ALKHATATBEH[2], N. KOPCHENKO[2], C. S. BROWER[3], E. NA[4];
[1]Texas Women's Univ., Denton, TX; [3]Biol., [2]Texas Woman's Univ., Denton, TX; [4]Texas Woman's Univ., Aubrey, TX

Abstract: Previous studies have shown that deletion or downregulation of Arginyltransferase 1 (ATE1) can lead to neurodegeneration, behavioral abnormalities, hyperphagia and lower body weight. To determine how the loss of ATE1 affects energy metabolism and food intake, ATE1-deficient and ATE1-containing mice were given high fat diet for 12 weeks. Based on our current data, we found significant sex differences in body weight and food intakes, with ATE1-deficient mice being less sensitive to the obesogenic effects of high fat diet even while consuming the same amount of high fat diet as controls. Interestingly, ATE1-deficient mice exposed to high fat diet displayed lower plasma leptin levels even after normalizing to white adipose tissue (WAT) weight. Leptin-treated ATE1-deficient mice show increased pSTAT3 expression in the arcuate nucleus of the hypothalamus relative to ATE1-containing mice, demonstrating that ATE1-deficient mice are still responsive to systemic leptin even after long-term exposure to high fat diet. Taken together, these data indicate that loss of ATE1 affects leptin levels independent of WAT and that leptin sensitivity is still preserved in ATE1-deficient mice exposed to a diet that typically leads to leptin insensitivity.

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Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.03

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: MICINN Spain PDI2019-106968RB-100
SENC Grant for SfN 2022

Title: Chemogenetic activation of astrocytes in vivo with Clozapine N-oxide produces alterations in brain glucose metabolism ([^{18}F]FDG-PET)

Authors: *N. HERNÁNDEZ MARTÍN[1,2], M. GÓMEZ MARTÍNEZ[3], P. BASCUÑANA[2,1], R. FERNÁNDEZ DE LA ROSA[4,1], L. GARCÍA GARCÍA[1,2], E. D. MARTÍN[5], M. A. POZO[1,2]; [1]Inst. Pluridisciplinar, Univ. Complutense, Madrid, Spain; [2]Inst. de Investigación Sanitaria San Carlos (IdISSC), Hosp. Clínico San Carlos, Univ. Complutense, Madrid, Spain; [3]BiomaGUNE, Guipuzkoa, Spain; [4]BIOIMAC, Univ. Complutense, Madrid, Spain; [5]Inst. Cajal, Madrid, Spain

Abstract: Astrocytes are one of the most abundant type of brain cells and they are considered to be crucial in brain metabolism, since they are involved in many relevant functions for cells survival such as the maintenance and regulation of neuronal activity. In recent years, chemogenetics has become one of the most revolutionary techniques since it allows modulating different populations of cells. However, the effect of Drug Receptors Exclusively Activated by Designer Drugs (DREADDs) activation in astrocytes remains unknown. Therefore, our study aimed to activate hippocampal astrocytes in CD1 male mice with Clozapine-N-oxide (CNO, 3 mg/kg, i.p.) to assess their impact on brain glucose metabolism using the Positron Emission Tomography (PET) combined with the glucose analog radiotracer 2-deoxy-2-[18F]-fluoro-D-glucose (FDG) imaging technique. Dynamic PET studies were performed in order to evaluate changes in glucose kinetics based on 2-tissue-compartment (2TC) model. With the aim to assess the effect of chemogenetic astrocyte activation in high requirement metabolic condition, the intrahippocampal kainic acid (KA, 2mM, 0.5 µl) mouse model of epilepsy was used, as it presents changes in glucose metabolism during epileptogenesis. In this experiment, three static FDG-PET studies were performed (three days before, one day after and 8 days after KA injection) as a chronic CNO treatment (1 mg/kg, i.p.) was administered during days 1 to 7. We have found that astrocyte activation by CNO increased the phosphorylation of glucose within the cells in the first 15-20 minutes and the effect disappeared after 60 minutes. In the KA epilepsy model, CNO chronic administration reversed the hypermetabolism at the first day and the hypometabolism at day 8, associated to the epileptogenesis process. Our findings provide the evidence for the effectiveness of acute CNO in activating astrocytes by DREADDs increasing brain glucose metabolism. In a pathological condition, such as epilepsy, sustained activation of astrocytes through chemogenetics shows the ability of these cells to regulate brain metabolic changes, which has been shown to be associated to epileptogenesis.

Disclosures: **N. Hernández Martín:** None. **M. Gómez Martínez:** None. **P. Bascuñana:** None. **R. Fernández de la Rosa:** None. **L. García García:** None. **E.D. Martín:** None. **M.A. Pozo:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.04

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Deficits in brain energy metabolism in a mouse model for Glut1 Deficiency Syndrome

Authors: *S. BURLET-GODINOT[1], M. SOYA[1], A. CARRARD[1], M. TANG[2], U. MONANI[2], D. DE VIVO[2], J.-L. MARTIN[1], P. MAGISTRETTI[1,3];
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Abstract: Glucose transporter type 1 deficiency syndrome (Glut1DS), also called De Vivo disease, is a rare genetic disorder caused by mutations in *SLC2A1* gene that encodes for glucose transporter type 1 (Glut1). In the brain, Glut1 is predominantly expressed in endothelial cells and astrocyte membranes. In human, this mutation results in deficient glucose transport to the brain and is characterized by early onset epilepsy, complex movement disorders and cognitive impairment. In order to assess the impact of Glut1 deficiency on brain energy metabolism, we measured glycogen, lactate, and glucose levels in the cerebral cortex and hippocampus of a mouse model for Glut1DS. Glut1DS mice were obtained from the De Vivo laboratory and mimic the major features of the classical phenotype of human Glut1DS. Measurements were performed in both male and female mice using standard biochemical assays. A significant decrease in glucose levels was already observed at 2 weeks of age both in the hippocampus and the cerebral cortex of GLUT1 +/- mice compared to WT mice (-37% in both structures). In addition to decreased glucose levels, we found a significant reduction in glycogen levels (hippocampus: -28.8%, cerebral cortex: -37.1%) and a decrease in lactate levels (hippocampus: -20.9%, cerebral cortex: -23.9%). In 10-week old mice, a decrease in glucose levels was observed in the hippocampus and cerebral cortex of GLUT1 +/- compared to WT mice (-31.9% and -40.8%, respectively). Decreases in glucose levels were accompanied by a significant reduction in cerebral glycogen content (hippocampus: -32.9%, cerebral cortex: -35.3%) and lactate levels (hippocampus: -15.7%, cerebral cortex: -17.9%) in GLUT1 +/- mice compared to WT mice. Differences in glucose, glycogen and lactate levels observed in the cerebral cortex and hippocampus were similar in male and female GLUT1+/- mice. No difference in glycaemia and lactatemia was observed between GLUT1+/- and WT mice. Together, these data show that Glut1DS has an early and marked impact on brain energy metabolism not only on glucose levels but also on other important energy substrates including lactate and glycogen. As astrocytes store glycogen and release lactate, an energy substrate for neurons and a signaling molecule, these data suggest that Glut1DS may affect the cooperation between astrocytes and neurons. Further

elucidation of the mechanisms underlying alterations in the astrocyte-neuron cooperation in GLUT1+/- mice should help to identify novel therapeutic targets for the treatment of De Vivo disease.

Disclosures: S. Burlet-godinot: None. **M. Soya:** None. **A. Carrard:** None. **M. Tang:** None. **U. Monani:** None. **D. De Vivo:** None. **J. Martin:** None. **P. Magistretti:** None.

Poster

308. Energy Regulation

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Program #/Poster #: 308.05

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: MOST 110-2320-B-002 -025 -MY3

Title: Roles of equilibrative nucleoside transporter-2 in energy homeostasis in the striatum of mice

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Abstract: Introduction
Adenosine is the building block of adenine nucleotides that participates in energy homeostasis. Equilibrative nucleoside transporter-1 (Ent1) and Ent2 are the major transporters responsible for the cellular uptake and homeostasis of adenosine and adenine nucleotides in the brain. A previous study has shown that the expression of Ent2 is higher than of Ent1 in the brain. Therefore, the role of Ent2 in energy homeostasis in the brain is worth an attention.
Objectives
The objective of the present study was to explore the roles of Ent2 in the modulation of energy homeostasis in physiological and pathological conditions.
Methods
$Ent2^{-/-}$ (C57BL/6-$slc29a2^{em1}$) mice and their littermate controls were generated by the CRISPR-Cas9 technique. $Ent2^{+/-}$ mice were crossed with R6/2 (B6CBA-Tg(HDexon1)62Gpb/3J) mice, a widely used mouse model of Huntington's disease, to obtain R6/2-$Ent2^{-/-}$ mice and the littermate controls. All animals were used at the age of 12 weeks. LPS treatment was used to induce neuroinflammation in $Ent2^{-/-}$ and $Ent2^{+/+}$ mice. To avoid gender effects, male mice were used in this study. The metabolomic assay was conducted by UPLC-MS/MS analysis. Acute brain slice metabolic analysis was performed by mitochondrial respirometry assay (the Seahorse assay) under glycemic and aglycemic conditions. Oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were recorded.
Results
Metabolomic results showed that Ent2 deletion didn't alter the striatal level of adenosine. However, the AMP/ATP ratio was significantly increased and the NAD^+/NADH ratio was

reduced in Ent2$^{-/-}$ mice. For mitochondrial function, the results of OCR showed that Ent2$^{-/-}$ mice had less basal respiration and less ATP production under glycemic condition. The change in basal respiration was also observed in R6/2-Ent2$^{-/-}$ mice, but not in the LPS-treated Ent2$^{-/-}$ mice. On the other hand, under aglycemic condition, only R6/2-Ent2$^{-/-}$ mice, but not Ent2$^{-/-}$ mice or LPS-treated Ent2$^{-/-}$ mice, exhibited less basal respiration than their littermate controls. In terms of the ECAR value, Ent2$^{-/-}$ mice showed less ECAR value than the controls, whereas the LPS-treated Ent2$^{-/-}$ mice showed a higher ECAR value. Ent2 deletion did not change the EACR value of R6/2 mice.

Conclusion

These findings suggest that Ent2 deletion alters energy homeostasis in the striatum of mice. The impacts of Ent2 deletion on mitochondria function were complicated by different pathophysiological conditions such as Huntington's disease and LPS-induced neuroinflammation.

Disclosures: C. Ho: None. **C. Chen:** None. **K. Wu:** None. **Y. Chern:** None. **C. Lin:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.06

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH/NINDS OD010996

Title: Involvement of the centrally projecting Edinger-Westphal nucleus in the brain circuitry controlling energy homeostasis

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Abstract: The centrally projecting Edinger-Westphal nucleus (EWcp) is anatomically and functionally distinct from the Edinger-Westphal nucleus that contains the parasympathetic preganglionic neurons involved in pupillary constriction. We have proposed that the EWcp contributes to the control of energy homeostasis via modulation of sympathetic outflow to multiple organs, including all adipose tissues, after integrating multimodal signals from other central systems involved in thermoregulation, food intake and metabolic control (Cano et al, 2021). To further characterize the central circuitry that involves EWcp in these functions, we injected the retrograde tracer cholera toxin beta subunit (CTb) in the EWcp of rats, which were later exposed to stimuli that activate EWcp and are known to challenge thermoregulation or metabolic status. Rats (n=6) were exposed to 4°C ambient temperature for 4 hrs to identify afferents to EWcp activated during cold-induced thermogenesis. Another group of rats (n=5) were fasted for 48 hrs to identify afferents activated during food deprivation. Immunohistochemical labeling for CTb and c-Fos was performed in brain sections. Co-

expression of both markers was scored using a 5-point rank scale to characterize EWcp afferents activated by each stimulus. EWcp afferents displaying higher activation (rank $\geq$ 3; $\geq$ 25 double-labeled cells) by both stimuli included the lateral septum, caudal lateral hypothalamus (LH), pretectal nucleus and Darkschewitsch nucleus. In addition, there was stimulus-specific activation in several EWcp afferents. Brain regions that were more activated after cold exposure were the median and medial preoptic nuclei, arcuate nucleus, dorsomedial hypothalamic and parabrachial nuclei, whereas the areas that displayed more activation after fasting included the habenula, rostral LH and nucleus incertus. Phenotypic characterization showed that some activated EWcp afferent neurons in LH contained orexin, known to be involved in thermoregulation and feeding, whereas some neurons in the nucleus incertus contained relaxin-3, a potent feeding suppressor. Our results identified specific EWcp afferents activated by different stimuli that challenge the metabolic status and require specific responses to maintain homeostasis. Moreover, these data provide anatomical evidence for a role of EWcp in the central control of energy expenditure in different conditions by virtue of integrating multiple inputs from regions differentially activated by distinct stimuli, and subsequently conveying the appropriate output response to presympathetic groups to modulate the sympathetic outflow to specific organs and tissues.

Disclosures: **S.L. Hernan:** None. **V.A. Legeza:** None. **A.F. Sved:** None. **G. Cano:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

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Program #/Poster #: 308.07

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: CONACYT Grant 284771
CONACYT fellowship 531683

Title: Sulpiride, a dopamine D2 receptor antagonist, counteracts hyperglycemia and promotes growth and hyperplasia in subcutaneous adipose tissue in diet-induced obese female mice

Authors: *D. I. VÁZQUEZ-CARRILLO, A. L. OCAMPO-RUIZ, A. BAEZ-MEZA, G. RAMIREZ-HERNANDEZ, E. ADAN-CASTRO, J. L. DENA-BELTRAN, J. F. GARCIA-RODRIGO, M. G. ORTIZ-ARBALLO, G. MATINEZ DE LA ESCALERA, C. CLAPP, Y. MACOTELA;
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Abstract: Second-generation antipsychotics such as sulpiride, a dopamine D2 receptor antagonist, associate with the development of metabolic disturbances, believed to derive from their hyperprolactinemic effect (Pillinger, et al. 2020). However, recent studies have shown that both low and very high levels of prolactin (PRL) are metabolically detrimental, whereas levels between 7 and 100 ng/mL are considered metabolically beneficial. In humans and rodents, low PRL levels are associated with obesity, hyperglycemia, and insulin resistance (Macotela, et al.

2020); and in obese male rats with low PRL the administration of PRL improves metabolic features (Ruiz-Herrera, et al. 2017), hence using drugs that increase PRL levels, like sulpiride, could been beneficial against metabolic alterations in low PRL-obesity. However, there are few studies in females and the results are contradictory regarding the effects of sulpiride on metabolic homeostasis. Female rats treated with sulpiride along with an obesogenic diet (a model of antipsychotics-enhanced metabolic alterations), showed increased weight gain and decreased insulin levels without alterations in glucose levels (Baptista, et al. 1998), in lactating female rats sulpiride treatment didn't produce changes in body weight, or food intake (Vieira, et al. 2013), meanwhile, in nulliparous female rats sulpiride treatment decreased body weight (Mustafapour, et al. 2014). Due to this wide range of effects of sulpiride in females, and its effect increasing PRL levels, our objective was to evaluate the effects of sulpiride on the metabolic homeostasis of obese female mice, to assess its possible therapeutic action against metabolic dysfunction. For this, C57BL/6 8-week-old female mice fed a high-fat diet (HFD) for 8 weeks to induce obesity, were administered daily with 30 mg/kg of sulpiride during the last 4 weeks of the diet. Sulpiride increased PRL levels to 112.7 ± 11.3 ng/ml on the control diet, and 147.3 ± 15.2 ng/ml on the obesogenic diet. In obese mice, sulpiride treatment decreased hyperglycemia, without alterations in insulin, triglyceride levels, body weight or caloric intake. In the adipose tissue (AT), sulpiride increased the weight of the subcutaneous AT (SAT) and the hyperplasia of the adipocytes. Also, the expression of Hif1a (a marker of hypoxia), and of InsR and Glut4 (markers of insulin sensitivity) was unchanged. Our results show that in obese females, sulpiride generates hyperprolactinemia without generating adverse metabolic effects associated with its use, and on the contrary, it improves glucose metabolism and increases SAT hyperplasia, a parameter associated with better metabolic outcomes.

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Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.08

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: PGC2018-098229-B-100 to JLF
19904/GERM/15

Title: Reduced adipose tissue contents after evening but not morning exercise and the role of the hypothalamus

Authors: *Y. KUTSENKO[1,2], A. BARREDA[2], L. IÑIGUEZ[3], L. PARDO[4], A. TOVAL[5], D. GARRIGOS[1,2], M. MARTINEZ-MORGA[1,2], S. PUJANTE[6], B. RIBEIRO DO-COUTO[6,2], J.

CERON[4], M. GARAULET[7], M. WISNIEWSKA[8], M. IRIMIA[3], J. FERRAN[1,2];
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Abstract: Many homeostatic processes show daily fluctuations and patterns that can be altered through stressors such as diet, sleep, or physical exercise. Many of these responses are regulated by different hypothalamic nuclei. However, the hypothalamic control and the metabolic changes of these interactions have been little explored. In a previous work we found that male rats showed decreased adipose tissue contents after a forced running with both “early” (ZT14) and “late” (ZT20) active phase sessions (2 sessions a day). Here, we analyzed the effects of forced running during either “early” (ZT13) or “late” (ZT23) sessions in the adipose and hypothalamic tissues of rats. 48 adolescent male and female Sprague-Dawley rats were trained with a forced running program (ZT13 cage control n=6; ZT13 running n=6, ZT23 cage control n=6, ZT23 running n=6, for males and for females). On P24 and P57, the whole body of the rats was analyzed through computerized tomography. Between P24 and P57, rats followed a specific forced running protocol. Body weight and food intake were measured every 24 hours. On P60, the hypothalamic region, the inguinal and the retro-renal fat were removed and stored at -80C until further analysis. Only the male late exercise group showed lower adipose tissue content ($p<0.05$). Some differences were observed in the food intake (especially after the second week of running, $p<0.05$) but not in the hypothalamic orexigenic/anorexigenic gene expression of Pomc, Agrp, Npy, Cartpt ($p>0.05$), nor sleep/wake regulating genes such as Hcrt or Pmch ($p>0.05$) from the hypothalamic region. The gene ontology analysis of the differentially expressed genes from the transcriptome of the inguinal and retro-renal fat revealed, among others, steroid biosynthesis, TCA cycle and glycerolipid metabolism as pathways that were enhanced by late exercise only. Our results suggest that the effects of early or late exercise on the body composition are not related to intake differences in the brain. The decreased gain of adipose tissue with late exercise was not related to a chronic imbalance of hypothalamic orexigenic/anorexigenic or sleep/wake gene expression. However, lipid mobilization and metabolism pathways in the inguinal and retro-renal adipose tissue were upregulated in late exercised rats. Further studies are required to elucidate the mechanistic connections between the brain and the adipose tissue that regulate the effects of physical exercise when performed early or during the late active phase.

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Poster

308. Energy Regulation

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Program #/Poster #: 308.09

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R01 AG047972001A1

Title: Neurometabolic and neurovascular changes with age and relationships to structural and functional integrity

Authors: *D. ABDELKARIM[1,3], Y. ZHAO[4,3], M. P. TURNER[2,3], P. LIU[6], H. LU[7], B. P. RYPMA[5];
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Abstract: Brain aging processes are complex, involving many interacting physiological systems. Age-related changes in metabolic processes arise from altered pathways and cellular damage that increase steadily over the lifespan. It is known that the resting cerebral rate of oxygen metabolism (CMRO2) increases with age while task-elicited CMRO2 activity decreases. These changes also correspond to known age changes in cerebral blood flow (CBF). In this study, we aimed to compare these divergent processes and their relationships to other processes of brain aging. In groups of younger (20-35) and older (55-70) adults, we used T2-relaxation under spin tagging (TRUST), along with arterial phase contrast-derived cerebral blood flow, to estimate resting CMRO2 and resting CBF. We then calculated CMRO2 and CBF during a simple visual task using a dual-echo sequence and hypercapnia gas challenge. During the visual task, participants viewed a checkerboard flickering at a rate of 2Hz, 4Hz or 8Hz. Volumetric measurements were calculated based on high-resolution anatomical scans. Results indicated resting CMRO2 differences between age groups. In older adults, there were significant anticorrelations between resting-CMRO2 and task-CMRO2 across flicker frequencies. No such relationships were observed in younger adults. Resting-CBF and task-CBF showed similar patterns. Age-differences were further explored using other physiological data such as atrophy estimates and cerebrovascular reactivity. These results suggest that aging imposes metabolic demands upon neural processes at rest and increases upon resource demand during task performance. Such resource demands compromise those hyperemic systems involved in guiding metabolic resources to active parenchyma in older adults.

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Poster

308. Energy Regulation

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Program #/Poster #: 308.10

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant R15 DK108668
NIH Grant R15 DK121246

Title: Applying chemogenetic technology and controlled physical activity to assess ventromedial hypothalamic regulation of skeletal muscle thermogenesis

Authors: *J. T. SMITH[1,2], R. GIACOMINO[2], D. WALTER[2], A. MALIK[2], C. M. NOVAK[2,3], C. A. WATTS[3];
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Abstract: Our research program has discovered that exposure to predator threat in the form of predator odor (PO; ferret odor) induces sympathetic activation of skeletal muscle thermogenesis in both rat and mouse models. In this study, we used designer receptor exclusively activated by designer drug (DREADD) technology and male and female SF1-Cre mice to investigate the underlying brain mechanism responsible for this induced thermogenesis, while controlling for physical activity as a potential confounder. Each mouse received a bilateral stereotaxic injection of an adeno-associated viral vector into the dorsomedial region of the ventromedial hypothalamus (dmVMH) containing either an mCherry control vector (n=14) or the hM3Dq excitatory DREADD vector (n=14) that allowed the expression of excitatory receptors of the steroidogenic factor-1 (SF-1) neurons. These receptors are selectively activated for burst firing of the SF-1 neurons using clozapine N-oxide (CNO) administered via intraperitoneal injection. All mice were also implanted with an IPTT-300 temperature transponder adjacent to the right gastrocnemius to allow instant temperature measurements using a remote reader. For the experimental protocol, mice were first acclimated to the testing area before receiving an injection of CNO or vehicle. One hour after injection, elevated muscle temperatures were observed after CNO-induced activation of SF-1 neurons. Mice were then placed under controlled activity for 30 minutes (i.e., treadmill walking; 5.2 m/min) where they were presented with either PO or a control odor. As expected, treadmill walking increased muscle temperatures in all groups by approximately 2.5°C after 10 minutes of walking, with chemogenetic activation of dmVMH SF-1 neurons resulting in greater temperature elevation. Muscle temperature remained elevated through the end of testing. This suggests that the CNO-induced activation of muscle thermogenesis meets or exceeds the contractile thermogenesis produced from sustained activity, regardless of odor context. Therefore, these data support the mechanistic role of dmVMH SF-1 neurons in skeletal muscle thermogenesis.

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Poster

308. Energy Regulation

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Program #/Poster #: 308.11

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: DFG-233886668/GRK1960

Title: Knock-out of mitochondrial fission factor changes calcium handling in AgRP neurons

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Abstract: Mitochondrial dynamics, or cycles of mitochondrial fusion and fission, are known to occur upon changes in the intracellular environment. The mitochondrial shape, size, and function are regulated by numerous molecules, including the mitochondrial fission factor (Mff). Mff is located at the outer mitochondrial membrane and, together with dynamin-related protein-1 (Drp-1), mediates mitochondrial fragmentation. The Mff was shown to be involved in the regulation of synaptic transmission by changing the mitochondrial calcium buffering at the sites of synapses and therefore modulating the release of synaptic vesicles in the pyramidal cortical neurons (Nat Commun 9, 5008; 2018). Here, we focus on the role of Mff in AgRP neurons, a key orexigenic neuronal population located in the arcuate nucleus of the hypothalamus and playing an important role in the regulation of energy homeostasis. For that, an AgRP specific Mff knock-out mouse line was generated using the cre/loxp system. Using immunocytochemistry, we show that $MFF^{\Delta AgRP}$ neurons have larger mitochondria in the soma and axon than control littermates. In contrast, smaller mitochondria were detected in a 16 h fasted group. Decreased food intake during the nighttime in $MFF^{\Delta AgRP}$ group was also observed. However, no differences in food intake upon ghrelin treatment were found between groups. To better understand how Mff deletion affects the intrinsic properties of the AgRP neurons, we performed perforated patch clamp recordings on acute brain slices. While we did not find a difference in spontaneous activity nor input resistance between the control and $MFF^{\Delta AgRP}$ groups, we observed increased excitability of AgRP neurons in the $MFF^{\Delta AgRP}$ group. To check if the excitability change is correlated with altered mitochondrial calcium handling in AgRP neurons, GCaMP6 calcium imaging was performed along with trifluoromethoxy carbonylcyanide phenylhydrazone (FCCP) application as an uncoupler agent to deplete mitochondrial calcium. These experiments have shown an increased mitochondrial capacity to accumulate calcium in the $MFF^{\Delta AgRP}$ group both in soma and axons and decreased mitochondrial capacity to accumulate calcium in the 16 h fasted group. Collectively, the present study brings more insights into the role of mitochondrial dynamics in AgRP neurons in response to different metabolic states.

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Poster

308. Energy Regulation

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Program #/Poster #: 308.12

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: 2021R1A2C3004572
2020R1A6A3A13075788

Title: Dgat2 prevents non-alcoholic fatty liver disease by activating insulin signal in the differentiated adipocytes

Authors: *S. KI, S. WON, H. JEONG, J. LEE;
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Abstract: Nonalcoholic fatty liver disease (NAFLD), characterized by the accumulation of hepatocyte triglycerides, is the most common chronic liver disease, but there is no effective treatment yet. Since dysfunction of white adipocyte tissue (WAT) is associated with NAFLD, it is important to understand the effect of adipocyte on lipid accumulation of hepatocyte. Diacylglycerol acyltransferase 2 (DGAT2), a triglyceride synthesis enzyme, is involved in development and maintenance of adipose tissue. In the present study, we investigate the effect of DGAT2 on dysfunctional WAT-induced NAFLD. We confirmed that depletion of *Dgat2* inhibited adipocyte differentiation and lipid accumulation in the murine pre-adipocyte line, 3T3-L1. We next identified Dgat2-mediated regulators involved in adipocyte differentiation by transcriptome analysis. The results revealed molecules associated with mitotic cell cycle process and microtubule polymerization were potential mediator by DGAT2. We found that the identified mediators suppress autophagy flux through the PI3K/AKT pathway to promote adipocyte differentiation. We further found the DGAT2 is essential for regulation of insulin signaling in adipocytes. Finally, we showed that insulin signaling regulated by DGAT2 in adipocytes is involved in the control of lipid accumulation in hepatocytes. Therefore, our data suggest that DGAT2-dependent insulin signaling in differentiated adipocytes is important for controlling lipid formation in hepatocytes. Finally, we propose that NAFLD can be treated by targeting DGAT2 acting in adipocyte differentiation.

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Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.13

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: 1R01AG076704-01
Massachusetts General Hospital Executive Committee on Research Shore Award
MGH ECOR Interim Support Funding (ISF)

Title: A ketogenic diet in rats decreases sevoflurane induced burst suppression compared to a standard diet

Authors: M. J. SIEGMANN[1], P. L. PURDON[2], ***C. J. NEHS**[1];
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Abstract: Metabolism is now a major focus of research in aging and dementia. The brains supply of energy is critical for neurons to support cognition and other brain functions. The ability of the brain to use glucose declines with age but the brain's ability to use ketones as fuel remains. Inhaled anesthetic drugs in particular are known to impair astrocyte function at the cellular level and to inhibit mitochondrial function. General anesthesia and burst suppression (BS), have been hypothesized to be directly tied to brain energy metabolism. Previous studies have shown that nutritional ketosis delays the onset of general anesthesia. Therefore, in this study, we investigated the relationship between metabolism and brain oscillations, using sleep and anesthesia as a means to manipulate brain states. We were able to measure oscillations and the metabolites lactate and glucose simultaneously at high temporal resolution, during different underlying metabolic states, in rats eating a standard diet (SD) or a ketogenic diet (KD), to understand more precisely the moment-to-moment relationships between oscillations and metabolism. Nine Fisher 344 rats were fed a KD for at least 60 days, while eight were fed the SD. Rats were implanted with electroencephalogram (EEG) and electromyogram (EMG) electrodes and bilateral guide cannulas in the prefrontal cortex for amperometric glucose and lactate biosensors. Spontaneous sleep and wake were recorded for 3 hours followed by 2 hours of 3% sevoflurane anesthesia. Five additional rats on the KD received an intraperitoneal injection of the adenosine A1 receptor antagonist DPCPX before 3% sevoflurane. Under sevoflurane anesthesia, glucose and lactate concentration had larger standard deviation than sleep/wake ($p<0.001$, $p=0.015$) and changes were closely tied to electrophysiological oscillations. Animals on the KD had reduced BS compared to the SD ($p=0.0072$) as well as increased time to loss of movement ($p=0.0032$). DPCPX reversed the ketogenic diet induced reduced burst suppression compared to paired controls during anesthesia exposure. DPCPX also reduced the time until BS started and the duration of BS ($p=0.0065$, $p=0.0458$). Manipulating the underlying metabolic state of an animal through the ketogenic diet, altered the depth of anesthesia as indicated by the amount of burst suppression present. These findings suggest that metabolic interventions could be useful tools to improve sleep and protect the brain from anesthesia.

Disclosures: M.J. Siegmann: None. **P.L. Purdon:** None. **C.J. Nehs:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.14

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Mount Holyoke College

Title: Reconstruction and neuroanatomical mapping of an adult Drosophila melanogaster tracheal brain network using the FAFB electron microscopy volume dataset

Authors: K. SCHOENBERG[1], L. AGBEDUN[1], S. HUNTRESS[1], R. KANG[1], B. WOLDETSADIK[1], Y. CRUZ[1], M. BUI[1], S. CHO[1], M. LIN[1], K. LIN[1], L. ZEBROWSKI[1], C. LEWIS[1], K. BHASIIN[1], C. ABRAHAM[1], F. REYNOLDS-CORNELL[1], A. M. DACKS[2,3], P. SCHLEGEL[4], D. D. BOCK[5], ***K. J. COLODNER**[1];
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Abstract: The tracheal system of the fruit fly, *Drosophila melanogaster*, is a network of epithelial tubules that functions as the respiratory organ. The fly breathes through spiracles on the side of the body that allow for oxygen intake and carbon dioxide output throughout the tracheal tubular network. In the embryonic and larval stages, target-derived signaling regulates branching, morphogenesis, and patterning of the tracheal system to ensure proper gas exchange throughout the body. While the stereotyped patterning of the *Drosophila* body's tracheal system during development is well established, less is known about tracheal patterning inside the brain. This study aims to establish a complete cytoarchitectural map of the adult brain tracheal network and analyze tracheal branching patterns and properties within its anatomical framework. To do this, we utilized an open access electron microscopy volume of a Female Adult Fly Brain (FAFB) to manually reconstruct and skeletonize the entire fruit fly brain's tracheal network using the Collaborative Annotation Toolkit for Massive Amounts of Image Data (CATMAID) software. We used Flywire, the online FAFB segmentation platform, to proofread meshes and assign volumetric data to the tracheal skeletons, and performed initial characterization and analysis of the tracheal network. This work defines the brain's tracheal system of one adult fruit fly brain, and will serve as a valuable resource for future studies aimed at understanding trachea/nervous system interactions in adult *Drosophila*.

Disclosures: K. Schoenberg: None. **L. Agbedun:** None. **S. Huntress:** None. **R. Kang:** None. **B. Woldetsadik:** None. **Y. Cruz:** None. **M. Bui:** None. **S. Cho:** None. **M. Lin:** None. **K. Lin:** None. **L. Zebrowski:** None. **C. Lewis:** None. **K. Bhasiin:** None. **C. Abraham:** None. **F. Reynolds-Cornell:** None. **A.M. Dacks:** None. **P. Schlegel:** None. **D.D. Bock:** None. **K.J. Colodner:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.15

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant DK112198 to CJM.

Title: Energy expenditure is suppressed by lateral parabrachial nucleus neurons in diet-induced obesity

Authors: *C. M. D. MOTA, C. J. MADDEN;
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Abstract: Oxidation of fat by the brown adipose tissue (BAT) is one of the mechanisms contributing to energy balance and heat production. When the body is exposed to cold ambient temperatures, BAT thermogenesis produces heat and warms the body. Obese subjects and rodents, however, show impaired BAT thermogenesis to the cold. Our previous studies suggest that a neural pathway, based on vagal afferents synapsing with neurons in the nucleus tractus solitarius (NTS) inhibits BAT thermogenesis to the cold in obese rats. NTS neurons send projections to the dorsal aspect of the lateral parabrachial nucleus (LPBd), which receives inputs from the thermal afferent pathway conveying warm signals from the periphery and inhibits BAT thermogenesis. This study investigated the role of the NTS-LPB pathway in suppressing BAT thermogenesis and the contribution of LPBd neurons in the impairment of BAT thermogenesis in obese rats. By using a targeted dual viral vector approach that allowed the expression of Gq-coupled DREADDs in LPB-projecting NTS neurons in male Sprague Dawley chow-fed rats, we found that chemogenetic activation of an NTS-LPB pathway inhibited BAT thermogenesis to the cold, as observed by reductions in BAT sympathetic nerve activity (SNA) (-270±68%baseline (BL) from a cold-evoked level of 478±95%BL, p=0.03) and BAT temperature (TBAT) (-0.7±0.2°C from a cold-evoked level of 33.7±0.4°C, n=4, p=0.04). To investigate if LPBd neurons are active in obese rats, thus suppressing BAT thermogenesis to the cold, other groups of rats were submitted to a high-fat diet or a chow diet. We found that the number of Fos-labelled neurons in the LPBd was higher in obese rats than in chow diet-fed rats (141±18 vs. 56±5, n=3, p=0.03) after exposure to a cold ambient temperature. Other groups of obese and control rats were anesthetized and received nanoinjections with a GABA receptor agonist into the LPBd. Inhibition of LPBd neurons rescued BAT thermogenesis to the cold in obese rats (BAT SNA: +494±157%BL from a prior level of 98±4%BL, p=0.03; TBAT: +1.7±0.7°C from a prior level of 33.2±0.5°C, n=6, p=0.04) but did not potentiate an ongoing BAT thermogenesis to the cold in control rats (BAT SNA: +194±400%BL from a prior cold-evoked level of 1291±649%BL, p=0.65; TBAT: +0.0±0.3°C from a prior cold-evoked level of 35.5±0.4°C, n=5, p>0.99). These data reveal a neural circuit (NTS-LPB) that reduces energy expenditure and a critical brain area (LPBd) that tonically suppresses energy expenditure in obesity. These findings reveal novel effects of high-fat diets in the brain and in the control of energy metabolism and can contribute to the development of therapeutic approaches to regulate fat metabolism.

Disclosures: C.M.D. Mota: None. **C.J. Madden:** None.

Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.16

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R15 DK121246
R15 DK108668

Title: Elevation of energy expenditure under perceived threat via steroidogenic factor-1 (SF-1) neurons

Authors: *C. A. WATTS[1], J. SMITH[2], A. MALIK[3], R. GIACOMINO[3], D. WALTER[3], E. WELCH[3], C. M. NOVAK[3];
[1]Sch. of Biomed. Sci., [2]Col. of Publ. Hlth., [3]Dept. of Biol. Sci., Kent State Univ., Kent, OH

Abstract: Homeostatic and allostatic influences on physiological regulation and stability of the internal environment find relevance in many diseases such as obesity and diabetes. Allostatic adaptations required under challenge may tap into mechanisms known to serve homeostatic control of energy balance. Though not in line with the homeostatic demands, allostatic physiological responses may be crucial to the animal's survival when faced with a perceived threat. Our research program has previously established that exposure to predator odor (PO) in rats significantly increases skeletal muscle thermogenesis, energy expenditure (EE), and oxygen consumption (VO_2) without significantly altering the respiratory exchange ratio (RER). Previous literature highlights steroidogenic factor-1 (SF-1) cells within the dorsal medial subregion of the ventromedial hypothalamus (dmVMH) as a prominent element in both energy homeostasis and defensive behaviors such as freezing, withdrawal, and avoidance. However, little is known about the brain mechanism at work driving elevated EE during PO exposure. We hypothesize that SF-1 neurons of the dmVMH play a critical role in regulating energy balance during predator odor exposure. To assess this hypothesis, we used the combined technology of designer receptors exclusively activated by designer drugs (DREADD) and SF1-Cre mice. Here, we evaluate data collected via indirect calorimetry of SF1-Cre mice bilaterally injected with an excitatory DREADD or mCherry control vector. Using clozapine-N-oxide (CNO), we remotely activated SF-1 neurons in the presence of a control or PO (i.e., ferret odor). We detected significant increases in EE and VO_2 with CNO activation of SF-1 neurons. No significant changes were identified in the RER or total activity, suggesting that physical activity is not responsible for the observed differences. However, there was no detectable DREADD-induced amplification of the EE and VO_2 due to perceived threat. These data support the importance of SF-1 neurons and their role in amplifying EE. This negative energy balance acts in conjunction with muscle thermogenesis, creating a whole-body response to the presence of a threat.

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Poster

308. Energy Regulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 308.17

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: MOST Grant 110-2314-B-001 -007 -

Title: Neuronal K_{ATP} channels are dispensable for glucose homeostasis in mice

Authors: *A. H. LI, H.-J. TSAI, S.-B. YANG;
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Abstract: The ATP-sensitive potassium (K_{ATP}) channels in pancreatic beta cells and hypothalamic glucose-sensing neurons are crucially involved in tight regulation of glucose homeostasis to maintain proper bodily functions. At the molecular level, K_{ATP} channels consisting of Kir6.2 and SUR1 subunits are the key molecular components required for the glucose-sensing properties in both cell types. Although whole-body Kir6.2 knockout (KO) mice exhibit severe disruption in glucose homeostasis, the impacts of cell-type-specific Kir6.2 KO have remained elusive. In this study, we utilized a sophisticated conditional Kir6.2 KO mouse strain (Kir6.2-Fc) to examine the respective outcomes of whole-body (Kir6.2-FcN), nervous system-dependent (Nestin-Cre;Kir6.2-$Fc^{F/F}$), and pancreatic beta cell-dependent Kir6.2 KO (Ins1-RFP-Cre;Kir6.2-$Fc^{F/F}$) on glucose homeostasis. First, we measured the alterations in blood glucose levels upon glucose, insulin, and 2-deoxy-glucose (2DG) challenges to assess glucose tolerance, insulin sensitivity, and glycopenia-sensing abilities, respectively. The Kir6.2-FcN mice demonstrated severe glucose intolerance and exacerbated glucose-releasing effects under glycopenic states, and these phenotypes were reciprocated only in Ins1-RFP-Cre;Kir6.2-$Fc^{F/F}$ but not Nestin-Cre;Kir6.2-$Fc^{F/F}$ mice. To further confirm that pancreatic but not neuronal K_{ATP} channels act as the systemic glucostat, a three-day time-course monitoring of blood glucose levels divided into three stages, 24-hour *ad libitum*, 24-hour fasting, and 4-hour re-feeding, was performed. As expected, Kir6.2-FcN and Ins1-RFP-Cre;Kir6.2-$Fc^{F/F}$ but not Nestin-Cre;Kir6.2-$Fc^{F/F}$ mice had higher glucose levels and wider fluctuation ranges (202.81 ± 51.44 mg/dL, 193.54 ± 44.11 mg/dL, and 166 ± 19.51 mg/dL) during *ad libitum*, and drastic decreases in blood glucose levels after a 24-hour fasting duration were observed also only in the former two groups (52.5 mg/dL, 51.71 mg/dL, and 89.71 mg/dL). Finally, Ins1-RFP-Cre;Kir6.2-FcN mice (n=3) were injected intraductally with AAV9-Kir6.2-Flex-GFP, and their glucose tolerance was rescued (area under curve, AUC, before and 8 weeks after rescue surgery: 33183.33 min·mg/dL and 21252.5 min·mg/dL). In conclusion, pancreatic K_{ATP} channels are sufficient to maintain the stability of circulating glucose levels in mice and should be treated as the primary target for therapeutic intervention to rescue glucose dyshomeostasis.

Disclosures: A.H. Li: None. **H. Tsai:** None. **S. Yang:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.01

Topic: F.07. Biological Rhythms and Sleep

Support: National Science Agency (ANPCyT) Grant
National Research Council (CONICET) Grant
National University of Quilmes (UNQ) Grant

Title: Food and light as circadian time cues: characterizing motivation behavior in mice under time-restricted feeding and chronic jet lag

Authors: *J. ACOSTA[1], M. CRESPO[1], S. A. PLANO[2,3], J. J. CHIESA[1,3], D. A. GOLOMBEK[1,3], P. V. AGOSTINO[1,3];
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Abstract: Most living organisms have a circadian timing system adapted to optimize the daily rhythm of exposure to the environment. This circadian system modulates several behavioral and physiological processes, including the response to natural and drug rewards. The main synchronizer agent of the circadian clock is the light-dark (LD) cycle. Changes in LD exposure can cause circadian disruption producing cognitive deficits. On the other hand, food can also act as a potent synchronizer when is temporally restricted. Under these conditions, animals display an anticipatory food activity (FAA) controlled by a food-entrainable oscillator (FEO). We have shown that motivation for food reward is regulated in a circadian manner in mice, with higher levels at night (active phase). In this work, we aim to continue characterizing the circadian modulation of motivation behavior in mice through two different approaches: when food is the main synchronizer (by time restricted feeding protocol, TRF) and when chronic phase advances in the LD cycle cause circadian disruption (by chronic jet lag protocol, CJL). In both experiments we assayed motivation through the Progressive Ratio (PR) schedule, in which subjects must increase the number of responses to earn subsequent food rewards. In the first experiment, we subjected two different groups of C57BL/6 male mice to a TRF protocol in which food was only available for 3 hours in the light (n=16) or dark (n=16) phase of the 12:12 LD cycle. Then, motivation behavior was assayed in each group at two different time points: during FAA - i.e., two hours before food availability - (n=8) and in the opposite phase to which the TRF was carried out (n=8). Our results showed that mice are highly motivated to work for food reward when FAA is present regardless of the time of day. More importantly, when FAA occurred during daytime mice presented high levels of motivation comparable to those observed at nighttime, opening the question of whether components related to reward pathways could be being synchronized to both light and food at the same time. In the second experiment, we submitted mice to a CJL protocol, which consisted of 6-hour phase advances of the LD schedule every 2 days. Then, we assayed motivation in the dark phase of CJL (n=12) and in the control group (12:12 LD, n=8). Our results showed that motivation in the CJL group was diminished compared to controls, suggesting that forced circadian desynchronization affects reward-related behaviors. Together, these findings contribute to gaining knowledge in potential mechanisms of

circadian modulation of motivational states in order to improve treatment related to psychiatric disorders or drugs of abuse.

Disclosures: **J. Acosta:** None. **M. Crespo:** None. **S.A. Plano:** None. **J.J. Chiesa:** None. **D.A. Golombek:** None. **P.V. Agostino:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.02

Topic: F.07. Biological Rhythms and Sleep

Support: TBD/inception study

Title: Inception: phase-amplitude coupling predicts incorporation of external stimuli into dreams

Authors: E. MONAI, F. DE PAIVA, B. RIEDNER, *B. SEVAK, G. TONONI, M. BOLY, B. BAIRD;
UW Madison, Madison, WI

Abstract: A hallmark of sleep is the sensory disconnection from the external world. This disconnection represents an unsolved paradox during the wake-like EEG pattern of REM sleep. Though, recent researches suggest that some processing of external stimuli occurs during REM sleep. However, the neurophysiological mechanisms underlying sensory incorporation versus disconnection during REM sleep are still unclear.To explore these mechanisms, we delivered visual stimuli (light pulses) using a custom-designed sleep mask during REM sleep. Nineteen healthy volunteers (7 female, mean age=23, range=19-27) were assessed with 20-channel electroencephalography (EEG) along with sleep polysomnography. For every REM cycle, participants received visual stimulation and were then immediately awoken and interviewed about their experience (i.e, perception of the light pulses). A total of 14 out of 19 participants achieved stable REM sleep and thus received visual stimulation. Sixteen out of 49 trials (32.6%) showed successful incorporation of the stimulus.Only recordings from participants who experienced both incorporation and no incorporation of visual stimuli (n=8) were analyzed. Recordings were band-pass filtered, and artifacts were manually rejected. Phase and power information were extracted from the occipital (Oz) EEG time series preceding the stimulus delivery, and the average phase-amplitude coupling (PAC) for each condition was estimated across a range of frequencies. The statistical significance of the observed PAC was assessed using a bootstrapping method. Our analysis revealed prominent coupling between the phase of high-alpha oscillations and the power of high-beta oscillations preceding the incorporation of visual stimuli. The strongest coupling was found between the phase of 11 Hz and the power of 21 Hz (Z score = 3.5645). Subjectively, incorporated stimuli were often reported as adapted to the content of the ongoing dream (i.e., “I was looking at my cell phone in the dream and the phone kept flashing”).These results show that sensory disconnection/connection during REM

sleep is a dynamic and flexible state rather than a static condition. Relevantly, incorporation is influenced by the spontaneous brain activity preceding the stimulus delivery. Particularly, for the first time we showed that pre-stimulus occipital PAC, a proposed mechanism for local computation as well as long-range information transmission, predicts trials with successful incorporation of visual stimuli. Furthermore the serial awakening paradigm revealed how external stimuli can be actually incorporated into the content of dreams.

Disclosures: E. Monai: None. **F. De Paiva:** None. **B. Riedner:** None. **B. Sevak:** None. **G. Tononi:** None. **M. Boly:** None. **B. Baird:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.03

Topic: F.07. Biological Rhythms and Sleep

Title: Differences in 24-hour mood rhythmicity between individuals with major depressive disorder and healthy controls: A preliminary analysis

Authors: *A. NEXHA[1], N. B. XAVIER[2], L. K. PILZ[2], M. A. B. OLIVERIA[2], M. P. L. HIDALGO[2], B. N. FREY[1,3];
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Abstract: INTRODUCTION: Major depressive disorder (MDD) is one of the most prevalent psychiatric disorders that affects over 300 million people worldwide. Abnormalities in circadian rhythms are thought to contribute to its symptomatology. Irregularities in behaviours, such as sleep and meal timing, may contribute to misalignment between central and peripheral clocks and the development of acute affective states. MDD has been associated with disrupted, excessive, or irregular sleep that reflects a disrupted circadian organization. This may affect subjective perception, such as the timing of mood-related symptoms in affective populations. The Mood Rhythm Instrument (MRhI) was developed to evaluate 24-hour rhythmicity of mood-related symptoms. The objective of this study is to test the MRhI in a psychiatric sample of Canadians with MDD. METHODS: Participants (52 MDD, 75% female, ages 17-70, mean age±SD=34±15 and 17 healthy controls (HC), 65% female, ages 18-72, mean age±SD=27±12) were recruited through online and community advertisements in the Greater Toronto and Hamilton Area, Canada. Recruitment is ongoing. Psychiatric diagnoses were assessed using the Mini International Neuropsychiatric Interview: we included MDD patients with a primary diagnosis of current or past MDD and HC not meeting criteria for any psychiatric disorder. The MRhI was administered to assess rhythmicity of affective, cognitive, and somatic mood-related symptoms for the previous 15 days. Group differences in frequency of peaks were assessed using Fisher's test, timing using the Watson-Wheeler test, and age and sex differences using logistic

regression. RESULTS: Individuals with MDD reported significantly more peaks than HC for anxiety (p=0.013), sadness (p=0.015), and energy (p=0.009). There were no significant differences between groups in age, sex, peaks of somatic symptoms, or timing of any symptoms. CONCLUSIONS: Individuals with major depression are more likely to perceive the 24-hour rhythmicity of affective symptoms, such as anxiety and sadness, as well as energy levels. Similar findings were seen in samples exploring the relationship between peak of symptoms against risk of psychiatric disorders in Spanish, Brazilian, Colombian, and Canadian populations. A limitation of the current analysis is the sample size, as recruitment is ongoing.

Disclosures: A. Nexha: None. **N.B. Xavier:** None. **L.K. Pilz:** None. **M.A.B. Oliveria:** None. **M.P.L. Hidalgo:** None. **B.N. Frey:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.04

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AG062883-01
NIH Grant GM121310-05

Title: A Novel Input to the Circadian System and its Painful Consequences

Authors: *A. WARFIELD, P. GUPTA, W. D. TODD, III;
Zoology and Physiology, Program in Neurosci., Univ. of Wyoming, Laramie, WY

Abstract: Chronic pain has long been thought to be influenced by circadian rhythms due to temporal clinical observations and, over the last twenty years, much research has begun to elucidate the interactions between these two systems. However, little to no work has addressed this question from the perspective of how chronic pain can disrupt rhythms. Additionally, there has not been a circuit identified and characterized that could underlie the observed effects. To address this, our work has focused on a neural circuit that connects the lateral parabrachial nucleus (LPB), a crucial node in the pain circuit, to both the suprachiasmatic nucleus (SCN), the master pacemaker of circadian rhythms, and the subparaventricular zone (SPZ), the major output of the SCN. We show that the cells projecting from the LPB to the SCN/SPZ heavily express prodynorphin mRNA as well as expressing c-Fos (a marker of neuronal activation) following hind paw injection of formalin, a painful stimulus. Additionally, we show that the cells of the SPZ express c-Fos to a greater extent after formalin injection than after saline injection. Furthermore, using models of both inflammatory and neuropathic pain, we show that circadian rhythms are significantly disrupted following the administration of painful stimulus. With Complete Freud's Adjuvant (CFA, an inflammatory agent), we see disruption in both acrophase (peak) and bathyphase (trough) of body temperature and locomotor activity rhythms. These effects on body temperature are taken away with global double knockout of transient receptor

potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1). In spared nerve injury (SNI) condition we see an advance in acrophase and bathyphase of body temperature in mice at 8-10 months of age for as long as 10 days after surgery, but we do not see this effect in younger mice. Additionally, mice receiving SNI experience hypothermia and reduced activity during the dark/active phase for 5 days following surgery but those receiving sham operation do not.

Disclosures: **A. Warfield:** None. **P. Gupta:** None. **W.D. Todd:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.05

Topic: F.07. Biological Rhythms and Sleep

Support: DARPA ADAPTER

Title: Modeling Circadian Rhythm Using Physiological and Behavioral Biomarkers

Authors: *R. JIANG, D. GRIFFIN, D. WEBER;
Carnegie Mellon Univ., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Circadian rhythm disruption is mostly caused by flights across multiple time zones and shift work. Prolonged circadian rhythm disruption can lead to sleep disorders, cognitive deficit, and higher risk of heart disease, diabetes, and obesity. Therefore, therapies for facilitating the maintenance of healthy circadian rhythms are crucial. However, current available therapies can yield different effects on entraining circadian rhythms when applied at different times. Therapies on circadian rhythm need to be delivered at the right phase of the rhythm to maximize effects, and thus an accurate predictive model of circadian rhythm is crucial . In animal models of circadian rhythm, melatonin and cortisol expression and core temperature are candidate biomarkers for constructing a predictive model. Many wearable sensors can collect heart rate, actigraphy, and electromyography (EMG) from humans. Whether these accessible biomarkers can provide an accurate estimation of the instantaneous phase of circadian rhythms is not certain. To determine whether these biomarkers provide an accurate phase estimate of circadian rhythm, we recorded biomarkers, including neck EMG, core temperature, electroencephalogram (EEG), electrocardiogram (ECG), and actigraphy, from four cynomolgus monkeys using an implantable telemetry implant (PhysioTel Digital L04, DSI). We selected cynomolgus monkeys as our subjects because they are diurnal animals exhibiting circadian rhythms similar to humans. Core temperature is used as the reference for circadian rhythm estimation. The animal is placed under a normal light-dark cycle for 5 days and shifted light-dark cycle for 10 days. We used cosinor analysis to extract circadian rhythms from neck EMG, heart rate from ECG, actigraphy, and core temperature. Under a stable light-dark cycle, neck EMG, heart rate, and actigraphy showed similar circadian phase fluctuation patterns across 5 days. However, when the animals were

placed in the new light-dark cycle, neck EMG, and heart rate showed different rates of entrainment in comparison to that of the core temperature. The core temperature required 3 days to entrain, while neck EMG and heart rate took only 1 day to do so. Our current finding suggested that successfully estimating circadian rhythm during the normal light-dark cycle might not entail the same results in the altered light-dark cycle. For future modeling, a combination of principal component analysis and Kalman filter could solve the inaccuracies in estimation in altered light-dark cycles.

Disclosures: R. Jiang: None. **D. Griffin:** None. **D. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power, Inc., BlackFynn, Inc, Iota Bioscience, Inc, Neuronoff, Inc, Neuroone, Inc, Reach Neuro, Inc.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.06

Topic: F.07. Biological Rhythms and Sleep

Title: Estradiol regulates circadian responses to acute and chronic light exposure in female mice

Authors: J. M. MICHAUD[1], C. T. WARING[1], **J. C. PRICE**[1], F. MEDEIROS CONTINI[1], M. E. BURNS[1], H. A. CONCEPCION[1], H. V. DEANE[1,2], *J. A. SEGGIO[1];
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Abstract: Sex hormones are well known to modulate circadian timekeeping. Sex differences also exist in the behavioral and physiological responses to circadian disruption, where males tend to be more sensitive to those negative effects. In this study, we decided to test whether ovaric estradiol (E2) plays a role in regulating the circadian rhythmicity in both a standard light:dark (LD) cycle and in constant light (LL) in female C57BL6/NJ mice. Mice were either ovariectomized or given sham surgery and given a placebo (P) or E2 pellet for hormone replacement, so that there were six groups: 1) LD/Sham, 2) LL/Sham, 3) LD/OVX+P, 4) LL/OVX+P, 5) LD/OVX+E2, and 6) LL/OVX+E2. OVX+P mice exhibited shorter circadian periods and were more likely to become arrhythmic in LL compared to mice with intact estradiol (sham or E2 replacement mice). Additionally, the period shortening exhibited by the OVX+P mice was gradual over the course of the experiment (i.e., the period became shorter over time), rather than being an initial difference when immediately placed in LL. OVX+P mice exhibited reduced circadian robustness (power) and reduced circadian locomotor activity in both LD and LL compared to sham controls or OVX+E2 mice, although these differences were found from the onset of placement into LL. OVX+P mice also exhibited reduced phase delays, but not advances, when given a 15-minute light pulse as well as later activity onsets compared to estrogen intact mice. LL led to reductions in estrogen receptor beta, but not estrogen alpha, in the

SCN regardless of surgery type. LL also lead to increases in novelty-induced activity in the open-field in all groups, regardless of estrogen status. Lastly, LL had no effects on serum or SCN estradiol levels, although OVX+P mice exhibited reduced serum (but not brain) estradiol. These results indicate that estradiol can mediate the effects of circadian disruption and that estradiol can provide protection against a loss of circadian robustness.

Disclosures: **J.M. Michaud:** None. **C.T. Waring:** None. **J.C. Price:** None. **F. Medeiros Contini:** None. **M.E. Burns:** None. **H.A. Concepcion:** None. **H.V. Deane:** None. **J.A. Seggio:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.07

Topic: F.07. Biological Rhythms and Sleep

Support: Owens Family Foundation

Title: Altered circadian re-entrainment in Alzheimer's disease mouse models does not require tauopathy or microglia

Authors: *T. K. WEIGEL[1], A. D. GÜLER[2,1], H. A. FERRIS[3,1];
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Abstract: Circadian symptoms have long been observed in Alzheimer’s disease (AD) and often appear before cognitive symptoms, but the mechanisms underlying circadian alterations in AD are poorly understood. We studied circadian re-entrainment in AD model mice using a “jet lag” paradigm, observing their behavior on a running wheel after a six hour advance in the light:dark cycle. Accelerated re-entrainment to this shifted light cycle can be an indication of impaired function in the suprachiasmatic nucleus (SCN), the circadian timekeeper of the brain. Female 3xTg mice, which carry mutations producing progressive amyloid beta and tau pathology, re-entrained significantly more rapidly than age-matched wild type controls at 8 and 13 months of age. This re-entrainment phenotype has not been previously reported in a murine AD model. Other aspects of circadian behavior, including free run period, were not altered. 3xTg mice reflect multiple aspects of AD pathology, including amyloid pathology, tau pathology, and microglia activation, which could all contribute to the circadian behavioral phenotype that we observed. To test whether tauopathy is necessary for this behavioral phenotype, we repeated the jet lag behavioral test with another AD mouse model, the 5xFAD mouse, which develops amyloid pathology but not tau pathology. As with 3xTg mice, 7-month-old female 5xFAD mice re-entrained significantly more rapidly than controls, demonstrating that tauopathy is not necessary for the re-entrainment phenotype. Because microglia are activated in AD and in the 3xTg and 5xFAD models, and inflammation can affect circadian rhythms, we hypothesized that microglia contribute to this re-entrainment phenotype. To test this, we used the colony

stimulating factor 1 receptor (CSF1R) inhibitor PLX3397, which rapidly depletes microglia from the brain. Microglia-depleted wild type and 3xTg mice were tested in the jet lag paradigm described above. Microglia depletion did not alter re-entrainment in either wild type or 3xTg mice, demonstrating that microglia activation is not responsible for the re-entrainment phenotype. Together, these experiments demonstrate a novel circadian behavioral phenotype in AD model mice which is not dependent on tauopathy or microglia.

Disclosures: T.K. Weigel: None. **A.D. Güler:** None. **H.A. Ferris:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 309.08

Topic: F.07. Biological Rhythms and Sleep

Support: NIH/NIGMS 1K99GM132557
Chan Zuckerberg Biohub
NIH 5T32NS95939-7
Molecular and Cell Biology, UC Berkeley

Title: Investigating the role of rhythmic cues in the parasite circadian clock

Authors: *B. PARRINGTON[1], R. PASCUAL[2], C. AGUDELO[2], V. ACOSTA RODRIGUEZ[4], F. RIJO-FERREIRA[3];
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Abstract: Malaria is famous for its rhythmic fevers, a consequence of the rhythms of the parasites in the bloodstream of the host. Parasites, despite never free living, such as the non-lethal malaria-causing parasite *Plasmodium chabaudi* (*P. chabaudi*), have intrinsic clocks. Parasite's rhythms are not generated by the vertebrate host's feeding rhythmicity nor the host's behavioral circadian rhythm (https://www.science.org/doi/abs/10.1126/science.aba2658). Nonetheless, our preliminary data suggest that the Plasmodium population relies on the host's circadian rhythm to be synchronous with each other. However the entrainment cues are mostly unknown. We set out to test whether feeding rhythms and thus nutrients or metabolic rhythms are an entrainment cue for the parasite population and how are the dynamics of synchronization. We infected both wild-type (WT) and arrhythmic circadian *Cry1/Cry2* knockout (KO) mice with *P. chabaudi*, either fed *ad libitum* (*ad lib*) or under 12-hour time restricted feeding (TRF). We measured parasitemia in blood smears of WT, *Cry1/Cry2* TRF, and *Cry1/Cry2 ad lib* infected mice to investigate whether the food rhythmicity impacted parasitemia and parasite rhythms. As expected, over 85% of WT mice survived. Surprisingly, we found that the arrhythmic mutants fed *ad lib* died by day 13 post-infection, whereas 50% of the *Cry1/Cry2* KO mice on TRF

survived. Rhythmic feeding may be sensed by the parasite and also entrain key immune lineages essential for controlling the infection. Understanding the molecular cues underlying malaria rhythms will lead to ways of disrupting the synchrony among parasites which is at the core of the major symptoms.

Disclosures: B. Parrington: None. **R. Pascual:** None. **C. Agudelo:** None. **V. Acosta Rodriguez:** None. **F. Rijo-Ferreira:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.09

Topic: F.07. Biological Rhythms and Sleep

Support: Internal funding for the University of Colorado, Boulder

Title: The effect of oral nicotine on diurnal sleep patterns and delta homeostasis in female C57BL/6J mice

Authors: *S. AKI, M. BROWN, J. STITZEL, H. MATHEWS;
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Abstract: Nicotine use has long been associated with a plethora of detrimental health effects, including sleep disruption. Our lab has previously published on the effects of nicotine administration on sleep in male C57BL/6J mice, yet no work has been done to examine the effects in females. This work is particularly important due to known sex differences in sleep patterns and responses to nicotine. Therefore, the current study aims to characterize sleep during a period of nicotine administration in female C57BL/6J mice. In initial experiments (n=4), mice were implanted with EEG/EMG recording devices and data was recorded continuously. During the baseline condition, mice had ad libitum access to a food and .2% saccharin water solution. To produce nicotine dependence, 200ug/ml of nicotine was added to the .2% saccharin drinking solution. EEG/EMG data was scored for two consecutive days of baseline and on nicotine administration days 1, 4, 8, & 12. EEG data were classified in 4 second epochs as either wake, NREM, or REM. The preliminary results indicate all significant alterations in sleep quantity and architecture during the nicotine administration period occurred during the inactive phase, no significant differences were observed during the normal active phase. Nicotine administration led to an increase in NREM sleep and a decrease in REM sleep during the inactive phase relative to baseline. NREM quantity increases were due to increases in NREM bout duration, whereas REM quantity decreases were due to decreases in REM bout frequency. Additionally, we observed decreases in the number of sleep and total stage shifts. These results suggest that nicotine leads to more consolidated NREM sleep (increased NREM bout duration and quantity) at the apparent cost of REM sleep. Power spectral analyses were also performed for each state and analyzed using a single component cosigner analysis. These analyses suggest no effect of nicotine

administration on delta (0.5-4hz) during NREM. However, diurnal alterations were observed for delta during wake. Future analyses will include a multicomponent cosigner analyses to further clarify these relationships as well as an analysis of the effects of nicotine withdrawal on vigilance states and the EEG spectra. Overall, these findings will shed new light on the consequences of nicotine on sleep in female mice.

Disclosures: S. Aki: None. **M. Brown:** None. **J. Stitzel:** None. **H. Mathews:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.10

Topic: F.07. Biological Rhythms and Sleep

Support: R00 HD084759

Title: Co-housing as a protective factor against depressive-like symptoms in response to light-induced circadian disruption in female mice

Authors: *B. M. DEVRIES[1], H. M. HOFFMANN[2];
[2]Michigan State Univ., [1]Michigan State Univ., East Lansing, MI

Abstract: Mistimed nocturnal exposure to light, such as in shift work or areas with high light pollution, alters various processes in the brain and body. Circadian, or daily, rhythms are sensitive to light, as the suprachiasmatic nucleus, the neurological master pacemaker, is in direct contact with the optic nerve. Alterations in light exposure have been shown to disrupt circadian rhythms and have negative effects on physiological and cognitive functions, including increasing the risk of depression and mood disorders. Our goal was to better understand the association between circadian disruption and mood disorders. To do this, we used a mouse model of light-induced circadian disruption, referred to here as the chronic advance shift (CAS) model, where the female mice experienced lights turning on 6 hours earlier every 4 days for 7 weeks. To understand how CAS affected depressive-like behaviors in mice, we examined despair-like behaviors via the forced swim test. When examining mice that were co-housed, we found no difference between the controls and CAS mice. However, CAS mice that were housed alone showed significantly higher levels of despair-like behavior when compared to single housed control mice. This suggests that socialization is a protective factor against depressive-like behavior in response to light-induced circadian disruption. To determine if socialization is a protective factor for other reported symptoms that often accompany depression, such as weight gain or reduced fertility, we examined weight and estrous cycles in the single and co-housed control and CAS mice. We found that socialization was not a protective factor for disrupted estrous cycles nor for weight gain in response to CAS. Together these data suggest that some of the symptoms associated with depression, such as irregular estrous cycles and weight gain, may

not be alleviated with socialization, but depressive-like behaviors may be reduced with social interaction.

Disclosures: B.M. Devries: None. **H.M. Hoffmann:** None.

Poster

309. Circadian Behaviors

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.11

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01DK119811

Title: Circadian desynchronization-induced metabolic disorder is ameliorated by endocannabinoid receptor knockout, without change in feeding or activity

Authors: *B. FALCY[1], G. L. PEARSON[1], T. L. LEISE[2], I. N. KARATSOREOS[1];
[1]Univ. of Massachusetts, Amherst, Univ. of Massachusetts, Amherst, Hadley, MA; [2]Mathematics and Computer Sci., Amherst Col., Amherst, MA

Abstract: Disruption of the circadian clock can lead to several changes in metabolic phenotypes, including weight gain, elevated plasma triglycerides, and increased hepatic lipid deposition. We have shown that environmental circadian desynchronization (ECD), by housing mice in 20h cycles (10h light - 10h dark), leads to weight gain, higher adiposity, and altered metabolic hormone levels in mice. While classical metabolic hormones certainly contribute to these phenotypes, understanding and treating metabolic disruption will require a well-rounded knowledge of all involved pathways. Endocannabinoid (eCB) signaling can affect metabolism from the cellular to behavioral levels via effects both in the periphery (e.g., liver) as well as centrally in the brain. Interactions between eCBs and circadian rhythms have been documented in humans, where circadian misalignment can lead to increases in eCBs measured in the blood. To explore these phenomena on a more mechanistic level, we determined the effects of global cannabinoid type 1 receptor (CB1r) knockout on the metabolic and behavioral consequences of ECD. We undertook detailed behavioral and metabolic phenotyping in CB1r WT and KO littermate mice of both sexes during baseline 24h and experimental 20h light-dark cycles. We found that in ECD, weight gain was significantly higher in WT male, but not female, mice compared to KO littermates, demonstrating that CB1r KO mice are resistant to the metabolic effects of ECD in a sex-dependent manner. We replicated this in two cohorts, for a total of n=8/sex/genotype. We additionally assessed levels of circulating metabolic hormones including insulin and leptin at different times throughout ECD for WT and CB1r KO mice. We also explored changes in several behaviors including locomotor activity, feeding, and drinking, as well as a detailed examination of changes in the respiratory exchange ratio, all of which was done blind to the groups. While there were genotype and sex differences in the organization of some of these behaviors, changes in overall activity or feeding levels do not explain the observed

weight differences, as no effect of genotype was found on overall locomotor activity or feeding. Together, our data support a potentially behavior-independent role for eCB signaling in ECD induced metabolic dysregulation.

Disclosures: B. Falcy: None. **G.L. Pearson:** None. **T.L. Leise:** None. **I.N. Karatsoreos:** None.

Poster

309. Circadian Behaviors

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Program #/Poster #: 309.12

Topic: F.07. Biological Rhythms and Sleep

Support: MOST-111-2636-B-002-021

Title: Visualizing the suprachiasmatic nucleus in vivo during jet lag by intracranial endoscope implantation

Authors: *Y.-Y. SHAN, S.-K. CHEN;
Dept. of Life Sci., Natl. Taiwan Univ., Taipei, Taiwan

Abstract: Living creatures on Earth rely on their intrinsic circadian rhythms to anticipate the day and night cycle, and hence to capitalize on environmental resources. The suprachiasmatic nucleus (SCN) in the mammalian hypothalamus serves as the central pacemaker to coordinate individual periphery oscillators and generate a coherent circadian output for optimal physiological functions critical to health. When rapidly crossing several time zones by flight, people often experience jet lag and suffer unsettled sleep-wake cycles. Previous study on jet lag *in vitro* has found that the expressions of *Per2* in the SCN neurons become less synchronized after jet lag and require couple days to gradually be re-entrained to the new phase (Davidson et al., 2009). However, further evidence about what happens in the SCN on single cell level *in vivo* during jet lag is still deficient. In this study, by implanting gradient index (GRIN) lens and infusing AAV9-hSyn-GCaMP7f into the murine SCN, we were able to record the SCN neuronal activities in head-fixed awake mice with two photon microscopy. We found that the mean correlations between Ca^{2+} activities ($\Delta F/F_0$) of each SCN neuron maintained relative high during the day but decreased after nightfall whereas this trend was not disrupted after a 4 hr-advanced jet lag exposure. On the other hand, we observed the basal Ca^{2+} levels (F_0) of most neurons originally exhibiting daily rhythms higher in the daytime in baseline became less synchronized after jet lag. By the changes of basal Ca^{2+} level patterns, we then identified several clusters that some neurons almost finished the re-entrainment of basal Ca^{2+} levels on day1, some clusters returned to baseline conditions more slowly until day3 and day4, which is similar to the behavior pattern, and one cluster which originally had higher basal Ca^{2+} levels at night was rapidly entrained to the similar pattern to of faster re-entrained cells. Furthermore, we uncovered that the mean correlations of Ca^{2+} activities among those neurons re-entrained faster were significantly greater than those of other clusters, indicating that the firing of these neurons may be strongly

organized. Together, these findings imply that Ca^{2+} activities and basal Ca^{2+} levels could be controlled by different mechanisms, with the latter probably mediated by clock genes. Moreover, the basal Ca^{2+} levels of those neurons with synchronized Ca^{2+} activities were re-entrained faster and might lead the re-entrainment process of other neurons, including those perhaps responsible for encoding behavior output.

Disclosures: Y. Shan: None. **S. Chen:** None.

Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.13

Topic: F.07. Biological Rhythms and Sleep

Title: Source regions of fMRI infraslow activity correlated with behavioral performance

Authors: *D. SIHN, J.-H. KIM, S.-P. KIM;
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Abstract: Previous studies have shown that brain infraslow activity (ISA: 0.01-0.1 Hz) is correlated with behavioral performance. Yet, the neural underpinnings of ISA remains unexplored. Due to the global coherence nature of ISA, a putative source of ISA has been assumed to be subcortical regions such as thalamus. However, previous studies on correlations between ISA and behavioral performance have been only conducted using electroencephalography (EEG), which limits precise examination of subcortical activity. Since it has been reported that ISA of functional magnetic resonance imaging (fMRI) is related to behavioral variability, the present study investigated potential sources of ISA that correlates with behavioral performance using fMRI. We analyzed the fMRI data recorded during visual detection and discrimination tasks. ISA was obtained by bandpass filtering (0.01-0.1 Hz) the preprocessed BOLD signals and its oscillation phase was extracted by Hilbert transformation. Similar to previous ISA studies using EEG, the behavioral performances of those tasks were the highest at certain oscillation phases of fMRI ISA and the lowest at the opposite oscillation phases. The voxels showing significant correlations with behavioral performance were distributed over broad brain regions including both cortical and subcortical regions. Effective connectivity analysis using Granger causality revealed that several cortical regions, including lateral orbitofrontal cortex and temporal pole regions, Granger-caused other brain regions showing correlations with behavioral performance, while no subcortical regions did so. Our results suggest that the sources of fMRI ISA that correlates with behavioral performance may be distributed over cortical regions rather than subcortical regions. This may help to understand the neural underpinnings of ISA and potential neurofeedback applications of ISA for behavioral performance regulation.

Disclosures: **D. Sihn:** None. **J. Kim:** None. **S. Kim:** None.

Poster

309. Circadian Behaviors

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Program #/Poster #: 309.14

Topic: F.07. Biological Rhythms and Sleep

Support: EPSRC EP/S515541/1

Title: Light at dusk delays daily torpor in Siberian hamsters (Phodopus sungorus)

Authors: *C. D. HARDING[1], A. WAITE[2], A. HERWIG[4], V. VAN DER VINNE[5], L. E. MCKILLOP[6], S. N. PEIRSON[3], V. V. VYAZOVSKIY[1];
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Abstract: Torpor is an strategy used by endotherms to save energy through a reduction in metabolic rate. Spontaneous daily torpor (SDT), the regular expression of torpor bouts lasting <24 hours in the absence of immediate energetic stress, is usually restricted to the animal's inactive phase and lesions to the SCN interfere with this process, suggesting that SDT is part of the normal circadian organisation. This is further supported by studies that show SDT entry time can re-entrain to large shifts in the photophase over a period of days. However, such studies do not allow for sensitive examination of the flexibility of circadian control of SDT on a daily basis and the importance of different inputs to this system. Here, we attempted to delay the phase of the circadian clock by shortening the scotophase on one night without shifting the photophase on the subsequent day. We recorded 8 (4M, 4F) with thermal imaging cameras (optris Xi 80, 80x80px) to measure spontaneous daily torpor under short photopheriod (8L:16D) conditions for 14 days with a light extension on day 8 between ZT9:ZT10 (10L:14D). Mixed sex adult (8-10 months) pairs matched in short photoperiod duration (17 weeks) were maintained in separated cage compartments (23x40x40cm) within a ventilated sound-proof chamber illuminated by warm white LED lighting (100 lux) at c.20°C. Skin temperature and XY position of each animal were measured from the pixel of maximum temperature within the compartment at 1Hz resolution. We defined torpor bouts as a decrease in skin temperature of >3 SD from mean skin temperature during the scotophase and torpor entry time as time at which skin temperature decreased to 1 SD below mean skin temperature. Activity was defined as an acceleration >0.5 pixels per second. 4/8 animals entered torpor prior to and following the light extension day, 3/8 entered torpor before the light extension day only and 1/8 never entered torpor. Torpor entry time was delayed more than 1.5 hours (mean = +100.7min, SD = 45.3, tstat = -4.5, $p<0.05$, paired t-test) on the day following the 2 hour light extension relative to pre-extension torpor bouts. Activity time from ZT0-ZT1 on the day after following light extension was increased relative to the previous week in 75% of subjects but the difference was not significant (mean = +283 s, SD = 506.7, tstat = -

1.58, p=0.15). This data suggests that torpor entry time can be shifted by extending the photophase on a single night, potentially via a shift in the animal's activity phase. This experimental paradigm can be used to further explore the relationship between SDT and the circadian system without the need for extreme light cycle shifts.

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Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.15

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant RO1 NS072337

Title: Circadian regulation of corticosterone and locomotor activity by the Subparaventricular Zone

Authors: *O. D. RAMÍREZ PLASCENCIA[1,2], N. MACHADO[1,2], M. A. KHANDAY[1,2], C. B. SAPER[3];
[1]Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Harvard Med. Sch., Boston, MA; [3]James Jackson Putnam Prof, Harvard Med. Sch. Dept. of Neurol., Boston, MA

Abstract: The circadian rhythm of physiology and behavior is regulated by a neural circuitry with the Suprachiasmatic Nucleus (SCN) as the master driving clock. SCN send densest innervation to the Sub Paraventricular Zone (SPZ), a GABAergic region that project to different hypothalamic areas to transmits and amplify the time information. Lesion of the SPZ disrupt the daily control of Locomotor Activity (LMA), Sleep/wake and corticosterone release, and the Body Temperature (Tb), when the lesion is in the dorsal part, but the lesions include all kind of neurons/cells, what maintain the question what specific neuronal population in the SPZ are involved in the circadian regulation of LMA, Cort and Tb. To evaluate the participation of the SPZ^{GABA} neurons in the circadian regulation of LMA, Tb and Cort, we placed injections of AAV-DIO-DTA or AAV-DIO-GFP into the SPZ in Vgat-ires-Cre mice. The deletion of SPZ^{GABA} reduces the percent of LMA during the dark phase from 70.57 ±2.03% in controls vs 61.19 ±2.38% (p=0.002) in SPZ mice under LD photoperiod, and in constant dark with 66.14 ±1.65% in controls and 52.29 ±0.93% (p<0.001) in SPZ mice, observing no differences between the subjective day and subjective. The increase of Tb at the beginning of the dark or subjective dark period was maintained, with mean temperatures at night in LD cycle of 36.94 ±0.04ºC in controls and 36.81 ±0.06ºC (p=0.24) in SPZ, and during the subjective night at 36.91 ±0.04ºC in controls vs 36,72 ±0.06ºC in SPZ mice. The corticosterone reaches at ZT13 in the controls, of 22.01 ±1.75 ng/ml in LD and 25.48 ng/ml ±3.47 in DD, but in the SPZ^{GABA} mice was reduced to 8.03 ng/ml ±2.16 in LD (p=<0.0001) and 6.05 ng/ml ±1.89 in DD (p=<0.0001). Additionally, to

evaluate GABA release from the SPZ neurons, we placed injections of AVV8-eSYN-EGFP-T2A-iCre or AAV-DIO-GFP into the SPZ in Vgat-Fl/Fl mice. The LMA during the dark period is reduced in the animals with the Vgat gene deletion, with the 77.1 ±1.29% in controls and 70.91 ±1.47% ($p=0.025$) in SPZ under LD cycle, and with 75.48 ±1.89% in control mice and 65.31 ±1.75% ($p<0.001$) in constant dark. The mean temperature during the night was reduced under LD photoperiod from 37.3 ±0.01ºC in Controls to 37.02 ±0.02ºC ($p<0.0001$) in SPZ, and in DD from 37.33 0.03ºC in controls to 36.94 0.03ºC ($p<0.001$) in SPZ mice. The cort peak at ZT13 in both groups, in controls 29.8 ±7.77 ng/ml in LD and 32.97 ±7.26 ng/ml in DD, while SPZ Vgat mice with gene deletion in 32.3 ±5.1 ng/ml in LD and 28.45 ±4.18 ng/ml in DD. Altogether, these results suggest that SPZ^{GABA} neurons are important for the circadian regulation of LMA, Tb and Cort, but other neurotransmitters or neuropeptides participates for the regulation.

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Poster

309. Circadian Behaviors

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Program #/Poster #: 309.16

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant MH126137
Portland Veterans Administration Research Fund
University of Michigan Neuroscience Scholars Fund

Title: Rem sleep has minute-scale rhythms in mice and humans: a non-binary continuum between phasic and tonic microstates

Authors: L. L. S. BUENO JUNIOR[1], M. RUCKSTUHL[2], M. M. LIM[3], ***B. O. WATSON**[2]; [1]Univ. of MIchigan, Ann Arbor, MI; [2]Univ. of Michigan, Ann Arbor, MI; [3]Veterans Affairs Portland Hlth. Care Syst., Portland, OR

Abstract: Rapid eye movement sleep (REM) is believed to have a binary temporal structure with “phasic” and “tonic" microstates, defined by motoric activity versus quiescence, respectively. Tonic/phasic structure has been a broad concept for understanding REM, having been observed across mammalian species and having been found altered in pathological conditions such as REM sleep behavior disorder and Major Depression. However, we report here that in mice REM theta frequency (a marker of rodent REM) fluctuates in a non-binary fashion, with the extremes of that fluctuation correlating with phasic-type and tonic-type facial motricity. This demonstrates that phasic and tonic REM are not binary, but rather represent ends of a continuous spectrum. These cycles of brain physiology and facial movement occurred at 0.01-0.06 Hz, or "infraslow" frequencies, and affected cross-frequency coupling and neuronal assembly activity in the

neocortex, suggesting network functional impact. We then found that humans also demonstrate non-binary phasic/tonic microstates, with continuous 0.01-0.04 Hz respiratory rate cycles matching the incidence of eye movements. Thus, we have found a new but fundamental property of REM, which can be relevant to our understanding of sleep health.

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Poster

309. Circadian Behaviors

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Topic: F.07. Biological Rhythms and Sleep

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NIH R01 Grant to RLS (MH115947)

Title: Sleep- and circadian-related calcium activity and diurnal clock gene expression in serotonergic neurons of the adult rat dorsal raphe nucleus

Authors: *H. STRNAD, A. OYLER-CASTRILLO, E. ROWE, W. STRITZEL, D. H. ROOT, R. L. SPENCER;
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Abstract: Transcriptional-translation feedback loops of clock genes (the molecular clock) drive autonomous 24-hour rhythms of physiology and behavior in mammals. These molecular clocks are tuned to environmental light:dark cycles and regulate cellular processes in a tissue-specific manner to promote health and survival. This is evident in people diagnosed with mood and anxiety disorders, which involve notable circadian disruption that is associated with symptom onset and severity. Mood and anxiety disorders feature a disruption of serotonergic signaling in the brain, which are known to have circadian rhythms in cell activity (*cFos*) and the rate limiting enzyme in serotonin synthesis (*Tryptophan hydroxylase 2* (*Tph2*)). It is unknown to what extent these observed circadian rhythms are impacted by decreases in serotonergic neuron activity during sleep, and/or are driven by local molecular clocks. This project used fiber photometry to measure calcium sensor (pGP-AAV-syn-FLEX-jGCaMP8s-WPRE) fluorescence in serotonergic neurons of the dorsal raphe nucleus (DRN), the primary source of extracellular serotonin in the forebrain. Fluorescent signal was sampled across 48 hours in male and female Tph2-Cre rats living in a 12:12 light:dark cycle and constant darkness. Transient peaks in calcium activity were quantified and compared to simultaneous recording of continuous transcranial electroencephalography to parse out the contribution of sleep versus circadian processes to changes in serotonergic neuron calcium activity throughout the day. Diurnal clock gene expression (*Bmal1, Per1, Per2*) in the DRN of adult male Sprague Dawley rats was assessed via radioactive (S-35) and double-labeled fluorescent in situ hybridization to confirm that

serotonergic neurons of the DRN have rhythmic clock gene expression, indicating the presence of a molecular clock.

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Poster

309. Circadian Behaviors

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 309.18

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant MH115947
NIH Grant MH119734
American Association for Laboratory Animal Science GLAS grant

Title: Nighttime red light sensitivity in laboratory rodents: suprachiasmatic nucleus activation, melatonin suppression, and phase shifted wheel running

Authors: *W. STRITZEL[1], C. T. LEVY[2], M. OSMAN[2], R. RAVENEL[2], H. K. STRNAD[1], E. D. PRÉVOST[1], J. REUTER[2], A. M. SLOAN[3], D. H. ROOT[1], R. L. SPENCER[1];
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Abstract: The vast majority of biomedical animal research uses nocturnal laboratory rats and mice. Laboratory animals, like humans, exhibit robust circadian rhythms in behavior and physiology. Therefore, the time of day is an important biological variable to consider when performing animal and human research. Experimental manipulations and measures performed on nocturnal animals should be performed during the nighttime, the animals' active phase, so that the results can be better generalized to the human active phase. Most laboratory rodents are dichromats: they have an ultraviolet-sensitive cone and a green-sensitive cone. Notably, these animals lack a cone that is sensitive to longer wavelengths of light, which humans possess. This has led to the widespread use of red light as a "safe" lighting condition for study of nocturnal rats and mice during their active phase, based on the assumption that red light is functionally invisible to these rodents. Recent studies, however, have begun to question this assumption. The aim of this study is to determine if there is a wavelength of red light where night-time exposure does not activate the suprachiasmatic nucleus (SCN) or affect circadian-related hormones. We measured melatonin and corticosterone concentration, as well as SCN neuronal activation (Per1 and c-Fos mRNA) following 1, 5, and 15 minute night-time exposure to 630nm red light, 15-minute exposure to 730nm red light (≤10 lux), or no light in male and female Sprague Dawley rats. We also measured melatonin, corticosterone, and SCN Per1 and cFos mRNA following nighttime exposure to 650nm red light or no light in male Sprague Dawley rats (10-minute exposure) and in male and female C57BL/6J mice (15-minute exposure). Next, we examined the

effect of a 15-minute nighttime exposure to white, 630nm red, 730nm red, or no light on wheel running activity in male and female Sprague Dawley rats. Last, we tested the rats' performance in an operant nose-poke task where a sucrose reward was gated by a red-light stimulus ranging from 635 to 850nm. Our results indicate that brief nighttime red light exposure results in rapid increases in SCN neuronal activity and suppression of melatonin secretion. Rats were also able to behaviorally detect red-light stimuli (635-730nm), indicating that red light is detected by both the non-image-forming and image-forming visual systems. Therefore, the disruptive effects of nighttime red light exposure on circadian rhythms, as well as its acute effects on physiology and behavior should be taken into consideration by animal researchers and veterinary staff.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.01

Topic: F.07. Biological Rhythms and Sleep

Support: NIH grant: NS091126
NIH grant: HL149630

Title: Noradrenaline in the ventrolateral preoptic area

Authors: *R. DE LUCA[1], C. CANO[2], F. RAFFIN[2], O. FANARI[2], S. NARDONE[2], B. FITZGERALD[2], L. ZHU[2], T. E. SCAMMELL[2], E. ARRIGONI[2];
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Abstract: Neurons in the ventrolateral preoptic (VLPO) area are responsible for initiating and maintaining sleep. During wakefulness, the sleep-promoting VLPO galanin neurons are thought to be under inhibition by arousal signals but the circuit base and the mechanisms by which this might occur are not fully understood. In this work, we investigated how noradrenaline (NA) inhibits the VLPO galanin neurons and how NA levels in VLPO change in sleep and wakefulness in WT and narcoleptic mice. We first tested by *in vitro* electrophysiology the effects of NA on VLPO neurons. We found that NA directly inhibits the VLPO galanin neurons by post-synaptic alpha-2 adrenergic receptors and indirectly by increasing the frequency of spontaneous inhibitory post-synaptic currents (sIPSCs), suggesting an additional pre-synaptic inhibitory mechanism. Optogenetic stimulation of local VLPO GABAergic neurons produced short-latency opto-evoked IPSCs in VLPO galanin neurons indicating a local inhibitory circuit controlling the sleep-active VLPO galanin neurons. NA increased the amplitude of these opto-evoked IPSCs via alpha-1 receptors. Our results demonstrate that NA inhibits VLPO galanin neurons directly and

indirectly at least in part through the activation of the local GABAergic interneurons. In addition, we expressed in the VLPO a G-protein-coupled receptor-activation-based NA ($GRAB_{NA}$) sensor to measure changes of NA levels across the sleep-wake cycle. NA signal in VLPO is higher in wakefulness compared to NREM and REM sleep. Interestingly, in narcoleptic mice in which the orexin/hypocretin neurons are ablated the NA tone in VLPO is lower compared to WT controls. The reduced NA signal in the VLPO of orexin/hypocretin deficient mice could be responsible for the sleep-wake instability, a clinical feature of the narcoleptic patients.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.02

Topic: F.07. Biological Rhythms and Sleep

Support: Canada Graduate Scholarships - Master's (CGSM)

Title: Intracranial correlates of microarousals in non-rapid eye movement sleep in the human brain

Authors: *Y. L. WANG[1], T. AVIGDOR[1], C. ABDALLAH[1], F. DUBEAU[1], L. PETER-DEREX[2], B. FRAUSCHER[1];
[1]Neurol. and Neurosurg., Montreal Neurolog. Inst. and Hosp., Montreal, QC, Canada; [2]Ctr. for Sleep Med. and Resp. Dis. of the Hospices Civils de Lyon, Lyon, France

Abstract: While sleep and wake are usually considered as two distinct states, short periods of awakening called microarousals, during which the brain activity becomes wake-like for 3-15 s, occur more than 100 times every night. Paradoxically, microarousals were proposed to crucially help shape sleep macrostructure rather than disrupt sleep. While they have been defined in an all-or-none fashion, they exhibit wide-ranging activity patterns on the scalp electroencephalography (EEG) which may reflect their different functions. This project thus characterizes the local activity during different microarousals in non-rapid eye movement (NREM) sleep across 14 brain regions, using intracranial EEG (iEEG) obtained from 29 patients with focal drug-resistant epilepsy. Only the iEEG channels located in healthy brain regions were analyzed. We specifically clustered and studied “slow” arousals, which exhibit predominant delta (0.3-4 Hz) activity on the scalp EEG, and “fast” arousals which show predominant alpha (8-13 Hz) and beta (13-30 Hz) activity. We did not explore the “intermediate” arousals which have mixed slow and fast activity. Performing spectral analysis, we quantified the activity change during microarousals in the delta, alpha, and beta frequency range by computing the ratio of the power during arousals versus that during baseline segments. The baseline for each arousal is the 10 s

sleep that ends at 2 s preceding the arousal. We then log-transformed the ratio and tested its significance in each region using a one-sample t-test corrected with the false discovery rate. Our results revealed a widespread delta increase in "slow" arousals ($p < 0.05$ in 12/14 regions in the frontal, parietal, occipital, and temporal lobes, and insula; min. - max. increase in the first 3 s of arousals (onset): 40% - 240% of the baseline power, after 3 s: 32% - 85%). In contrast, a widespread delta decrease was seen in "fast" arousals ($p < 0.05$ in 13/15 regions; decrease in onset: 25% - 33%, after 3 s: 23% - 65%). Alpha and beta activity increased during both types of arousals but in fewer regions, mainly in the parieto-occipital areas (alpha: 24% - 68%; beta: 10% - 79%) . The anterior and middle cingulate gyrus did not display any alpha or beta increase during neither "slow" nor "fast" microarousals. Our results also indicated a gradual process approaching wakefulness during both types of microarousals despite their short duration. After the first 3 s of "slow" microarousals, the delta increase became weaker and the beta increase became more widespread. These findings can help us further understand the intracranial mechanisms of NREM microarousals and sleep-wake regulation.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.03

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS KAKENHI Grant Numbers JP22H01107
JSPS KAKENHI Grant Numbers JP20K22297

Title: Reading out sleep spindles from cortical and subcortical brain circuits during sleep

Authors: ***M. UJI**[1], X. LI[1], A. SAOTOME[1,2], R. KATSUMATA[1,3], S. ARITAKE[1,2], C. SUZUKI[1], K. UENO[1], M. TAMAKI[1,4];
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Abstract: While sleep is known to be crucial for learning and memory (Tamaki et al., 2020), how cortical and subcortical brain regions during sleep play a role in learning facilitation remains elusive. One line of research has demonstrated that activities of the cortical brain regions involved in the acquisition of learning increase regionally during nonrapid eye movement (NREM) sleep in correlation with the amount of learning. Another line of research has shown that subcortical regions, including the thalamus and hypothalamus, play an essential role in wake-sleep regulations. However, how subcortical regions alone, or the interactions between cortical and subcortical regions, could facilitate sleep-dependent offline learning has been unclear. Here, by employing a functional magnetic resonance imaging (fMRI) decoding

technique on sleeping brain activation pattens, we tested whether sleep spindles, a key neural oscillation for brain plasticity during sleep, are represented in subcortical brain regions. Simultaneous polysomnography and fMRI measurements were conducted during a 90-min nap in six young and healthy subjects. Sleep spindles (12-15 Hz, Mölle et al., 2011) were detected from NREM sleep. A binary sleep-spindle fMRI classifier was constructed using a sparse logistic regression (Yamashita et al., 2008). The regions of interest (ROIs) were the supplementary motor area, involved in motor learning (Tamaki et al., 2013), and subcortical regions (thalamus, hypothalamus), involved in the generation of spindles and sleep-wake regulations. The classification accuracy for sleep spindles was above chance level not only in the supplementary motor area (72% ± 0.07, one-sample t-test, $p < .001$) and the thalamus (69% ± 0.07, one-sample t-test, $p < .001$) but also in the hypothalamus (65% ± 0.05, one-sample t-test, $p < .001$). A label-shuffled decoder showed a chance-level performance (hypothalamus, 49.5% ± 0.02, one-sample t-test, $p=0.537$). These suggest that learning processing during sleep recruits not only local cortical brain circuits used for acquisition of learning but also dynamic cortical-subcortical brain modulations and that the hypothalamus, which has been widely regarded as the centre for wake-sleep transitions, could play a pivotal role in offline processing of learning during NREM sleep.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.04

Topic: F.07. Biological Rhythms and Sleep

Support: NARSAD Young Investigator grant (27799)

Title: Prefrontal cortical regulation of REM sleep

Authors: *J. HONG[1], K. T. BEIER[2], S. CHUNG[1], F. WEBER[1];
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Abstract: Rapid-eye-movement (REM) sleep is characterized by intense cortical activity underlying its wake-like electroencephalogram (EEG). The neural activity inducing REM sleep is thought to originate from subcortical circuits in the brainstem and hypothalamus. However, despite the functional importance of REM sleep in cortical synaptic plasticity and memory processing, whether cortical neurons also possess the capability to regulate REM sleep has remained overlooked. Here, we show that the medial prefrontal cortex (mPFC) strongly promotes REM sleep. Bidirectional optogenetic manipulations demonstrate that excitatory mPFC neurons or their projections to the lateral hypothalamus (LH) induce REM sleep and regulate phasic events, reflected in intensified theta oscillations in the EEG during REM sleep. Calcium

imaging reveals that the majority of LH-projecting mPFC neurons are maximally activated during REM sleep and a subpopulation of these neurons is recruited during phasic events. Our results delineate a cortico-hypothalamic circuit for the top-down control of REM sleep and thus demonstrate that cortical neurons are directly involved in the regulation of REM sleep.

Disclosures: **J. Hong:** None. **K.T. Beier:** None. **S. Chung:** None. **F. Weber:** None.

Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

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Program #/Poster #: 310.05

Topic: F.07. Biological Rhythms and Sleep

Support: Israel Science Foundation
ERC-2019-COG 864353

Title: Sleep deprivation leads to NREM sleep-like neuronal activity changes in early auditory cortex

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Abstract: Insufficient sleep is commonplace in modern lifestyle and can lead to grave outcomes ranging from lapses and accidents to increased epileptiform activity, yet the changes in neuronal activity accumulating over hours of extended wakefulness remain poorly understood. Specifically, which aspects of cortical processing are affected by sleep deprivation (SD), and whether they also affect early sensory regions, remains unclear. Previous non-invasive studies examined the effect of insufficient sleep on neurophysiological activity, yet only few studies examined the effects of SD on spiking activities in local neuronal populations. Here, we recorded spiking activity in rat auditory cortex along with polysomnography while presenting sounds (tones and click-trains at different rates) during SD followed by recovery sleep (n=7 rats, 19 experimental sessions). We found three main results. First, certain aspects of auditory processing are invariant to SD while others are especially sensitive. Specifically, frequency tuning width, onset response magnitude and spontaneous firing rates were minimally affected by SD, when comparing last vs. first 1.5h of SD (-1.9±2.1%, -0.5±1.2% and -5.1±1.1% change, respectively. mean±sem. n=198, 324, 324 units). By contrast, SD significantly reduced entrainment to rapid (≥20 Hz) click-trains (-17.7±1.5%, n=324), increased population synchrony (17±1.6%, n=324), and increased sleep-like stimulus-induced silent periods (7.1±1.4% in vigilance vs. 25.4±1.6% following SD, n=126 channels). Second, recovery NREM sleep was associated with similar effects as SD with even greater magnitude. Accordingly, click entrainment (-47.4±1.7%), population synchrony (48.3±1.3%, n=324), and stimulus-induced silent periods (42±1.6% vs. 7.1±1.4% in NREM vs. vigilance) were strongly modulated by sleep. Third, auditory processing

during REM sleep was similar to vigilant wakefulness, implying that changes in auditory cortical processing were not characteristic of sleep in general but are associated specifically with synchronized brain states. In conclusion, our results show that specific aspects of auditory cortex processing, including sensory adaptation (entrainment to rapid click trains), population synchrony and stimulus induced silent periods are strongly modulated by sleep pressure. Such changes are qualitatively similar to those observed during NREM, but not REM sleep, implying that processes akin to those in NREM sleep invade the activity of cortical circuits during SD in wakefulness, already in early sensory cortex.

Disclosures: A. Marmelshtein: None. **Y. Nir:** None.

Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

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Program #/Poster #: 310.06

Topic: F.07. Biological Rhythms and Sleep

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JSPS KAKENHI JP16H06280
JSPS KAKENHI 20H03552
JSPS KAKENHI 21H05674
MEXT World Premier
International Research Center Initiative

Title: Fully automatic sleep stage-specific intervention using single EEG in mice

Authors: *I. KOYANAGI[1,2,7], T. TEZUKA[3], J. YU[1,2], S. SRINIVASAN[1], T. NAOI[1], S. YASUGAKI[1,7,4], A. NAKAI[1,2], S. TANIGUCHI[1,5], Y. HAYASHI[1,8,9], Y. NAKANO[10], M. SAKAGUCHI[1,2,4,5,6];
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Abstract: Sleep stage-specific intervention is widely used to elucidate the functions of sleep and their underlying mechanisms. For this intervention, it is imperative to accurately classify rapid eye movement (REM) sleep. However, the proof of fully automatic real-time REM sleep classification in vivo has not been obtained in mice. Previously, we reported an artificial

intelligence model, UTSN-L, which can classify sleep from single-channel electroencephalogram (EEG) in a simulation setting for real-time analysis. Here, we report the in vivo implementation of its derivative model, UTSN-L2. The integration of this model into a commercially available sleep recording system enabled REM sleep-specific intervention with 90% sensitivity and 86% precision without prior configuration to each mouse. We further derived models capable of classification with higher frequency sampling and time resolution. Finally, we explored the possibility of distinguishing the high-theta wake period, in which EEG characteristics resemble those of REM sleep. This attach-and-go sleep staging system provides a fully automatic accurate and scalable tool for investigating the mysterious functions of sleep.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.07

Topic: F.07. Biological Rhythms and Sleep

Title: Isoflurane-induced loss of responsiveness and the physiological transition from wakefulness to NREM-sleep display similar neuronal features

Authors: L. JOYCE, R. NUTTAL, M. KREUZER, G. SCHNEIDER, *T. FENZL;
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Abstract: The neuronal mechanisms of loss of responsiveness (LR) prior to unconsciousness, induced by general anesthesia (GA) are still highly contended. The ‘bottom-up’ theory postulates primary modulation of subcortical nuclei including parts of the sleep/wake promoting pathways (SW) with a cascade effect on cortical regions. In contrast, the ‘top-down’ theory suggests a primary breakdown of cortical connectivity and hence impaired information transfer in cortical and subcortical regions. A systematic delineation of functional connectivity between SW and thalamocortical circuits during GA could be the key to understanding the fundamental processes underlying GA-induced LR. We recorded local field potentials (LFP) in C57BL/6N male mice (n = 12, age = 12-18 weeks) at various subcortical/cortical sites (ventrolateral preoptic nucleus VLPO: a non-rapid eye movement (NREMS) sleep-promoting nucleus; locus coeruleus LC: a wake-promoting nucleus; thalamic ventral posteromedial nucleus VPM). Parallel EEG and EMG recordings complemented the data acquisition. Baseline recordings were performed across 2 days. On day 3, a 1-hour anesthesia protocol was applied. During the protocol, isoflurane concentration was increased by 0.1% every 2 min until the first silent second, followed by a reduction of Isoflurane (0.1% every 2 min) until recovery of consciousness (ROC). The experiments were finalized with 24h of EEG/EMG and LFP-recordings following ROC. Baseline

recordings were scored (WAKE, NREMS, REMS) and neuronal signatures of functional connectivity between nuclei pairings (VLPO-LC, VLPO-VPM, VPM-LC) at baseline WAKE/NREMS transitions were established. Spectral coherence and phase lag in the 0.5 - 40Hz frequency band of nuclei pairings across baseline were analyzed. At baseline, we found a significant increase in coherence between all 3 nuclei pairings across all frequencies during WAKE to NREMS transition. Maximum increase was observed for VLPO-LC pairings at 4-12Hz. Additionally, we found a robust shift in phase lag towards zero specifically between VLPO-LC and VPM-LC (4-12Hz) accompanying the increase in coherence. NREMS to WAKE transitions showed the opposite effect. Under Isoflurane administration, higher doses triggered high coherence and near-zero phase lag at 4-12Hz, followed by a transient decrease in coherence and increase in phase lag around loss of righting reflex for VLPO-LC and VPM-LC pairings. Our data point towards a relationship between the sleep/wake promoting pathway and LR, induced by Isoflurane and potentially also contribute to the question of cortical versus subcortical dominance during anesthetic-induced loss of consciousness.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

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Program #/Poster #: 310.08

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01HL149133
NIH Grant F31HL154855
NARSAD Young Investigator Grant #27799
Margaret Q. Landenberger Foundation Grant

Title: A medullary hub for controlling REM sleep and pontine waves

Authors: *A. SCHOTT[1], J. BAIK[2], S. CHUNG[1], F. WEBER[1];
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Abstract: REM sleep is a distinctive behavioral state associated with vivid dreaming and memory consolidation. In addition to tonic features such as a desynchronized EEG and skeletal muscle paralysis, REM sleep is characterized by phasic bursts of electrical brain activity, which manifest as spike-like field potentials (P-waves) in the pons. Though P-waves are thought to contribute to memory processing during REM sleep, little is known about their underlying neural circuits, nor the extent to which these circuits interact with networks generating REM sleep itself. Here, we show that an excitatory population of medullary neurons expressing corticotropin-releasing hormone (CRH) is important for regulating both REM sleep and P-waves. Optogenetic and chemogenetic experiments revealed that dmM CRH+ neurons strongly promote

REM sleep, and fiber photometry recordings showed that this population is selectively active during REM sleep, with phasic increases in activity that temporally correlate with P-waves. Brief optogenetic activation of dmM CRH+ neurons reliably elicited P-waves, while chemogenetic manipulations bidirectionally modulated P-wave frequency before and during REM sleep. Our results demonstrate that dmM CRH+ neurons regulate both P-waves and REM sleep initiation, suggesting that this population serves as a common hub for controlling REM sleep and its phasic components.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.09

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant MH117155

Title: Cortical ripples during NREM sleep and waking in humans

Authors: *I. A. VERZHBINSKY[1], C. W. DICKEY[1], X. JIANG[1], B. Q. ROSEN[1], S. KAJFEZ[2], E. N. ESKANDAR[4], J. A. GONZÁLEZ-MARTÍNEZ[5], S. S. CASH[6], E. HALGREN[2,3]; [1]Neurosciences Grad. Program, Univ. Of California San Diego, La Jolla, CA; [2]Dept. of Radiology, [3]Dept. of Neurosciences, Univ. of California San Diego, La Jolla, CA; [4]Dept. of Neurolog. Surgery, Montefiore Med. Ctr., Albert Einstein Col. of Med., Bronx, NY; [5]Neurosurg. and Epilepsy Ctr., Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; [6]Dept Neurol, Mass Genl Hosp, Boston, MA

Abstract: Hippocampal ripples index the reconstruction of spatiotemporal neuronal firing patterns essential for the consolidation of memories in the cortex during non-rapid eye movement sleep (NREM). Recently, cortical ripples in humans have been shown to enfold the replay of neuron firing patterns during cued recall. Here, using intracranial recordings from 18 patients (12 female), we show that cortical ripples also occur during NREM in humans, with similar density (~8/min), oscillation frequency (~90 Hz), duration (~70ms), and amplitude (~6 microvolts) to waking. Ripples occurred in all cortical regions with similar characteristics, unrelated to putative hippocampal connectivity, and were less dense and robust in higher association areas. Cortical ripples were smaller in amplitude than hippocampal ripples, but were similar in density, frequency, and duration. Cortical ripples during NREM typically occurred just prior to the upstate peak, often during spindles. Upstates and spindles have previously been associated with memory consolidation, and we found that cortical ripples grouped co-firing between units within the window of spike-timing-dependent plasticity. Furthermore, putative pyramidal and interneuron spiking phase-locked to cortical ripples during NREM, with phase delays consistent with ripple generation through pyramidal-interneuron feedback. Thus, human

NREM cortical ripples are: (1) ubiquitous and stereotyped with a tightly focused oscillation frequency; (2) similar to hippocampal ripples; (3) associated with upstates and spindles and (4) associated with unit co-firing. These properties are consistent with cortical ripples possibly contributing to memory consolidation and other functions during NREM in humans. This work was supported by MH117155.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.10

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant MH117155

Title: Ripples occur in the human cortex, hippocampus and amygdala during REM sleep

Authors: *S. KAJFEZ[1], I. A. VERZHBINSKY[3,4], C. W. DICKEY[3,4], B. Q. ROSEN[3], J. A. GONZÁLEZ-MARTÍNEZ[5], S. S. CASH[6], E. HALGREN[1,2];
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Abstract: Brief high frequency oscillations, termed ‘ripples’, occur in the rodent hippocampus during Non-Rapid Eye Movement (NREM) sleep, and are associated with replay of firing patterns from the preceding waking, and memory consolidation. Recently, ripples have been recorded in the human hippocampus, cortex and amygdala during NREM and waking, but their occurrence in Rapid Eye Movement (REM) sleep has been little studied. Here, we use human intracranial recordings to demonstrate that cortical, hippocampal and amygdala ripples also occur during REM sleep, with similar basic properties to those observed in NREM and Waking, including frequency (~90Hz), duration (~70ms), and density (~20/min). Sawtooth waves (STW), 2-5Hz oscillations in frontocentral scalp EEG, are characteristic of phasic (active) REM and the transition from tonic to phasic REM. We show that cortical REM ripples commonly occur on the rising phase of STW, associated with increased cortical activation, perhaps analogous to cortical NREM ripples, which often occur on upstates. We found that STW with ripples primarily occur in the sensorimotor cortex and associated areas suggesting a possible relationship to action sequences while dreaming. Additionally, STW with ripples were also found in limbic areas and the frontal lobe, indicating there might be a relationship between dreaming and actively problem-solving emotional situations. More generally, the REM ripples reported here provide a possible

substrate for facilitating emotional memory consolidation, which has previously been associated with REM sleep in humans and animals.

Disclosures: S. Kajfez: None. **I.A. Verzhbinsky:** None. **C.W. Dickey:** None. **B.Q. Rosen:** None. **J.A. González-Martínez:** None. **S.S. Cash:** None. **E. Halgren:** None.

Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.11

Topic: F.07. Biological Rhythms and Sleep

Support: NINDS R01 NS-096177

Title: Quantitative characterization of sleep spindle dynamics

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Abstract: Sleep spindles, transient oscillatory waveforms in the sleep electroencephalogram (EEG), have been linked to numerous factors in health and disease, including memory consolidation, neurological dysfunction and disorders, and natural aging. Spindle activity is known to continuously evolve over time as a function of sleep stage, slow oscillation (SO) activity, and other covariates. Typical sleep spindle analyses, however, focus on differences in average spindle density (event rate), ignoring rich information about spindle dynamics and limiting their potential use as a clinical EEG biomarker. Here, we develop a statistical modeling framework based on point process theory that allows us to characterize temporal patterns of spindles unique to individuals, as well as patterns common across populations. By doing so, we can evaluate the relative influences of multiple factors on the moment-to-moment rate of spindle events. We apply this framework to large cross-sectional databases of heterogeneous general population data and to clinical databases of patients with neurological disorders. Our results show that most adults have a characteristic event pattern that involves a refractory period followed by a period of increased probability of spindle occurrence. This event pattern varies substantially from subject to subject, but night-to-night variability within subjects is small. An analysis of spindles/SO coupling corroborated previous studies showing shifts in preferred phase with age, while providing a novel characterization of shifts in preferential phase with depth of sleep. Statistical goodness-of-fit analysis suggests consistent and substantial improvements in our ability to predict individual spindle times over current approaches. Overall, we demonstrate a more statistically robust approach to characterizing sleep spindle dynamics, which provides key insights into the mechanisms of healthy aging and neuropathology and can potentially serve as a robust biomarker for neurological disorders.

Disclosures: S. Chen: None. **T.J. Possidente:** None. **U.T. Eden:** None. **M.J. Prerau:** None.

Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.12

Topic: F.07. Biological Rhythms and Sleep

Support: MH117155

Title: A general role of cortical ripples in binding is supported by increased co-rippling during correct lexical, semantic, executive, and response integration

Authors: *J. C. GARRETT[1], I. A. VERZHBINSKY[1], E. KAESTNER[2], O. DEVINSKY[3], W. DOYLE[4], T. THESEN[5], E. HALGREN[6];
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Abstract: Perception, decision, and action require the binding of information which is highly distributed across the cortex. A prominent theory proposes that binding is facilitated by synchronous oscillations in participating cortical sites. We hypothesized that cortical ripples, 90 Hz oscillations which occur widely across cortical regions and commonly co-occur, subserve cortico-cortical binding during all stages of task performance. We analyzed patterns of cortical rippling and ripple coordination in intracranial EEG data during a task where subjects were presented with written words or non-words, and responded to words representing animals. We observed increased co-rippling to letter-strings that could be bound into words versus consonant-strings or false-fonts which could not. The increase occurred at a latency (200-400ms post-stimulus onset) in cortices required for reading and understanding words, including the visual wordform area in the left ventral fusiform gyrus, and semantic areas in the left middle temporal and supramarginal gyri. Subsequently, all these areas co-rippled with dorsolateral prefrontal and then Rolandic areas time-locked to the keypress, i.e., during the binding of response-guiding semantic information with task-instructions and response-execution. Critically, correct as opposed to incorrect task responses were associated with distinctly higher co-rippling within and between engaged areas. These results show that cortical ripples co-occur within and between areas critical for lexical, semantic, executive and response processes, at the times when information from these areas needs to be integrated for successful performance, and are thus consistent with the hypothesis that they play a general role in cognitive binding. Supported by MH117155.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.13

Topic: F.07. Biological Rhythms and Sleep

Support: MH117155

Title: Widespread ripples synchronize human cortical activity during sleep, waking, and memory recall

Authors: *C. W. DICKEY[1], I. A. VERZHBINSKY[1], X. JIANG[1], B. Q. ROSEN[1], S. KAJFEZ[2], B. STEDELIN[6], J. J. SHIH[3], S. BEN-HAIM[4], A. M. RASLAN[6], E. N. ESKANDAR[7], J. GONZALEZ-MARTINEZ[8], S. S. CASH[9], E. HALGREN[5];
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Abstract: Declarative memory encoding, consolidation, and retrieval require the integration of elements encoded in widespread cortical locations. The mechanism whereby such 'binding' of different components of mental events into unified representations occurs is unknown. The 'binding-by-synchrony' theory proposes that distributed encoding areas are bound by synchronous oscillations enabling enhanced communication. However, evidence for such oscillations is sparse. Brief high-frequency oscillations ('ripples') occur in the hippocampus and cortex, and help organize memory recall and consolidation. Here, using intracranial recordings in humans, we report that these ~70 ms duration 90 Hz ripples often couple (within ±500 ms), co-occur (≥25 ms overlap), and crucially, phase-lock (have consistent phase-lags) between widely distributed focal cortical locations during both sleep and waking, even between hemispheres. Cortical ripple co-occurrence is facilitated through activation across multiple sites, and phase-locking increases with more cortical sites co-rippling. Ripples in all cortical areas co-occur with hippocampal ripples but do not phase-lock with them, further suggesting that cortico-cortical synchrony is mediated by cortico-cortical connections. Ripple phase-lags vary across sleep nights, consistent with participation in different networks. During waking, we show that hippocampo-cortical and cortico-cortical co-ripples increase preceding successful delayed memory recall, when binding between the cue and response is essential. Ripples increase and phase-modulate unit firing, and co-ripples increase high-frequency correlations between areas, suggesting synchronized unit spiking facilitating information exchange. Co-occurrence, phase-synchrony, and high-frequency correlation are maintained with little decrement over very long distances (25 cm). Hippocampo-cortico-cortical co-ripples appear to possess the essential properties necessary to support binding-by-synchrony during memory retrieval, and perhaps generally in cognition. This work was supported by MH117155.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 310.14

Topic: F.07. Biological Rhythms and Sleep

Support: MH117155
Dept of Veterans Affairs Office of Academic Affiliations Advanced Fellowship Program in Mental Illness Research and Treatment
Medical Research Service of the Veterans Affairs San Diego Health Care System/UCSD
Dept of Veterans Affairs Desert Pacific Data Science Fellowship within MIRECC

Title: Human sleep spindles show an anterior-posterior frequency gradient across the cortex, thalamus, hippocampus, and amygdala

Authors: *C. E. GONZALEZ[1], X. JIANG[2], B. Q. ROSEN[3], J. A. GONZÁLEZ-MARTÍNEZ[4], H. BASTUJI[5], M. REY[6], S. PATI[7], S. S. CASH[8], E. HALGREN[9];
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Abstract: In humans, sleep spindles are 10-16 Hz neural oscillations lasting approximately 0.5-2s. Spindles, along with cortical slow oscillations, facilitate memory consolidation by enabling synaptic plasticity. Although cortical spindle dynamics are well characterized, less is known regarding whether spindles are also generated in human subcortical structures such as thalamus, hippocampus, and amygdala, and whether they have similar characteristics to cortical spindles. Using a large dataset of semi-chronic intracranial recordings from patients with intractable epilepsy (5 sites, 40 patients, 805 total bipolar recordings with 687 cortical), we tested for differences in spindle characteristics between cortical and subcortical structures as well as differences between anterior and posterior locations within each structure (with the exception of the amygdala which was considered anterior). Electrode contacts were excluded from analysis if they were involved in seizure initiation or had frequent interictal activity. Spindles were automatically detected, quality controlled, and characterized as described in previous

publications. Brain structures significantly varied by duration, amplitude, intra spindle frequency variability, and frequency change, but not by overall frequency. Interestingly, anterior structures had overall slower frequency than posterior structures, as seen in cortex (0.67 Hz), hippocampus (0.27 Hz), and thalamus (0.79 Hz). The average cortical spindle duration was greater than spindles in amygdala and hippocampus, but shorter duration than thalamus. The average amplitude at cortical sites was larger than amygdala and thalamic sites, but smaller than hippocampal. While cortex and thalamus showed significant spindle frequency slowing, amygdala and hippocampus did not. Interestingly, for both cortex and thalamus, more posterior structures slowed less than anterior structures. These findings highlight spindles are generated in anterior and posterior subcortical structures and differ from cortex in duration, amplitude, and intra-spindle frequency dynamics. Furthermore, spindle properties such as overall frequency and within-spindle frequency dynamics vary along the anterior-posterior axis for cortical and subcortical structures. Frequency changes occur in parallel across structures consistent with topographical organization of thalamo-hippocampo-cortical circuits. Clarifying such regional differences in spindle dynamics could inform mechanisms of sleep dependent memory consolidation. Supported by MH117155.

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Poster

310. Integrative Physiology and Behavior: Sleep Systems I

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Program #/Poster #: 310.15

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01 NS118440
NSF 1757574
Department of Physics UM

Title: Differential roles of NREM and REM during sleep consolidation of multiple memory traces

Authors: M. SATCHELL[1], A. SIMMONS[4], S. J. ATON[2], *M. R. ZOCHOWSKI[3];
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Abstract: Across vertebrate species, sleep states are known to cycle consistently from non-rapid eye movement (NREM) to REM sleep. However, the functional significance of these transitions is unknown. Critically, NREM and REM sleep states are associated with dramatically different neuromodulatory milieu resulting in vastly different physiological features and network

activation patterns, with acetylcholine (ACh) being an important factor in differentiating network activity between these two states. We have built a reduced computational model of sleep dependent memory consolidation to probe how state-specific changes in ACh alter network dynamics and consequently engram consolidation via synaptic, spike timing dependent plasticity (STDP). We show that the sequential, bidirectional changes in ACh neuromodulation during NREM and REM states plays a vital role in consolidation, particularly when multiple memory traces are being stored simultaneously. In the low-ACh (NREM-like) state, synapses to and between slow firing plastic engram neurons are primarily potentiated via spike timing-dependent plasticity, resulting in rapid recruitment of new cells into the memory engram and its consequent enlargement. In the subsequent high-ACh (REM-like) state, ACh mediates activation of GABAergic interneurons that competitively suppress activity among newly recruited excitatory pyramidal neurons during prior NREM, leading to the orthogonalization of newly enhanced representations of different memory traces. Consequently, the sequence of changes to ACh signaling is vital to the generation of two segregated, but expanded, memory traces. These results are contrasted to NREM-like only and, separately, REM-like only sleep.

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Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.01

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH065961-17A1

Title: Chemogenetic activation of the ventral hippocampus selectively increases intertrial avoidance responses during signaled active avoidance in rats

Authors: *C. OLEKSIAK[1], D. CARRIAGA[2], S. PLAS[3], K. VASUDEVAN[1], S. MAREN[1], J. M. MOSCARELLO[1];
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Abstract: Learning to avoid danger is critical for survival. This form of learning can be modeled in the laboratory with instrumental avoidance conditioning procedures, such as two-way signaled active avoidance (SAA). In this task, rats learn to shuttle from one compartment to another to avoid a footshock signaled by an acoustic warning stimulus (WS). We have previously found that SAA is context-dependent and mediated by the ventral hippocampus (VH). Specifically, well-trained rats tested in a novel context show a significant decrease in avoidance responding and this performance deficit is rescued by reversible inactivation of the VH. We therefore

hypothesized that chemogenetically activating the VH in the familiar, conditioning context would similarly reduce shuttling. To test this hypothesis, we used an adeno-associated viral vector (AAV) to express an excitatory designer receptor exclusively activated by designer drugs (*n*=13) (DREADD; AAV8-CaMKII-hM3Dq-mCherry or AAV8-CaMKII-GFP as a control) in the VH of male and female rats. Contrary to our hypothesis, chemogenetic activation of VH neurons using the DREADD ligand, clozapine-*N*-oxide (CNO), in the conditioning context did not affect avoidance responses to the WS. However, CNO-treated rats exhibited a marked increase in inter-trial responding (ITR) in the 2-min period between each WS compared to vehicle and blank-GFP (*n* = 12) rats. Preliminary work indicates that ITRs are mediated by the conditioning context insofar as they are reduced by context extinction. Collectively, these results suggest that the VH activation increases ITRs by promoting context-mediated shuttling. Future work will explore the neural circuits underlying this effect and the differential role for the VH in the contextual modulation of avoidance responses to cues and contexts.

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Poster

311. Fear and Stress

Location: SDCC Halls B-H

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Program #/Poster #: 311.02

Topic: G.01. Fear and Aversive Learning and Memory

Title: Single prolonged stress impairs acquisition and extinction recall of avoidance responses in male rats

Authors: *S. L. PLAS[1], C. R. OLEKSIAK[2], J. M. MOSCARELLO[2], S. MAREN[2], I. LIBERZON[3];
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Abstract: Post-traumatic stress disorder (PTSD) is a debilitating disorder characterized by excessive fear and dread, hypervigilance, and deficits in extinction learning. Here, we used single prolonged stress (SPS), a well-validated rodent model of PTSD that recapitulates symptomology and physiology analogous to PTSD patients, to test the effect of stress on acquisition and extinction of avoidance behavior in the two-way signaled active avoidance paradigm (SAA). SAA is a proactive problem-solving paradigm where rats learn to prevent an aversive outcome (shock) by performing a shuttling response when exposed to a warning signal (tone). Importantly, excessive expression of fear (freezing) impairs avoidance learning. Previous work demonstrates that lesions or pharmacological inhibition of the infralimbic (IL) prefrontal cortex results in impaired acquisition of SAA and increased freezing across SAA training. Additionally, rodents subjected to SPS exhibit impaired engagement of the IL, a region required

to suppress freezing. We therefore hypothesized that rodents subjected to SPS would demonstrate impaired acquisition of SAA. To test this hypothesis, we submitted male Sprague-Dawley rats to SPS or control procedures. SPS consisted of a two-hour immobilization period, 20 minute forced swim, 15 minute rest period, and then loss of consciousness by ether followed by a seven day quiescent period. This was followed by four days of SAA training (30 trials/day, 2 min ITI) and two days of SAA extinction (30 WS-alone trials). We found that SPS significantly increased the number of animals unable to acquire ARs ("poor avoiders"). Additionally, the SPS rats that did acquire ARs were resistant to extinction and maintained ARs at higher rates than controls. Collectively, this evidence demonstrates that SPS induces a learned helplessness-like phenotype in the SAA paradigm. Further investigation into the contribution of the IL to these deficits is needed.

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Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH065961

Title: Post-reactivation hippocampal rapamycin attenuates subsequent extinction retrieval deficits after inactivation of the thalamic nucleus reuniens in male and female rats.

Authors: *J. E. HASSELL, Jr, K. VASUDEVAN, V. V. M. VIERKANT, S. MAREN; Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Extinction learning is central to behavioral therapies for treatment of stressor- and trauma-related disorders including posttraumatic stress disorder (PTSD). Work in both rats and humans suggests that extinction learning recruits the medial prefrontal cortex (mPFC) to suppress the expression of conditioned fear encoded in the amygdala. However, we have recently discovered that mPFC projections to the thalamic nucleus reuniens (RE), which projects strongly to the hippocampus (HPC), are critical for extinction learning and retrieval. After extinction, pharmacological inactivation of the RE results in a relapse of extinguished fear. We therefore hypothesize that the RE promotes extinction retrieval by suppressing the retrieval of hippocampus-dependent fear memories. To test this idea, we sought to determine whether post-retrieval attenuation of hippocampal fear memories would reduce the relapse of extinguished fear associated with RE inactivation. Adult male/female Long-Evans rats first underwent auditory fear conditioning. Twenty-four hours later they were briefly re-exposed to the conditioning context and received an immediate, intra-HPC infusion of either vehicle (DMSO) or the protein synthesis inhibitor rapamycin (10 ug/ul, 0.5 µl per side) (n = 8 per group). The next day rats

underwent fear extinction in a novel context, and this was followed 24 hours later by an extinction retrieval test. Prior to the retrieval test, rats received intra-RE infusions of either vehicle (saline) or muscimol. Consistent with prior findings, inactivation of the RE increased freezing to the CS in the extinction context. However, prior hippocampal protein synthesis inhibition attenuated the RE-inactivated induced increase in freezing. These data are consistent with the hypothesis that the RE is involved in suppressing inappropriate contextual fear memories and increases our understanding of trauma- and stressor-related psychiatric disorders.

Disclosures: J.E. Hassell: None. **K. Vasudevan:** None. **V.V.M. Vierkant:** None. **S. Maren:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

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Program #/Poster #: 311.04

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH065961

Title: Optogenetic inhibition of the thalamic nucleus reuniens impairs the retrieval of fear extinction memories in male and female rats

Authors: *T. TUNA, M. TOTTY, S. MAREN;
Texas A&M Inst. for Neuroscience, Dept. of Psychological & Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Interactions between the medial prefrontal cortex (mPFC) and the hippocampus (HPC) are known to be crucial for memory retrieval. The nucleus reuniens (RE) of the thalamus interconnects mPFC and HPC. Reversible inactivation of the RE with the $GABA_A$ agonist muscimol or chemogenetic inhibition of mPFC afferents in RE impairs the retrieval of extinction memory. These results reveal a critical role for the RE in memory retrieval, but do not delineate whether RE inactivation during specific phases of the retrieval test (pre-CS baseline, CS, or intertrial intervals) is critical for these effects. To address this issue, we adopted an optogenetic approach. Adult male and female Long-Evans rats were injected either with an AAV expressing the red-light inhibitory opsin, Jaws (n=13), or a blank control (n=10) virus; optical fibers were implanted in the RE. Following 3-4 weeks of recovery, rats underwent auditory fear conditioning with five tone (CS) and foot shock (US) pairings and extinction with 45 CS-alone trials. An additional control group (n=8) received Jaws but was not conditioned. Twenty-four hours after extinction, all animals underwent a within-subjects extinction retrieval where they were tested with five CS-alone trials under either continuous red light during the entire retrieval session (ON) or no laser illumination (OFF) conditions. Confirming earlier RE inhibition effects, animals injected with Jaws showed poor extinction retrieval under continuous red light. Blank and nonconditioned controls did not differ between red light ON vs OFF conditions. Next, we

examined the temporal window around which RE inhibition impairs extinction retrieval. We optogenetically silenced RE during different time periods of the extinction retrieval test. We hypothesized that the inhibition of RE would impair extinction retrieval when administered during the CS, but not during the pre-CS baseline (BL) or interstimulus intervals (ISIs). Rats expressing Jaws in RE were assigned to three retrieval testing groups based on red light ON period: continuous (n=9), CS-only (n=11), or baseline and interstimulus interval (BL+ISI) (n=11). They were tested under counterbalanced light ON and OFF conditions. Optogenetic inhibition of RE modestly impaired extinction retrieval in all groups, and this effect was most pronounced in the continuous and BL+ISI groups. These results suggest that RE may coordinate recognition and processing of the extinction context that is required for extinction memory retrieval.

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Poster

311. Fear and Stress

Location: SDCC Halls B-H

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Program #/Poster #: 311.05

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01MH117852

Title: Pharmacological stimulation of the infralimbic cortex during fear memory consolidation facilitates future extinction learning

Authors: *H. BAYER, S. MAREN, C. R. OLEKSIAK;
Psychological and Brain Sci. and Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: The infralimbic cortex (IL) has a critical role in extinction learning, including the extinction of Pavlovian fear conditioning. Interestingly, recent evidence suggests that pharmacological inhibition of the IL immediately after fear conditioning (during consolidation) impairs subsequent extinction learning. Although the IL is not required for fear conditioning, these data suggest that it nonetheless encodes information during conditioning that can modulate later extinction learning. Hence, the IL may encode inhibitory associations during or after fear conditioning that promote later extinction learning. To test this hypothesis, we examined whether post-conditioning pharmacological stimulation of the IL would facilitate the extinction of conditioned fear. We examined this both under standard extinction conditions and when extinction was conducted under stress, a procedure known to impair extinction learning. Adult male and female rats were implanted with guide cannulae targeting the IL and one week after they underwent auditory fear conditioning consisting of 5 tone-footshock pairings in context A. Immediately after conditioning they received intra-IL infusions of either picrotoxin (PIC) or vehicle (VEH). Two days later, the subjects received a fear extinction session, which consisted of 45 tone-alone presentations in context B; in half the animals this was conducted after a

footshock reminder in a novel context. Freezing behavior was used an index of conditioned fear. In non-stressed rats, PIC-treated animals exhibited less freezing both during extinction learning and retrieval, suggesting that IL stimulation during consolidation might lead to retrograde amnesia of the fear memory. Rats in the stress group, however, did not benefit from IL stimulation. These results suggest that IL stimulation during consolidation either weakens fear memory or potentiates extinction learning. In either case, this work agrees with previous studies showing that increased IL activity leads to less fear, and further confirms that IL's activity during early stages of fear memory consolidation modulates memory fate bidirectionally.

Disclosures: H. Bayer: None. **S. Maren:** None. **C.R. Oleksiak:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

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Program #/Poster #: 311.06

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH065961

Title: The thalamic nucleus reuniens mediates fear extinction retrieval by coordinating prefrontal-hippocampal theta oscillations

Authors: *M. TOTTY, K. R. RAMANATHAN, J. JIN, S. PETERS, T. TUNA, S. MAREN; Psych and Brain Sci. and Inst. for Neurosci., Texas A&M Univ., College Station, TX

Abstract: The nucleus reuniens (RE) is a thalamic hub coordinating activity in the medial prefrontal cortex (mPFC) and hippocampus (HPC). Recent work from our laboratory has found that inactivating the RE, and mPFC projections to the RE, impairs the encoding and retrieval of extinction to an auditory conditioned stimulus (CS) (Ramanathan et al., 2018). One mechanism by which the RE may facilitate fear extinction is by coordinating mPFC-HPC theta oscillations (6 - 12 Hz). Synchronized corticolimbic theta oscillations (Lesting et al., 2013) accompany the retrieval of fear and extinction memories, and reversible inactivation of the RE impairs mPFC-HPC theta synchrony (Hallock et al., 2016). We thus hypothesize that the RE coordinates mPFC-HPC theta coherence to promote the context-dependent retrieval of extinction memories. To test this, we used a standard auditory fear conditioning and extinction procedure in both male and female rats. Using *in vivo* electrophysiology, we found that mPFC and HPC theta oscillations are strongly synchronized during the retrieval of both fear and extinction memories, however, the retrieval of extinction memories is uniquely associated with mPFC-to-HPC theta directionality. Pharmacological inactivation of the RE induced fear relapse, impaired mPFC and HPC function as evidenced reduced immediate-early gene (c-Fos) expression, and disrupted mPFC-HPC theta synchrony. Importantly, RE inactivation did not impair c-Fos expression in control regions (i.e., lateral geniculate nucleus) and did not increase freezing in rats that were never conditioned. This suggests that inactivation of RE causes relapse by reducing coordinated mPFC-HPC activity.

Because extinction retrieval is associated with ~8 Hz theta rhythms in the mPFC-HPC circuit, we hypothesized that theta-paced stimulation of the RE might be sufficient to block fear relapse. Indeed, we found that optogenetic stimulation of the RE using 8 Hz sinusoidal waves blocked the context-dependent renewal of fear in rats expressing the excitatory opsin, channelrhodopsin-2, but not in rats expressing a control fluorophore, mCherry. Collectively, these results show for the first time the RE enables the retrieval of fear extinction memories by coordinating mPFC-to-HPC theta oscillations.

Disclosures: **M. Totty:** None. **K.R. Ramanathan:** None. **J. Jin:** None. **S. Peters:** None. **T. Tuna:** None. **S. Maren:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH R01MH117852
NIH R01MH117852S01

Title: The contribution of parvalbumin-positive interneurons in the medial prefrontal cortex to stress-impaired fear extinction in male and female rats

Authors: ***A. N. BINETTE**[1], J. LIU[2], K. CRAYTON[1], L. MELISSARI[1], H. BAYER[1], S. MAREN[1];
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Abstract: Stress has deleterious consequences on fear extinction, a type of learning that underlies behavioral therapies for trauma- and anxiety-related disorders. We have found that delivering extinction trials 15 minutes after fear conditioning impairs extinction learning, what we term the “immediate extinction deficit” (IED). The IED is associated with decreases in neuronal activity in the infralimbic (IL) cortex, a brain region essential for extinction learning. Considerable work suggests that stress may recruit prefrontal cortical (mPFC) interneurons, including parvalbumin (PV)-positive cells, to dampen the excitability of mPFC output. To test this hypothesis, adult male (n = 12) and female (n = 12) Long-Evans rats were exposed to footshock and Fos expression was quantified in PV^+ and PV^- cells in the infralimbic and prelimbic (PL) subdivisions of the mPFC, with no-shock and home cage controls. Footshock induced Fos expression in PV^+ and PV^- cells in the IL and PL of both sexes. Interestingly, the IL contained a greater proportion of double-labeled PV^+ cells relative to the PL. To determine the contribution of mPFC PV^+ interneurons to stress-impaired fear extinction, we expressed an inhibitory designer receptor exclusively activated by designer drugs (DREADDs) in the mPFC of adult male (n = 45) and female (n = 38) transgenic PV-Cre rats. Animals were treated with

vehicle or the DREADD ligand clozapine-*N*-oxide (CNO; 3 mg/kg, i.p.) prior to conditioning (a stressor) and subsequent immediate extinction (conducted 15 min after conditioning); extinction retrieval was tested 48-hr later. CNO treatment had no effect on conditioned freezing during the conditioning and extinction procedures. However, chemogenetic inhibition of PV interneurons during immediate extinction influenced extinction retrieval and this was dependent on the pattern of DREADD expression in the IL and PL. In rats with the highest levels of PL DREADD expression, CNO treatment during extinction exacerbated extinction retrieval in both male and female rats. In contrast, in rats with the highest levels of IL DREADD expression, CNO produced a modest facilitation of extinction in the earliest retrieval trials, but in male rats only. These results demonstrate that mPFC PV interneurons are engaged by stress and contribute to the regulation of stress-induced extinction impairments in a sex-dependent manner.

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Poster

311. Fear and Stress

Location: SDCC Halls B-H

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Program #/Poster #: 311.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: BBRF 30637
NSF 2143910
R21 MH126278

Title: The temporal dynamics of encoding fear memories in the BLA, and how they change following experience with rewards.

Authors: *L. DIFAZIO[1], R. JIA[1], Z. GREER[1], M. SHARPE[2];
[2]Psychology, [1]UCLA, Los Angeles, CA

Abstract: Decades of research has shown that lesions or inactivation of the basolateral amygdala (BLA) disrupts the acquisition and storage of memories during Pavlovian fear learning (Cousens & Otto, 1998, *Behavioral Neuroscience*; Maren, Aharonov & Fanselow, 1996, *Behavioral Neuroscience*; Phillips & LeDoux, 1992, *Behavioral Neuroscience*). As a result, current models of fear learning posit that information about the stimuli and aversive event arrive in the BLA and become an associative fear memory (Maren & Quirk, 2004, *Nature Reviews Neuroscience*; Pitkänen, Savander & LeDoux, 1997, *Trends in Neurosciences*). Optogenetics allows us to investigate the temporal dynamics of the BLA's involvement in fear learning in a way that was not previously possible. We investigated the effect of optogenetic inhibition of glutamatergic neurons in the BLA at different time-points during Pavlovian fear conditioning (with tone-shock pairings). We found that inhibition of BLA neurons during the tone, but not the shock disrupted learning. This demonstrates that the BLA is necessary for learning about the shock-predictive

tone, but processing of the shock itself remains intact when BLA activity is inhibited. Following recent evidence that the lateral hypothalamus becomes necessary for fear learning following experience with reward learning (Sharpe et al., 2021, *Nature Neuroscience*), we investigated how reward learning experience might impact the role of the BLA in fear learning. In a new experiment, one group of rats learned a light-food association, while the "naïve" control group had the same experience without the light-food pairing. Then, in a different but familiar context, rats experienced tone-shock conditioning. Here, we inhibited pyramidal neurons in the BLA during the tone. We found that BLA inhibition disrupted fear memories in naïve rats, replicating our prior result. However, in rats with prior reward learning experience BLA inhibition did not affect fear memories. These results show that reward learning shifts the fear circuit towards the lateral hypothalamus and away from the BLA.

Disclosures: L. DiFazio: None. **R. Jia:** None. **Z. Greer:** None. **M. Sharpe:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

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Program #/Poster #: 311.09

Topic: G.01. Fear and Aversive Learning and Memory

Support: T32-MH125786
P50-MH115874
R01-MH108665
Jonathan Edward Brooking Mental Health Research Award
McLean Frazier Fund

Title: An infralimbic-lateral entorhinal cortex pathway modulates cue-context discrimination in threat memory encoding and retrieval

Authors: *E. HISEY, K. RESSLER;
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Abstract: The ability to form and retain memories of safety in the presence of formerly threatening cues and contexts is critical for survival, while the inability to do so can result in threat-related disorders. The medial prefrontal cortex (mPFC) and hippocampus are both well known to play critical roles in cued and contextual threat memory processing, respectively. However, the circuits that mediate prefrontal-hippocampal modulation of context discrimination in cued threat processing are unknown. Here we demonstrate the role of a previously unexplored projection from the infralimbic region of mPFC (IL) to the lateral entorhinal cortex (LEC) in modulating the gain of behavior in response to contextual information during threat retrieval and encoding. Using *in vivo* calcium imaging, we find that subpopulations of IL-LEC projecting cells respond only to the cue or only to a specific context while other subpopulations selectively respond to cues in specific contexts during threat processing. We then employ optogenetics to

manipulate IL-LEC activity in response to threat-associated cues in different contexts. We find that IL-LEC cells can flexibly shape behavior during threat expression, shaping sensitivity to contextual information to increase or decrease the gain of behavioral output in response to a threatening or neutral context, respectively. Additionally, we find that IL-LEC cells bidirectionally modulate encoding of threat memory, differentially shaping the behavioral response to threat during contextual recall, namely activation or inactivation during encoding increases or decreases threat responding during contextual recall. Finally, we investigate the inputs to, and outputs from, IL-LEC cells, finding that IL-LEC cells receive dense input from prefrontal and salience processing networks and provide excitatory drive onto layers 2/3 and 5 of LEC. Overall these results suggest that glutamatergic IL-LEC cells are a key player in behavioral gain control in response to contextual information during threat processing and may provide a future target for pharmacological intervention in threat-based disorders.

Disclosures: E. Hisey: None. **K. Ressler:** F. Consulting Fees (e.g., advisory boards); Bioxcel, Bionomics, Acer, Takeda, Jazz Pharma, Sage, Alto Neuroscience, Brainsway.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.10

Topic: G.01. Fear and Aversive Learning and Memory

Support: Mina Rees Dissertation Fellowship
Provosts University Fellowship
R21MH114182
PSC-CUNY Award
R01MH118441

Title: Chronic stress disrupts safety learning and theta-based circuit communication

Authors: I. S. GRUNFELD[1], L. E. DENHOLTZ[2], S. HANIF[3], I. NAHMOUD[4,5], S. DATTA[6], S. CHATTARJI[6], N. BURGHARDT[3], ***E. LIKHTIK**[4,2];
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Abstract: We have previously shown that chronic stress leads to generalization of fear to non-threatening cues, a key symptom in numerous psychiatric disorders. Here, we used the chronic social defeat stress (CSDS) model to investigate whether this might reflect an effect of stress on safety learning. Following 10 days of CSDS, male 129Sv/Ev mice underwent two days of salient safety conditioning, where they were exposed to 5 unsignaled shocks (0.5mA) intermixed with five presentations of a safety stimulus. The safety stimulus was a 30s tone that turned on at the same time as a 1s house light (CS_{safety}) to signal the explicit absence of shock. The next day,

animals were tested with 5 presentations of the CS_{safety} cue in the same context without shock. We found that non-stressed control mice (n= 15) exhibited defensive freezing to the context, which was reduced during the CS_{safety} cue. In contrast, stressed mice (n = 15) showed high levels of freezing behavior regardless of the presence of the CS_{safety} cue until the last trial, indicating that chronic stress impairs safety learning. To gain insight into the underlying circuits, we used local field potential (LFP) recordings in the prelimbic cortex (PL), basolateral amygdala (BLA) and dorsal hippocampus (HPC) and compared activity in the presence and absence of the CS_{safety} cue. In the PL of control mice, the theta (4-8Hz):delta (0.1-3.9Hz) ratio was lower when CS_{safety} was on. In stressed mice this process was disrupted, as evidence by the lack of change in the theta:delta ratio and the increase in theta and delta power during the CS_{safety} signal, indicating increased drive onto the PL. In the BLA, controls also had a lower theta:delta ratio during the CS_{safety} cue, whereas the ratio in stressed mice did not change. Furthermore, stressed mice showed significantly lower overall PL-BLA theta coherence than control mice, indicating that chronic stress decreased synchronous theta-based communication in this circuit. Notably, by the end of the retrieval session (trial 5), when stressed animals first suppress defensive freezing to the CS_{safety} cue, they showed a significantly lower PL theta:delta ratio and lower BLA theta power when the CS_{safety} signal was on, consistent with a delay in dynamic changes in the circuit underlying retrieval of a safety cue. In a separate cohort of mice, we evaluated spine density in the PL after CSDS, and found that CSDS decreased the number and density of PL spines. Currently, we are investigating the contributions of HPC theta-oscillations to CS_{safety} processing in this circuit.

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Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.11

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH114961

Title: Context Fear Discrimination Learning in Juvenile and Adult Female and Male Rats

Authors: *I. GUPTA[1], A. M. POULOS[2];
[1]Psychology, Univ. of Albany, Albany, NY; [2]Dept. of Psychology, Univ. At Albany, SUNY, Albany, NY

Abstract: Fundamental to the survival of any foraging species is the ability to discriminate spatial environments offering harbor versus danger. While some sex differences in contextual-spatial processing have been studied, relatively little work has focused on examining the ability of juvenile females and males to perform context-spatial discrimination. We aim to examine

fear-motivated context discrimination learning in juvenile and adult female and male Long-Evans rats through two experiments where postnatal day (P) 24(juvenile) and P90 (adult) rats underwent multiple sessions of one-trial context fear conditioning, where we assessed freezing behavior in two distinct contexts, A and B.In experiment 1, subjects were administered a single footshock (1 mA; 2 sec) in contextA for five consecutive days and then tested in context A (day 6) and novel context B(day 7). In experiment 2, we administered an extensive context fear discrimination procedure in three phases. In phase 1, subjects received a single footshock (0.65 mA; 2sec) for three consecutive days. Freezing was assessed prior to footshock. In phase 2, subjects were tested for freezing, shock-free, in both contexts A and B on two consecutive days. We counterbalanced the testing order with half of the subjects placed in context A, and then B with the order reversed the next day. In phase 3, subjects were returned to context A, where they once again received a single footshock (0.65mA; 2sec), and context B, where they never received a footshock, for 12 days.Preliminary results from experiment 1 reveal freezing significantly increased across successive days of context fear conditioning ($p < .0001$), indicating successful conditioning. Moreover, we identified a significant difference in conditioning between juvenile males and adult females ($p = .014$). In experiment 2, preliminary results indicate all groups except adult females consistently discriminated between contexts A and B.We also found a significant difference in context fear discrimination between adult females and adult males ($p = .010$).These results suggest adult female rats exhibit a reduced capacity in discriminating context-spatial environments associated with safety and threat compared to other experimental groups. Long Evans females have a higher reproductive burden than males, resulting in greater caretaking than foraging responsibilities. This emphasis likely results in diminished context-spatial learning in females. Further studies evaluating sex and developmental differences in contextual processing and threat discrimination may include an assessment of the impact of rodent estrous cycles on these processes.

Disclosures: I. Gupta: None. **A.M. Poulos:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.12

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01
BP Endure

Title: The basal forebrain as a mediator of infralimbic-amygdala communication in fear extinction

Authors: *C. FERNANDES-HENRIQUES[1,3], R. ZHANG[1], L. YUSUFOVA[1], Y. GUETTA[2], E. LIKHTIK[1,3];

[1]Biol., [2]Psychology, Hunter College, CUNY, New York, NY; [3]Biol., Grad. Center, CUNY, New York, NY

Abstract: Projections from the infralimbic region (IL) of the medial prefrontal cortex to the basolateral amygdala (BLA) are needed to suppress fear responses after extinction. However, which IL afferents serve as a teaching signal during extinction remains unknown. Given the importance of the basal forebrain (BF) in learning, we investigated whether BF-IL communication contributes to extinction and to IL-BLA physiology. To this end, we used multisite local field potential recordings in the IL, BF, and BLA during behavior to identify patterns of communication during extinction learning and recall. Retrograde neuroanatomical tracers were then used to study IL projections to the BLA and BF, and combined with functional immunohistochemistry to assess the activity of IL-BF versus IL-BLA projectors during fear and extinction recall. These experiments showed that during extinction learning and recall, cue-evoked IL theta power (4-8Hz) changes resembled the BF more than the BLA. Directionality analyses showed that IL-BF theta communication was bidirectional during extinction acquisition, but IL-to-BF directionality prevailed during extinction recall, whereas the IL-to-BLA directionality remained throughout. Furthermore, partial correlations showed that IL-BLA theta power correlations dropped dramatically when the BF signal was removed. Anatomical data confirmed IL-BLA projections arising in LII-III and showed IL-BF projections from LII-III and LIV-V. Immunohistochemical analyses showed that the IL-BLA projectors didn't exhibit preferred activation during fear vs extinction recall, whereas IL-BF projectors from LIV-V showed higher activity during extinction recall. We are currently investigating the functional significance of the BF-to-IL projection to extinction learning using an optogenetic approach. We posit that BF input to the IL is a critical teaching signal during extinction acquisition that influences IL-BLA communication, and IL-to-BF signaling during extinction recall.

Disclosures: C. Fernandes-Henriques: None. **R. Zhang:** None. **L. Yusufova:** None. **Y. Guetta:** None. **E. Likhtik:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.13

Topic: G.01. Fear and Aversive Learning and Memory

Support: RO1MH114961

Title: The Role of Stress Severity upon the Persistence of SEFL in Juvenile and Adult Male and Female Rats.

Authors: *S. R. HETHERINGTON[1], A. M. POULOS[2];
[1]State Univ. of New York, Univ. of Albany, Albany, NY; [2]Dept. of Psychology, Univ. At Albany, SUNY, Albany, NY

Abstract: Post-Traumatic Stress Disorder (PTSD) is a stress- and trauma-related disorder characterized by persistent changes in affect and cognition, including an exaggerated fear response. More than half of the US population will experience at least one traumatic event in their lifetime. Of those, around 6% will develop PTSD. It is well established that females (10%) are more than twice as likely to be diagnosed with PTSD than males (4%) as adults. PTSD typically has an onset during early to middle adulthood but is seen in children and adolescents, with 8% of females and 3% of males being affected by PTSD.
To better understand PTSD and open new avenues for treatment, it is crucial to adopt animal models that capture key characteristics of PTSD. The Stress Enhanced Fear Learning (SEFL) paradigm captures some of these complexities, including the sensitization of fear. Although PTSD disproportionately affects women, relatively few studies have examined SEFL in juvenile and adult female rats. In this study, we examine how the severity of stress affects the persistence of SEFL in juvenile and adult male and female rats. P24 (juvenile) and P90 (adult) rats were initially exposed to no (context alone), low (1 footshock), moderate (3 footshocks), or high (15 footshocks) levels of stress, followed by a session of extinction at a recent (1 day) or remote (60 days) interval. Next, to assess fear sensitization in a novel context, all subjects received a single footshock (1 mA; 2 sec) and were tested in this context the following day.
In adult female rats, our results indicate that all levels of stress induction (low, moderate, and high) produced SEFL at the recent test interval. However, at the remote test interval, only rats that experienced high levels of stress induction exhibited a sustained enhancement of fear learning. In adult male rats, our results indicate that all levels of stress induction produced SEFL in both recent and remote test groups. In juvenile female rats, our results indicate that all levels of stress induction produced SEFL in both recent and remote test groups. In juvenile males, our results indicate that all levels of stress induction produced SEFL at the recent test interval; however, this effect was not sustained at the remote test interval..
These results suggest that there are sex and age difference in the persistence of SEFL, particularly at the remote interval, where SEFL was detected with all levels of stress induction only in adult males and juvenile females. In adult females, this persistence was only evident with high stress induction. Strikingly, in juvenile males, we failed to find any evidence for the persistence of SEFL.

Disclosures: S.R. Hetherington: None. **A.M. Poulos:** None.

Poster

311. Fear and Stress

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 311.14

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01MH114961

Title: Robust anterograde amnesia of contextual fear memory induced by ventral hippocampal inactivation in adult male and female rats

Authors: *E. PERU, E. GROOM, A. M. POULOS;
Psychology, Univ. at Albany SUNY, Albany, NY

Abstract: Mounting evidence has demonstrated that the hippocampus does not act as a single functional unit in processing contextual information during fear learning; instead, it can be divided into three independent yet interconnected units that process different aspects of this learning. These regions have been described as the dorsal, intermediate, and ventral hippocampus (DH, IH, and VH). Currently, more is known about the role of the DH during context fear conditioning, leading to increased interest in the role of VH in more recent years. Lesions and inactivation studies of the DH demonstrate retrograde and anterograde amnesia for context fear memories. When it comes to the VH, however, there are more nuanced findings that seem to depend on the method used to test this region. Lesion studies demonstrate robust retrograde amnesia with negligible anterograde amnesia, while pharmacological inactivation creates a more pronounced anterograde amnesia. The difference between these methods raises the question of whether the inactivation of VH during both training and test may contribute to the strength of anterograde amnesia. To address this question, we infused the GABAa receptor agonist, Muscimol, into the VH of adult male and female rats either before training (M-S), retrieval testing (S-M), or both (M-M), thus allowing assessment of retrograde and anterograde amnesia and possible interference of VH independent retrieval of memory. Our preliminary results demonstrate no significant effects of Muscimol infusion prior to training on learning. At retrieval, a significant interaction between the training drug (Muscimol vs. saline) and the test drug was observed. Post hoc comparisons revealed a significant difference in freezing between the S-S control and M-S group. However, as identified by previous findings, we did not observe a significant difference between Sal-Sal and Sal-Mus groups. In addition to confirmation of cannula placement, we will also be using atlas-based mapping to determine if spatial differences in cannulae placement can account for the variance in some of our groups. Overall, our preliminary data suggest a role of the VH in the encoding of contextual fear memories. However, disruption of VH function at both training and test indicates that other brain region(s) can support conditioning independent of the VH.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Program #/Poster #: 312.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: ERC Consolidator Grant (772994, FeedHypNet, to T.K.
DFG (233886668-GRK1960, to R.F.)
DFG (Project-ID 431549029 – SFB 1451, to T.K.)
DFG (EXC2030 CECAD, to T.K.)

Title: Complementary lateral hypothalamic populations resist hunger pressure to balance nutritional and social needs

Authors: A. PETZOLD[1,2], H. E. VAN DEN MUNKHOF[1,2], ***R. FIGGE**[1,2], T. KOROTKOVA[1,2];
[1]Inst. for Vegetative Physiol., Univ. of Cologne, Cologne, Germany; [2]Neuronal Circuits and Behaviour Group, Max Planck Inst. for Metabolism Res., Cologne, Germany

Abstract: Animals continuously weigh hunger and thirst against competing needs, such as social contact and mating, according to state and opportunity. However, the neuronal mechanisms of sensing and resisting metabolic pressure remain poorly understood. Combining calcium imaging in freely behaving mice, opto-, and chemogenetics, we show that two distinct neuronal populations of leptin receptor- (LepR) and neurotensin-expressing cells (Nts) of the lateral hypothalamus (LH) guide increasingly hungry animals through behavioural choices between nutritional and social rewards.
We found that LepRLH neurons encoded food, whereby the magnitude of food-elicited responses increased with food intake under moderate, but not under strong hunger pressure. Failure to resist moderate hunger pressure was encoded by escalating inhibition of a leptin-sensitive LepRLH subpopulation at a fast time scale. Similarly, LepRLH neurons of thirsty animals encode water. Optogenetic stimulation of LepRLH neurons suppressed food intake in moderately hungry animals and water intake in thirsty animals. Further, it promoted interaction with females but not males. During social interaction LepRLH neurons exhibited stronger responses to conspecifics of opposite sex. Growing hunger pressure led to an increase of proportion of food-selective and a decrease of social-selective LepRLH neurons, suggesting need-dependent competitive coding of these orthogonal stimuli by LepRLH neurons.Conversely, hunger pressure intensified water encoding of NtsLH neurons, whereby the magnitude of water-elicited responses increased with food intake. NtsLH neurons tracked food intake to scale water intake accordingly, ensuring the balance between feeding and drinking. In contrast, NtsLH neurons did not differentiate the sex of conspecifics and chemogenetic activation of NtsLH neurons reduced social interaction.
In summary, hunger pressure gates LepRLH and NtsLH populations in a complementary manner. LepRLH and NtsLH populations exert opposite effects on social interaction with LepRLH neurons promoting and NtsLH neurons restraining social drive. This complementary control of innate drives enables the flexible fulfilment of orthogonal needs according to current opportunities and gated by physiological demands.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 312.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA IRP

Title: New tools for studying neuronal ensembles that encode operant social reward in mice

Authors: *L. A. RAMSEY[1], F. M. HOLLOMAN[1], M. VENNIRO[2], Y. SHAHAM[1], B. T. HOPE[1];
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Abstract: Background: We recently developed a mouse model of operant social self-administration and choice (Ramsey et al. Biol Psychiatry, 2021). Using this model, we found that outbred female CD1 mice, but not C57BL/6J female mice, showed reliable social interaction self-administration, strong social-seeking behavior during isolation, preference for social interaction over food . Current neurobiological investigations of social behavior are performed almost exclusively using C57BL/6J mice, the most common background strain of transgenic mice. Given that female C57BL/6J mice are not suitable for studying operant social reward, we created new transgenic lines to study activity-dependent neuronal ensembles that encode social self-administration. We tested whether breeding outbred female CD1 mice with FosGFP, FosTRAP2, and FosTRAP2 x Ai14 transgenic C57BL/6J male mice will maintain the operant social reward phenotype in the hybrid F1 female offspring. **Methods:** First, we crossed male C57BL/6J transgenic mice from each of the three strains (FosGFP, FosTRAP2, FosTRAP2 x Ai14) with female CD1 mice. We trained the F1 generation to lever-press for palatable food pellets and then to lever-press under increasing fixed-ratio response requirements for access to a female social partner. Next, we tested their motivation to seek social interaction after 15 days of social isolation. We compared the three transgenic strains on CD1 background to wild-type CD1 female mice. **Results:** Female mice from the three newly bred hybrid transgenic mouse lines showed reliable social self-administration and social-seeking behavior after isolation, similar to wild-type CD1 female mice. **Conclusion:** Our data indicate that the social phenotype is maintained in the F1 generation in all three strains tested using the hybrid breeding scheme. This will enable us and other researchers, to identify, characterize, and manipulate activity-dependent neuronal ensembles involved in operant social reward. Funding: This work was supported by the National Institute on Drug Abuse Intramural Research Program

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Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Center for transformative research on health behaviors at Virginia Tech

Title: Macronutrient modulation of food reward: Content and sex-specific effects on striatal dopamine dynamics

Authors: *A. HARTLE, C. SALLEE, A. DOFAT, K. MARSHCHALKO, K. RUNYON, J. SISCO, S. SENGUPTA, A. DIFELICEANTONIO, W. HOWE;
Virginia Tech. Neurosci. PhD Program, Blacksburg, VA

Abstract: Emerging evidence suggests that the nutritional profile of food modulates output from the gut to the brain to influence its rewarding properties, in part by affecting the excitability of midbrain dopamine (DA) neurons to contribute to learning and motivation. Interestingly, carbohydrates and fats appear to engage distinct ascending pathways from the gut to midbrain DA cells. Furthermore, recent human research has demonstrated foods that contain both carbohydrates and fats are overvalued, calorie-for-calorie, compared to equally liked foods made of just one macronutrient. Building off these observations, we hypothesized that foods made of combinations of fat and carbohydrate simultaneously recruit each ascending pathway, ultimately augmenting such post-ingestive DA release to modulate food reward. To test this hypothesis, we investigated the influence of single and combination macronutrients on food preference and DA release in mice. We found that when given the option to freely choose between foods made of fat, carbohydrate, or fat/carbohydrate combined, mice consumed far more combination compared to either individual macronutrient (n=52). This preference was independent of satiety state. Next, we used the fluorescent DA reporter, dLight, and fiber photometry to measure DA release across the striatum as mice consumed isocaloric amounts of fat, sugar, or combination food. We found that consuming a single calorie of combination food nearly doubled DA release in the nucleus accumbens (NAc) relative to fat or carbohydrate alone (n=7). In contrast, DA release in the dorsal striatum (DS) scaled with caloric density, not preference (n=7). Fat-evoked DA release in the DS was also greater in females than in males, and corresponded with an increase in the capacity of fat to create a conditioned place preference (n=10 male, 7 female; see also Dofat et al.). These ongoing studies support a model in which caloric density and reward value of foods are differentially encoded by meso-striatal DA circuits. Additionally, interactions between nutrient composition, food reward, and DA release are region and sex-specific. Our ongoing studies employ predictive modeling and optogenetics to identify and selectively manipulate the relay of nutrient content from the gut to the midbrain to test its necessity and sufficiency for food reinforcement.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: G.02. Reward and Appetitive Learning and Memory

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Title: VTA dopamine neurons signal phasic and ramping reward prediction errors for goal-directed navigation

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Abstract: Phasic midbrain dopamine responses readily fit with the reward prediction error (RPE) hypothesis, where the magnitude of the response reflects the difference between expected and observed outcome. Some recent studies have observed pre-reward ramping of dopamine activity and release, but its relationship to RPE signalling is currently under debate. We set out to explain the function of this ramp and its relationship to phasic RPEs, using both experimental and theoretical methods. We chose to examine goal-directed navigation, as it requires learning to accurately estimate location and select optimal actions in each location. Given that ventral tegmental area (VTA) dopamine neurons are involved in value learning, action selection, and reward location learning, they are ideally placed to provide teaching signals for goal-directed navigation.

We characterised VTA dopamine neuron activity by performing calcium imaging using cre-dependent GCaMP6m and a Miniscope in mice (DAT-cre; n=8; 5F) as they learned to navigate in a closed-loop virtual reality corridor and lick to report a reward location. We show that phasic and ramping signals are concurrently observable in dopamine neuron activity, with the pre-reward ramp and cue responses developing across learning. The gradient of this ramp was modulated by both learning stage and task engagement, and was inversely correlated with locomotor speed. These results suggest that the ramp is similar to RPE responses rather than reflecting motor vigour.

Given these observations, we decided to test whether ramping VTA dopamine activity could represent a form of RPE in an action-dependent framework. We therefore devised a Q-learning model that incorporated noisy state inference and an eligibility trace to simulate the representations that mice could use to solve the task. This model recapitulated our behavioural findings and produced simultaneous phasic and ramping prediction error. The model predicted that a ramp should improve task performance (licking in the reward zone) on the subsequent trial, which we confirmed in our experimental data, and additionally showed that this effect occurred on a trial-to-trial basis. These results indicate that the ramp played a teaching role in the selection of accurate location-specific action during navigation. We therefore find that pre-reward ramping of VTA dopamine neuron activity is most consistent with an RPE signal. Our findings provide neural evidence and a theoretical framework to explain phasic and ramping dopamine neuron activity as RPE signals that improve goal-directed navigation.

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Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: 5R01DA036612-09

Title: Local connectivity of non-dopamine projection neurons in Ventral Tegmental Area

Authors: *L. ORIOL, M. CHAO, S. URAN, S. WARLOW, S. SINGAL, T. S. HNASKO; Neurosciences, Univ. of California San Diego, La Jolla, CA

Abstract: The ventral tegmental area (VTA) is central to brain reward circuits and addictive drugs share a common effect of increasing dopamine released from VTA projections, most famously in nucleus accumbens (NAc). Understanding the VTA's functional organization is therefore important toward understanding the mechanisms underlying addiction. For example, opioids reduce GABA-mediated inhibition in the VTA, thereby disinhibiting dopamine neurons, which contributes to the reinforcing properties of those drugs. While classical models suggest that dopamine disinhibition in VTA is mediated by GABA interneurons (defined as neurons only making local connections such that their soma and axon are contained in the same brain region), there is no direct evidence that interneurons of this type exist in VTA. Instead, GABA projection neurons in VTA could make intra-VTA collaterals. To determine whether VTA GABA and glutamate projection neurons make local connections, we used optogenetics-assisted electrophysiology to functionally identify local GABA and glutamate connections made by projection-target defined VTA neurons. We selectively expressed ChR2 in VTA projection neurons (to NAc, prefrontal cortex, ventral pallidum, and lateral Habenula successively) and performed whole-cell patch clamp recordings of VTA neurons. We detected opto-evoked excitatory postsynaptic currents, presumably mediated by glutamate, and opto-evoked inhibitory postsynaptic currents, presumably mediated by GABA. Our results suggest that VTA projections neurons collateralize to make intra-VTA connections (as well as distal ones) which could mediate functions prior attributed to VTA interneurons.

Disclosures: L. Oriol: None. **M. Chao:** None. **S. Uran:** None. **S. Warlow:** None. **S. Singal:** None. **T.S. Hnasko:** None.

Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R00MH118422
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Title: Mesolimbic dopamine release conveys causal associations

Authors: *H. JEONG, M. ZHOU, B. WU, D. A. BURKE, V. K. NAMBOODIRI;
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Abstract: Learning to predict rewards based on environmental cues is essential for survival. It is widely believed that animals learn to predict rewards by updating predictions whenever the outcome deviates from expectations. Such violations of predictions are called reward prediction errors (RPEs) and thought to facilitate learning. RPEs are the critical teaching signal in the most widely accepted model for cue-reward associative learning—temporal difference reinforcement learning (TDRL). TDRL RPE has been successful at explaining the activity dynamics of dopaminergic cell bodies and release in the nucleus accumbens. Hence, TDRL RPE has become the dominant theory of dopamine's role as the critical regulator of behavioral learning. An alternative approach to learn cue-reward associations is to infer the cause of meaningful outcomes such as rewards. Since causes must precede outcomes, a viable approach to infer whether a cue causes reward is to learn whether the cue consistently *precedes* reward. This approach is advantageous as predicting the future is highly demanding in a cue-rich environment but inferring the cause of a rarer meaningful outcome simply requires a memory of previous experience. In other words, if an animal knows that a stimulus it just received is meaningful (e.g., a reward), it can look back in memory to infer its cause. Using this intuition, here we propose a causal inference algorithm that infers whether a cue is a cause of reward. Based on this algorithm, we denote stimuli (cues or rewards) whose cause should be learned by the animal as "meaningful causal targets" and propose that mesolimbic dopamine signals whether a current event is a meaningful causal target using an adjusted net contingency for causal relations (ANCCR) of that event in relation to other meaningful causal targets. We found that ANCCR makes similar predictions as RPE under commonly studied experimental settings. Hence, to distinguish between the two hypotheses (RPE or ANCCR signaling by mesolimbic dopamine release), we performed eight experimental tests by measuring dopamine release in nucleus accumbens core. We found that mesolimbic dopamine conveys causal associations but not RPE in every case, thereby challenging the dominant theory of reward learning in the brain. Our results provide a new conceptual and biological framework for associative learning.

Disclosures: H. Jeong: None. **M. Zhou:** None. **B. Wu:** None. **D.A. Burke:** None. **V.K. Namboodiri:** None.

Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: Revson Foundation Senior Postdoctoral Fellowship

Title: Subregion specific processing of Pavlovian reward cues in the nucleus accumbens

Authors: *C. BAIMEL[1], K. MANOOCHERI[2], A. G. CARTER[2];
[2]Ctr. for Neural Sci., [1]New York Univ., New York, NY

Abstract: The neural circuits that guide motivated behavior converge in the nucleus accumbens, which translates salient environmental stimuli into action. The nucleus accumbens is a heterogeneous brain region made up of multiple anatomically and functionally distinct subregions, which receive and process inputs from many parts of the brain onto multiple cell types. How these circuits are organized and how information about reward is relayed through these circuits to drive motivated behaviour is still not well understood. Here we use a head-fixed Pavlovian reward conditioning task and multi-site *in vivo* fibre photometry to examine how reward-predicting cues are encoded by subregion specific circuits within the nucleus accumbens. We observe distinct patters of activity in the nucleus accumbens medial and lateral shell across the learning and expression of Pavlovian reward conditioning. Our results suggest that cues that drive motivated behaviour are differentially routed to networks of cells in distinct locations within the nucleus accumbens.

Disclosures: C. Baimel: None. **K. Manoocheri:** None. **A.G. Carter:** None.

Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH R01DA035943

Title: Distinct dopamine signaling in action sequence learning driven by reward predictive stimulus

Authors: *R. MAGNARD[1], Y. CHENG[1], J. ZHOU[1], L. CASTELL[1], P. H. JANAK[1,2], Y. VANDAELE[1];
[1]Psychological and Brain Sci., Johns Hopkins Univ., Baltimore, MD; [2]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Shall we consider habits as Stimulus-Response associations or as automated behavioral sequences executed without deliberation? To determine which of these two mechanisms best capture the concept of habit, we tested male and female WT rats in two instrumental procedures, the lever insertion fixed-ratio 5 (LI5) task (n=7 males; 8 females) and the lever retraction fixed-ratio 5 (LR5) task (n=7 males; 8 females). In these paradigm the lever could either serve as the cue that triggers the initiation of a chain of actions or as the cue that

signals its completion. The lever insertion (LI) cue could promote Stimulus-Response habit by serving as the stimulus that triggers the response. In contrast, the lever retraction (LR) cue could serve as response feedback predicting reward delivery and alleviating any requirement for behavioral monitoring. Following training in the LR5 or LI5 task, we then tested whether behavior was goal directed or habitual using satiety-induced devaluation. Finally, given the role of dopamine (DA) in the ventral tegmental area (VTA) in sequence learning, cue, and reward processing, we monitored DA neuron activity using calcium fiber photometry imaging after VTA infusion of Cre-dependent GCaMP6f virus in adult TH-Cre rats, performing the LR5 (n=5 males; 5 females) or LI5 (n=3 male; 5 females) task. We found substantial task differences with no effect of sex. The LR cue predicting immediate reward delivery in the LR5 task, was sufficient to promote automaticity, behavioral chunking and a relative lack of behavioral flexibility. In contrast, when trial onset was signaled by the LI cue in the LI5 task, behavior was more flexible and goal directed with an absence of behavioral chunking as rats kept seeking for the reward in between lever presses. We observed distinct changes in DA signaling consistent with task differences in behavior. In the LR5 task, we found a rapid shift in the activation of DA VTA neurons from reward retrieval to the earlier LR cue, followed by a decrease in cue-evoked DA neurons activity across repeated trials as skill learning elaborates. In contrast, in the LI5 task, cue- and reward-induced DA activation remained relatively constant across trials and sessions. These results show manifest task differences in DA signaling that contribute to better delineate the dopaminergic correlates of action sequence learning in response to reward predictive cues.

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Poster

312. Neural Basis of Reward I

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Title: Basal forebrain-hippocampus cholinergic signaling facilitates learning for behavioral adjustment in the absence of expected reward

Authors: *H. MUKOHIRA, S. ISHINO, T. KAMADA, M. OGAWA;
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Abstract: How efficiently animals can adjust their behavior to changing environments is critical for survival. In the face of multiple times of the absence of expected reward, animals need to

withhold their behavior to obtain the reward. Acetylcholine (ACh) neurons in the basal forebrain (BF) have been implicated in arousal, attention, learning, and memory. Recent studies have shown that ACh neurons in BF respond to reward and punishment in a phasic manner, and enhances the association between conditioned stimuli and those reinforcers through projection to basolateral amygdala (BLA) and cortex (Hangya et al., 2015; Crouse et al., 2020; Guo et al., 2019; Jiang et al., 2016). However, the role of ACh neurons in modulating the efficiency of learning to adjust behaviors in the face of reward omission remains unclear. We hypothesized that ACh neurons projecting to hippocampus (HPC), which is important for learning and memory, might be critical for the modulation. To address the potential role, we trained head-restrained rats to push forward a lever to trigger presentation of an auditory cue and then pull back the lever toward their mouth to obtain a probabilistic reward from the tip of the lever. One of three auditory cues was presented on each trial, associated with 100, 50, or 0% probability of reward. As rats learned the contingency, they tended to pull the lever slowly in response to cues predicting 0% reward. We recorded ACh transients in the HPC and BLA using an ACh fluorescent sensor $GRAB_{Ach}$ (Jing et al., 2020). We found that ACh levels in HPC increased in response to reward omission, whereas those in BLA decreased. ACh levels in HPC positively correlated with the amount of time rats kept pulling the lever in the face of reward omission. Further, the correlation was stronger in the earlier stage of learning. The higher the ACh levels in HPC in the early stage, the better the discrimination of cues predicting 100% reward and 0% reward. Next, we recorded activity of ACh neurons with single-cell level calcium imaging in behaving rats. Most of ACh neurons in the medial septum (MS), which mainly project to HPC, showed the similar activity patterns as observed in ACh levels in HPC. Finally, we examined the causality of the optogenetic stimulation of MS to HPC cholinergic pathway. The stimulation of cholinergic axon terminals in HPC at the time of the absence of reward facilitated the behavioral adjustment to repetitive reward omissions. These results demonstrate crucial roles of ACh neurons in the MS projecting to HPC in facilitating learning to adjust behavior in the absence of expected reward.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Program #/Poster #: 312.10

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Exploration of the functional role of recurrent connectivity between dopamine neurons in VTA with a recurrent neural network

Authors: *C. MASSOT;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The Ventral Tegmental Area (VTA) is one of the main regions of dopamine signaling in the brain. It has been established that the main function of dopamine neurons is to compute a reward prediction error (RPE), a variable involved in reward-based reinforcement learning. However, how RPE is generated within VTA neural circuit remains an open question. Here, to tackle this question, we trained a recurrent neural network (RNN) to reproduce the population averaged output of the VTA based on its main inputs. Using a supervised learning scheme, we aimed to reverse engineer the network in order to study the temporal activity of individual artificial units and their similarity with real dopamine neurons. We designed a single layer RNN with two inputs corresponding to the 'value' and the 'reward' signals. The 'value' signal represents the input generated by a reward predicting cue appearing at the beginning of the trial. The 'reward' signal represents the input due to the reward delivery towards the end of the trial. The target 'dopamine response' was represented by two temporally separated phasic activity: a first transient response matching the input 'value' signal and a second transient response matching the difference between the reward and the value inputs (i.e., the RPE). The network is trained to minimize the mean square error between the output produced by the network and the simulated dopamine response. By constraining the recurrent weights to be positive (to reproduce the excitatory nature of dopamine neurons), the trained network is stable and is consistently able to reproduce the input-output relationship of a simulated VTA. The activity of individual artificial units plotted against RPE reveal that their activation function is linear, with varying rates and display saturation for negative and high RPE. These results show that a neural network with a simple recurrent architecture can reproduce some of the main features of VTA dopamine neurons. Based on these modelling results, we looked at the physiology and connectivity of dopamine neurons in VTA. We derive hypotheses about the role of the axon-carrying dendrites and dendro-dendritic reciprocal connections to form recurrent connections within the population of dopamine neurons. Finally, we suggest future experiments to test the validity of these hypotheses and to better understand the formation of the reward prediction error signal within VTA before its broadcast to other brain regions.

Disclosures: C. Massot: None.

Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.11

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Entropy of Caudate Nucleus increasing during reward task

Authors: *D. YOON[1], B. JEONG[2];
[1]KAIST, KAIST, Yuseong- gu, Korea, Republic of; [2]KAIST, Daejeon, Korea, Republic of

Abstract: Depression is one of most increasing disease during the covid-19 era. One feature of depression is anhedonia, inability to experience reward, so there is lots of study about relation

reinforcement tasks with anhedonia. But research on comparing the response to the reward of both negative and positive directly is insufficient. 54 healthy subjects enrolled and we acquired functional MRI data during the subjects are doing the task. 2-armed bandit task was done. It consists of two types whether subjects get or lose the reward by selection and we called “reward task” and “loss task” respectively. Each type includes 40 trials, so total 80 trials took about 30 minutes. Subjects need to learn which arm is better to get the reward or avoid loss. After preprocessing, we acquire time series from cortex with Schaefer 100 Atlas(Schaefer et al., 2018) and from subcortex with CIT Atlas (Pauli et al., 2017). With the development of network neuroscience, there are lots of method to get the subnetwork between brain regions. We made multilayer functional connectivity matrices acquired from 13seconds of time window(Betzel et al., 2018) and used the GenLouvain algorithm(Lucas et al., 2011) to get communities. Then we calculate the entropy measure to calculate how various communities one node be assigned to. We compare the average entropy of reward task and loss task. There is no significant difference(Permutation test; P = 0.40). Then we cluster the nodes into the systems(Yeo et al., 2011). And compare the entropy in each system. Nodes from subcortex aren’t included in those system, so they are clustered into same one system. And we also separate each type of task into two part, because this 2-armed bandit task is so simple that subjects tend to choose just habitually after they have learned some extent. After 10 trials the correct rate rose sharply, so we devide the data into earlier part and later part by 10th trial and analyze separately. Only the subcortex system show little difference but not significant level(P = 0,05) in first 10th trials. Subcortical regions have own specific function, so we compare the entropy in each 11 subcortical structures. Only caudate nucleus during first 10 trials show the significant difference between reward and loss task(P=0.015). And entropy is higher during reward than loss task. Caudate efferents to various subcortical structures and receives input from cortex and has important function in reward system. Higher value of entropy in reward means caudate forms many connections during dealing with reward so it can be treated as hub region and this result can be utilized when we apply neuromodulation to treat the symptoms related to rewards like depression or addiction.

Disclosures: **D. Yoon:** None. **B. Jeong:** None.

Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: IRP/NIH/NIMH

Title: Doxycycline inducible expression and monitoring of small RNAs for CRISPRi and RNAi in the old-world monkey brain

Authors: L. SALHANI, *W. LERCHNER, A. LUZ-RICCA, B. LI, M. A. ELDRIDGE, B. J. RICHMOND;
Lab. of Neuropsychology, Natl. Inst. of Mental Health, NIH, Bethesda, MD

Abstract: Genetic reporters, like the chemogenetic receptor hM4Di_CFP, can be used to monitor expression of small RNAs in the monkey brain *in vivo* or *ex vivo* when co-expressed from the same lentiviral promoter. We previously showed a linear positive correlation between the expression of hM4Di_CFP and RNAi-mediated suppression of Choline Acetyltransferase (ChAT). We are now working on strategies to induce the expression of small RNAs via Doxycycline (Dox), while also being able to monitor this expression during on and off cycles of Dox administration. Two methods of interfering with endogenous protein expression are being tested: a) RNAi, in which single shRNAs are expressed via microRNA scaffolds in the 3' UTR of the reporter gene, and b) CRISPRi, in which multiple guide RNAs are expressed from the 3' UTR of the reporter gene, along with the essential dCas-KRAB transcription inhibitor expressed from an additional lentivirus construct to silence the endogenous ChAT promoter. These strategies allow us to place the reporter and small RNAs behind a Dox-inducible promoter and correlate expression cycles (Dox-on and Dox-off) with the effects of endogenous protein suppression via RNAi or CRISPRi. Using histology, we already confirmed a linear/gradual correlation between reporter expression and ChAT protein suppression when RNAi is used. CRISPRi experiments are ongoing, but we expect that induced guide RNAs against ChAT, as part of the CAS-KRAB complex, act more as an on-off switch for ChAT protein levels, rather than a linear suppression. Here, we present our progress with testing these constructs in cell culture and postmortem histology.

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Poster

312. Neural Basis of Reward I

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MEXT KAKENHI 20H05955 (to HA)

Title: Distinct patterns of dopamine responses to reward association in primate caudate nucleus and putamen as revealed by dLight signals

Authors: *G. YAN, H. AMITA, S. NONOMURA, K.-I. INOUE, M. TAKADA;
Ctr. for the Evolutionary Origins of Human Behavior, Kyoto Univ., Aichi, Japan

Abstract: The midbrain dopamine (DA) projections to the striatum play essential roles in reward association. In primates, DA neurons are more activated by unpredicted reward than by predicted reward, while they are suppressed by unpredicted no-reward (Schultz et al. 1997). Here, a major question arises as to whether DA neurons convey the same reward signal to different striatal regions. Some previous studies, in which the DA dynamics were measured in task-performing monkeys by using in vivo voltammetry, reported a certain difference in the response pattern between the caudate nucleus and putamen (Yoshimi et al. 2011, 2015; Schwerdt et al. 2019). However, this method suffers from physical fragility of probes, susceptibility to noise artefacts, and low chemical specificity. Therefore, a novel approach should be required to measure the DA signal. A newly-developed fluorescent dopamine sensor (dLight) exhibits a high affinity for DA and permits analyzing the DA dynamics with high spatiotemporal resolution (Patriarchi et al. 2018). In the present study, we applied the dLight1.1 to the monkey striatum for measuring DA signals in relation to reward association. The dLight was expressed in the striatum via viral vector injections thereinto, and DA signals were measured in response to two types of Pavlovian conditioning tasks. First, we tested the dLight signal responding to a reversal learning task. Either of two visual stimuli was followed by reward delivery. The stimulus-reward contingency was reversed across blocks. We found that DA signals detected in the putamen were enhanced by unpredicted reward but suppressed by unpredicted no-reward, while those in the caudate nucleus were not modulated by the outcomes. Second, we tested the dLight signal responding to a probabilistic reward task. In this task, three types of visual stimuli were followed by reward delivery with 0%, 50%, or 100% probability. We observed that DA signals in the putamen were accentuated by the unpredicted reward per se but dampened by the unpredicted no-reward in the 50% reward probability condition. However, DA signals in the caudate nucleus were more largely enhanced or suppressed by stimuli associated with the reward with 100% or 0% probability. These data suggest that the midbrain DA neurons transmit reward-related signals differentially to striatal regions which are involved in distinct patterns of reward information processing. As evidenced by the successful dLight recording from task-performing monkeys, our study provides a powerful tool for elucidating diverse functional roles of DA signals in the primate brain.

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Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01 MH126178

Title: G-protein coupled receptor activation based (GRAB) photometery reveals serotonin release during reward consumpition in the dorsal striatum

Authors: M. G. SPRING, K. M. NAUTIYAL;
Dartmouth Col., Hanover, NH

Abstract: Serotonergic signaling throughout the brain is involved in reward processing, threat detection, behavioral inhibition, and other facets of motivation and behavioral control. In the Dorsal Striatum (DS), pharmacological manipulation of the serotonin system disrupts behavioral control and the prospective encoding of rewards. However, the precise role of serotonin signaling in the DS during reward related behavior has been difficult to determine. This difficulty is due, in part, to a lack of available techniques suitable for measuring serotonin release on a timescale compatible with single reward trials. Recent advances in G-protein coupled Receptor Activation Based (GRAB) fluorescent proteins enable monitoring such dynamics in real time. Seeking to parse the involvement of serotonin in striatal reward processing, we monitored serotonin levels using GRAB-5-HT in the DS of adult mice (n=10; 6 male, 4 female) during reward presentations of evaporated milk using a Davis Lickometer. Following an initial training period to establish licking behavior, fluorescent activity was recorded through an optic fiber implanted in the DS at the site of viral expression of GRAB-5HT (AAV9-hSyn-5-HT3.0). Serotonin release was quantified by identifying serotonin transients (i.e. brief periods of increased activity) according to threshold criteria and measuring their alignment to behavior. Given access to 100% evaporated milk in discrete trials of 5 seconds, 83% of transients overlapped with licking behavior, with 96% of licks occurring within 2 seconds of the onset of the transient. During reward availability, lick bouts and transients were 48% overlapping. In two additional sessions, mice were provided alternating access, in discrete 10 second trials, to a low (20%) and a high (100%) concentration of milk and also access to six concentrations of milk (0-100%). Transient frequency (in events/second) was unaffected by concentration in either the 2 or 6 bottle preference paradigms, while transient magnitude, duration, and, to a lesser extent, overlap-with-licking tended to increase with milk concentration. The timing and association of serotonin signaling with licking behavior suggests that serotonin is involved in some aspect of anticipation, motivation, or approach. Ongoing studies are focused on disentangling these processes from value encoding and motor output. This work, characterizing real-time serotonin release, is an important first step in furthering our understanding of serotonin's involvement in reward-related processing and behavioral control.

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Poster

312. Neural Basis of Reward I

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Title: Neural excitations and inhibitions in the nucleus accumbens are differently related to sign-tracking and goal-tracking behavior

Authors: K. DUFFER, *S. E. MORRISON;
Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The ability of reward-associated cues to produce approach and/or interaction varies widely among individuals. For example, if a cue (e.g., extension of a lever) predicts a reward in a different location (e.g., a sugar pellet delivered to a food cup), some rats will preferentially approach and interact with the lever - a behavior known as sign tracking (ST) - and others will approach the site of reward delivery, a behavior known as goal tracking (GT). A propensity towards ST has been linked to susceptibility to drug-taking, relapse, and related behaviors. Both ST and addiction are highly dependent on dopaminergic transmission in the mesocorticolimbic circuit, and especially on dopamine release in the nucleus accumbens (NAc). Moreover, sign trackers and goal trackers show distinct patterns of NAc dopamine release during the acquisition of reward-seeking behavior, implying that ST and GT behavior may engage different circuits - one dopamine-dependent, and one not - for associative learning.
We previously showed (Gillis & Morrison, 2019) that cue-evoked excitations in the NAc encode the vigor of both ST and GT behavior. At the same time, among sign tracker individuals (but not goal tracker individuals), reward-related excitations showed a sharp decrease over the course of training, which may reflect the decreasing reward prediction error encoded by phasic dopamine release. However, a substantial subset of NAc neurons respond to reward-predictive cues with inhibitions that, similar to excitations, encode the vigor of approach during instrumental tasks (Morrison et al., 2017). We therefore analyzed the activity patterns of cue-inhibited neurons during the acquisition, maintenance, and extinction of ST and GT behavior. We found that both the proportion of inhibited cells and the magnitude of inhibitory responses increase over the course of learning in both sign tracker and goal trackers. Unlike excitations, reward responses, which are predominantly (but not exclusively) inhibitory, do not diverge between sign tracker and goal tracker individuals. Finally, during extinction, some cue-inhibited cells "extinguish" their response, but many do not, providing a possible neural substrate for the maintenance of cue-reward associations and recovery/relapse after extinction.

Disclosures: K. Duffer: None. **S.E. Morrison:** None.

Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 312.16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: VT College of Science

Title: Functional neuroanatomy of ChAT+ neurons in the dorsal parabrachial nucleus.

Authors: *W. TATERA, J. SISCO, K. MARSCHALKO, W. HOWE;
Virginia Tech., Blacksburg, VA

Abstract: The parabrachial nucleus stands at the interface of peripheral and central nervous systems, and is known to contribute to central modulation of a diverse array of functions including respiration, thermoregulation, gustation, and affect. This pontine structure is composed of a number of anatomically and molecularly distinct glutamatergic projection neurons. Among these is a population in the dorsal-lateral parabrachial nucleus (DLPB) that innervate both forebrain and midbrain structures, express choline acetyltransferase (ChAT) as well as produce and release acetylcholine during neurodevelopment. Given this pattern of connectivity, we reasoned that these neurons may control multiple aspects of appetitive behavior, though which, and through what specific circuits, is unclear. Here we employed transgenic mice in combination with viral circuit mapping and fiber photometry to explore and characterize the functional neuroanatomy of this population of "transiently" cholinergic cells. To examine anatomical connectivity, we unilaterally injected a CRE-dependent adeno-associated virus that generates a synaptophysin-fused mRuby protein into the DLPB of ChAT-CRE mice (n=6). In agreement with previous studies, we noted the highest density of pre-synaptic release sites in the central amygdala (CEN), but also notably observed considerable expression in the bed nucleus of the stria terminalis (BNST), and the interstitial nucleus of the posterior limb of the anterior commissure (IPAC). Within the CEN, we observed a medial to lateral gradient of synaptic inputs, with lateral regions being most heavily innervated. To gain insight into how the activity of these cells co-varies with appetitive behavior, next we labeled these cells with GCaMP7f, and used fiber photometry to monitor changes in their activation in freely moving mice foraging for food. Preliminary analysis (n=3) suggests a general inverse correlation between Ca^{+2} activity in DLPB ChAT cell bodies and locomotor output, though this was not specific to periods of food intake. Our ongoing experiments seek to parse out role of DLPB ChAT projections to terminal fields using pathway-specific DREADD manipulations, as well as direct measurements of Ca^{+2} activity in DLPB-ChAT terminals in the CEN, BNST, and IPAC. We will combine these with open-source behavioral segmentation pipelines (DeepLabCut/BSOID) to analyze how specific behavioral motifs and behavioral transitions are modulated by these DLPB circuits. Combined, these experiments will help characterize the unique and overlapping functions of DLPB circuits in appetitive behavior.

Disclosures: W. Tatera: None. **J. Sisco:** None. **K. Marschalko:** None. **W. Howe:** F. Consulting Fees (e.g., advisory boards); Takeda, Inc..

Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Program #/Poster #: 312.17

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant F30 DA051159
NIH Grant P50 DA046373
NIH Grant R01 DA012513
NIH Grant R25 DA033680

Title: Accumbens Ca^{2+} Dynamics Underlying Natural Reward Seeking Behavior

Authors: *R. M. CHALHOUB[1], C. CARTHY[2], J. L. HOPKINS[3], P. W. KALIVAS[3]; [2]Summer Undergraduate Res. Program, [3]Neurosci., [1]Med. Univ. of South Carolina, Charleston, SC

Abstract: The nucleus accumbens plays a central role in controlling reward seeking behavior across species. Its constituent D1R- and D2R- expressing medium spiny neurons (D1- and D2-MSNs) regulate motivated behaviors by integrating glutamatergic inputs from various cortical and subcortical areas, including dopaminergic inputs from the ventral tegmental area. However, how these projection neurons encode reward-specific information remains unknown. Here, we used optogenetics and single-cell resolution calcium imaging in a freely behaving mouse model (Drd1-cre or Drd2-cre) of cued sucrose seeking to analyze the physiological role of the D1- and D2-MSNs. Animals were trained to self-administer sucrose by pressing a nosepoke, associated with a complex cue, over 12 days; and reward seeking was tested after 10 days of abstinence. While time-locked optogenetic stimulation of D1- or D2-MSNs (n=4 per group) respectively potentiated or halted reward seeking activity, overall recorded calcium activity shows an increase in D2-MSNs activity, compared to D1-MSNs following reward seeking. At a single cell level, both D1- (n=5, ~200-250 cells) and D2- (n=4, ~180-200 cells) MSNs showed a highly heterogenous response around a seeking epoch (defined as 15 seconds surrounding nosepokes). Our preliminary analysis shows that 30-40% of D1- or D2-MSNs exhibit a stable divergent (equally distributed between excitatory and inhibitory ensembles) time-locked response associated with cued-seeking across trials. This activity profile is absent during early self-administration and develops over days of training. This data challenges the functional dichotomy of the direct/indirect pathways hypothesis and suggest a dynamic interaction between both cell types to regulate reward seeking.

Disclosures: R.M. Chalhoub: None. **C. Carthy:** None. **J.L. Hopkins:** None. **P.W. Kalivas:** None.

Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 312.18

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH NIDA R01 DA025634

Title: Post-ingestive feedback alters the dopamine response during sodium appetite

Authors: *P. BAZZINO, M. K. LOH, M. F. ROITMAN;
Psychology, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Sodium appetite is an innate, natural behavior where sodium deficit promotes vigorous seeking and consumption of sources of sodium at concentrations that would otherwise be avoided. Sodium appetite can be induced in the laboratory after by sodium depletion using diuretic treatment and 24hr maintenance on a sodium deficient diet. We have previously shown that intraoral infusion of hypertonic sodium chloride (NaCl) recruits dopamine release in the nucleus accumbens while rats are sodium depleted. However, it remains unknown whether dopamine responses decrease as the appetite is sated following sodium consumption. We used in vivo fiber photometry to measure real time dopamine transients. To do so, we injected a viral vector expressing the genetically encoded dopamine sensor GRABda in the nucleus accumbens lateral shell. We recorded activity while performing intra-oral infusions of either hyper- (0.45M) or hypotonic (0.1M) NaCl in sodium deplete rats for a total of 50 trials, where in each trial rats received 400 microliters over 10 seconds . Relative to pre-infusion baseline period, intra-oral infusions evoked a significant increase in dopamine that was initially similar in magnitude across the two concentrations of NaCl. However, regression analysis showed a significant interaction between sodium concentration and dopamine response to intra-oral infusion across trials such that dopamine responses to hypertonic NaCl decreased at a significantly faster rate relative to the hypotonic solution. Thus, dopamine responses to NaCl integrate post-ingestive feedback during sodium appetite. Ongoing work is aimed at identifying signals that provide negative feedback to tune dopamine signaling as homeostatic needs are met.

Disclosures: P. Bazzino: None. **M.K. Loh:** None. **M.F. Roitman:** None.

Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Program #/Poster #: 312.19

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Intramural Research Program at the National Eye Institute, 1ZIAEY000415

Title: Amygdala to basal ganglia circuit drives contextual learning and action

Authors: *K. MAEDA, O. HIKOSAKA;
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Abstract: From the standpoint of behavior, environmental context is everything. Animals must decide which actions are helpful and which are harmful given their current circumstances. For humans, behaving in a useful or appropriate manner demands an accurate reading of the social environment. This abstract form of social cognition is a common point of failure in mental disorders. What brain circuits mediate the regulation of behavior by environmental context? For decades our laboratory has studied how the basal ganglia implement the selection of good (e.g.

rewarding, valuable) objects and the avoidance of bad (e.g. harmful, useless) objects. More recently, we developed a task in which monkeys learned to either choose or avoid specific objects in a manner contingent on environments. We found that neurons in the amygdala encode environmental context and apparently govern choice-related neural activity via projections to the basal ganglia. In the current study, we used optogenetics combined with multichannel electrophysiological recording methods to probe the functional organization of the amygdala - basal ganglia circuit. Whereas dopamine (DA) neurons are known to respond to both primary rewards and secondary reinforcers with phasic spike bursts, we found that rewarding environments induce tonic firing rate changes in DA neurons. Crucially, these tonic changes in DA cells are mediated by disinhibition via GABAergic projections from substantia-nigra pars reticulata (SNr) neurons, which in turn are suppressed by inhibitory projections from the amygdala. Thus, the amygdala provides an additional source of learning signals to the basal ganglia circuit, namely contingencies imposed by the environment. We propose that the amygdala regulates two distinct circuits within the basal ganglia. First, the amygdala-SNr-superior colliculus circuit mediates moment-to-moment control of action in the form of eye movements guiding exploration and foraging behaviors. Second, the amygdala-SNr-DA circuit mediates learning of rewarding actions via plasticity induced by dopaminergic feedback projections.

Disclosures: K. Maeda: None. **O. Hikosaka:** None.

Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.20

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01DK103676

Title: Effects of glucose modulation in lateral hypothalamus on motivation for sucrose pellets in an operant task.

Authors: *J. STAMOS[1], K. STALNAKER[2], S. TEEGALA[3], V. H. ROUTH[4], K. D. BECK[1,4]; [1]VA New Jersey Hlth. Care Syst., East Orange, NJ; [2]Wellman Ctr. for Photomedicine at Massachusetts Gen. Hosp., Boston, MA; [3]Pharmacology, Physiol. and Neurosci., Rutgers, The State Univ. of New Jersey, Newark, NJ; [4]Pharmacol, Physiol & Neurosci, New Jersey Med. Sch. Dept. of Neurol. & Neurosci., Newark, NJ

Abstract: Orexin neurons in the Lateral Hypothalamus (LH) play an important role in in the control of feeding behavior. Approximately 60 percent of these neurons are known to increase their activity in response to decreases in extracellular glucose levels. Previously we showed that elevated LH extracellular glucose decreased conditioned place preference for food. In this study we build upon these results by showing how modulation of LH extracellular glucose effects

motivation to work for a food reward. Here we used reverse microdialysis to modulate extracellular glucose levels in LH during an operant task for sucrose pellets. In a progressive ratio paradigm, it was shown that a perfusion of 4mM glucose significantly decreased consumption of sucrose pellets. Behavioral economics modeling showed that 4mM glucose perfusion increased behavioral elasticity but not consumption at zero price, indicating that LH glucose levels modulate motivation to work for food and not hunger per se. In a third experiment we demonstrated that increased motivation for sucrose pellets brought on by low LH glucose persisted after LH glucose levels had been raised to a satiety level. Taken together these experiments indicate that LH glucose sensing neurons play an important role in motivation to initiate feeding. However, once consumption has begun it is likely that feeding is controlled by different circuitry.

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Poster

312. Neural Basis of Reward I

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Title: A dopamine circuit for active switching toward the pursuit of future reward

Authors: *S. ISHINO[1], T. KAMADA[1], G. A. SARPONG[2], J. KITANO[1], R. TSUKASA[1], H. MUKOHIRA[1], F. SUN[3], Y. LI[4], K. KOBAYASHI[5], N. HONDA[6], N. OISHI[7], M. OGAWA[1]; [1]Kyoto Univ., Kyoto Univ., Kyoto, Japan; [2]Okinawa Inst. of Sci. and Technol., Okinawa, Japan; [3]UCLA, Los Angeles, CA; [4]Peking Univ., Beijing, China; [5]Natl. Inst. For Physiological Sci., Aichi, Japan; [6]Hiroshima Univ., Hiroshima, Japan; [7]Kyoto Univ. Grad. Sch. of Med., Kyoto, Japan

Abstract: Midbrain dopamine neurons are thought to signal reward prediction errors (RPEs). RPE-type dopamine neurons are thought to be critical for learning based on value. The signal induces negative learning when expected reward is unavailable. However, to obtain probabilistic reward, even after it is unavailable, one must actively switch to pursuing the next opportunity. Dopamine neurons that may serve to this switching remains unclear. To examine a potential role

of dopamine neurons for the switching, here we developed an operant cue-reward association task for rats. Head-restrained rats were required to continue to pursue a probabilistic reward by repeating a specific sequence of actions: pushing forward a spout-lever to initiate a trial and pulling back the lever toward their mouth in order to potentially receive a water reward dispensed from the tip of the lever. By monitoring the position of the lever after no-reward, we were able to precisely observe active behavioural switching from the previous reward seeking to the next reward seeking. We combined the task with recording of spiking activity of dopamine neurons in the ventral tegmental area (VTA), dopamine measurement in the nucleus accumbens (NAc), and circuit-specific optogenetics. We found that some dopamine neurons in lateral VTA exhibited increased responses to unexpected no-reward and decreased responses to unexpected reward, which was also reflected in the dopamine level in a region of the NAc. The responses were opposite to and slower than those of the RPE-type dopamine signals. The higher the responses of some dopamine neurons and dopamine levels in the NAc to no-reward, the faster the behavioral switching toward seeking the next reward. During a reward extinction process and transition to a Pavlovian task, this response was initially apparent but weakened as learning to cope with no-reward progressed. Optogenetic activation of the dopamine inputs to the NAc after no-reward supported the next reward seeking as long as the reward can be expected in a reward extinction task. We propose that the dopamine circuitry signals errors in processing of no-reward, providing a mechanism for active switching toward future reward.

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Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: P50 MH096889
T32 DA05055802
F30 MH126615

Title: The paraventricular nucleus of the thalamus contributes to early-life adversity-induced disruptions in reward-related behaviors

Authors: *C. L. KOOIKER, M. T. BIRNIE, Y. CHEN, Q. DING, T. Z. BARAM;
Univ. of California, Irvine, Univ. of California, Irvine, Irvine, CA

Abstract: Background: Early-life adversity (ELA) is associated with poor cognitive and emotional health and increased risk for a variety of affective disorders, such as major depressive disorder and substance use disorders. Many of these disorders are characterized by impairments

in reward-related behaviors, and we find that these same deficits are provoked by rodent models of ELA. However, the brain regions and processes underlying these long-term consequences of ELA remain largely unknown. The paraventricular nucleus of the thalamus (PVT) is an important component of the reward circuit that encodes remote emotionally salient experiences to influence future motivated behaviors. We hypothesize that the PVT encodes adverse experiences as remote as the early postnatal period in mice, and that ELA-engaged PVT neurons subsequently contribute to deficits in reward-related behaviors in adults. **Methods**: We employ TRAP2 mice, which we exposed to a week of simulated ELA in a limited-resource cage between postnatal days 2-9. We induce the TRAP2 system using tamoxifen on P6, triggering Cre-dependent recombination in neurons activated during P6-P8. This leads to permanent tagging of neurons activated during this epoch. We validated our findings using routine cFos immunohistochemistry in WT mice. We then chemogenetically inhibit these ELA-engaged neurons during an adult reward-seeking task with the goal of ameliorating ELA-induced changes in reward-seeking behavior. **Results**: ELA robustly and selectively activates PVT neurons to a degree much higher than typical rearing conditions, and a large proportion of these ELA-engaged PVT neurons express CRFR1. Silencing ELA-engaged PVT neurons during reward-related tasks in adult female mice ameliorates the observed ELA-induced changes in reward-seeking behaviors. **Conclusions**: The PVT is robustly and almost uniquely activated in response to emotionally salient events in neonatal mice, and inhibition of these ELA-engaged neurons ameliorates ELA-induced changes in reward-seeking. The PVT is thus poised as a potential contributor to deficits in reward-related behaviors following ELA.

Disclosures: C.L. Kooiker: None. **M.T. Birnie:** None. **Y. Chen:** None. **Q. Ding:** None. **T.Z. Baram:** None.

Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.23

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Brain stimulation reward supports high discriminability and stable responding in delay discounting tasks

Authors: *R. M. DONKA[1], M. K. LOH[2], M. F. ROITMAN[2], J. D. ROITMAN[2];
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Abstract: Delay discounting tasks measure reward processing and impulsivity, determining the depreciation of an individual's perceived reward value based on delay to delivery. To assess temporal discounting, subjects choose between a large but delayed and a small immediate reward. For non-human animals, high value food rewards are typical. However, previous research has demonstrated that in tasks utilizing food reward, subject motivation declines across the session as satiety is presumably develops. Additionally, subjects must be food deprived to

ensure motivation, potentially unintendedly altering choice. We sought to remedy these challenges by utilizing brain stimulation reward (BSR) in a delay discounting paradigm. Rats (n = 10) were implanted with stimulating electrodes in the medial forebrain bundle at the level of the lateral hypothalamus and were trained to lever press for stimulation. Using a rate-frequency protocol, we determined the threshold (theta) and maximal (alpha) stimulation frequencies. We conducted magnitude discrimination training, pseudo-randomly assigning small and large rewards to right and left levers. Individual small and large reward values were set to 50% and 95% of alpha respectively. Each reward delivery consisted of five 500 ms trains of stimulation. Magnitude discrimination training with both delays set at 1 s resulted in a high preference for the large lever. We then tested a modified delay discounting task with 6 blocks of increasing delay for the large reward (1, 3, 5, 7, 9, 11 s). Each block contained 10 forced and 10 free choice trials. Subjects displayed a declining preference for the large reward when delay was greater than 3 s. To determine if subjects were flexibly adapting decision-making strategies based on recent information, we conducted the same structured task but with all large delays set to 1 s, equal to the small delay. Subjects initially persisted in decreasing large lever choices across blocks, but by the third session were nearly exclusively choosing the large reward. We then altered the task to 5 blocks with an increasing large delay schedule (1, 2, 4, 8, 12 s). This schedule resulted in a declining preference for the large reward when delays were 4 s or greater. Finally, we randomized block order. Block one always consisted of a 1 s delay on the large lever. When block order was randomized, a significant preference for the large lever was demonstrated in block one, but the small lever was preferred in all subsequent blocks. Overall, these results support the use of BSR in delay discounting tasks. BSR avoids issues with food rewarding while supporting a high level of discrimination and motivating behavior.

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Poster

312. Neural Basis of Reward I

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Support: NSERC Discovery grant (M.L., V.B.-P.)
NARSAD Young Investigator Award (V.B.-P.)
FRQS Jr 1 (V.B.-P.)
FRQS Senior (M.L.)

Title: Regulation of learning-related dopaminergic signal along the mesocorticolimbic pathway

Authors: ***S.-J. BOUCHARD**[1], L.-M. GAUTHIER[2], J. OBERGASTEIGER[1], Y. ZHUO[3], C. PROULX[1], M. VOLTA[4], Y. LI[3], M. LEVESQUE[1], V. BRETON-PROVENCHER[1];

[1]Psychiatry and Neurosciences, Univ. Laval, Québec, QC, Canada; [2]Univ. de Montréal, Québec, QC, Canada; [3]Sch. of Life Sci., Peking Univ., Beijing, China; [4]Eurac Res., Bolzano, Italy

Abstract: The dopamine system carries critical learning signals through the mesocorticolimbic pathway, in which dopaminergic neurons from the ventral tegmental area send dopamine to the ventral striatum and the cortex. How homogeneous dopamine release along the mesocorticolimbic pathway is during reinforcement learning remains unknown. Here, we measured dopamine signals in two critical areas of this pathway: the nucleus accumbens (NAc), known for its role in reward processing, and the medial prefrontal cortex (mPFC), known for its role in associative learning. To compare fast kinetics of dopamine release, fiber optics were simultaneously implanted above the NAc and mPFC to measure fluorescence of a novel and improved dopamine sensor (GRAB-DA3) with multi-site fiber photometry. Head-fixed mice were conditioned over several sessions to obtain a reward following an auditory tone of a fixed frequency. Following these initial sessions, we varied the task contingencies to measure the dopamine reinforcement signal to reward prediction error, reward magnitude and reinforcement stimuli of variable valence. Our preliminary data show that dopamine linked to reward prediction error and reward magnitude is signaled homogeneously throughout the mesocorticolimbic pathway. However, negative reinforcement produces relatively larger dopamine release in mPFC compared to NAc. To understand the origin of these heterogeneous signals, we are carrying anatomical tracing studies to measure how many neurons from the ventral tegmental area have mPFC-only projections. Since recent evidence shows a discrepancy between somatic activity of dopaminergic neurons and the release of dopamine in target regions, our ongoing experiments are also testing cellular mechanisms that could locally regulate dopamine release. One important candidate could be the protein Rin, coded by the Rit2 gene, which we found to interact with the dopamine transporter DAT and could play a role in dopamine release. Together, our results indicate that dopamine release signals aversive stimuli primarily in mPFC, yet dopamine release linked to reward is a prevalent feature of the mesocorticolimbic pathway.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Program #/Poster #: 312.25

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DP2 MH119425-01
RF1 MH117055-01

Title: Gabaergic synaptic control of dopamine as an expertise signal in error-based learning

Authors: *S. C. BURWELL[1], H. YAN[1], S. S. X. LIM[2], B. C. SHIELDS[2], M. R. TADROSS[2]; [1]Dept. of Neurobio., [2]Dept. of Biomed. Engin., Duke Univ., Durham, NC

Abstract: Ventral tegmental area dopamine (VTA_{DA}) neurons fire in a manner consistent with Reward Prediction Error, with unexpected successes and failures correlating with bursts and pauses, respectively. With regard to causal experiments, it has been particularly difficult to interrogate the necessity of VTA_{DA} pauses for behavior, as these events are synaptically mediated and occur in a sparse subset of VTA_{DA} neurons. Here, we explore this question by manipulating inhibitory $GABA_A$ receptors on VTA_{DA} neurons with DART (drugs acutely restricted by tethering). We studied mice performing a multiplexed cue-reward classical conditioning task designed to assess positive-valence learning (conditioning) and negative-valence learning (extinction) simultaneously, within-mouse. Our key finding is that blocking $GABA_A$ receptors on VTA_{DA} neurons (i.e., disinhibiting VTA_{DA} cells) accelerates extinction learning in response to unexpected reward omission. This confirms that inhibitory afferents onto VTA_{DA} cells regulate negative-valence learning, albeit in a manner opposite to the canonical hypothesis. In the same mice, the manipulation had no impact on conditioning to a novel cue-reward pairing, indicating that positive-valence learning was unperturbed. *In vivo* fiber photometry and single unit recordings are underway to examine how blocking $GABA_A$ receptors on VTA_{DA} neurons impacts their activity dynamics. While preliminary, we propose a model in which GABA inputs to VTA_{DA} neurons convey expertise, encoding a strong prior achieved through extensive cue-reward association. Blocking $GABA_A$ receptors on VTA_{DA} neurons thus weakens the prior, allowing outlier events (unexpected failures) to drive rapid negative-valence learning. This work provides critical insight into the circuitry underlying adaptive behaviors.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 312.26

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Uniformed Services University

Title: Activation of thalamic inputs to the dorsomedial striatum drives place preference and locomotor activity

Authors: C. BOUSLOG[1], M. HOCHSTEIN[3], D. CHANG[3], *K. A. JOHNSON[2]; [2]Dept. of Pharmacol., [1]Uniformed Services Univ., Bethesda, MD; [3]Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Glutamatergic thalamic inputs to the striatum produce dopamine release via local actions on dopaminergic terminals. There is increasing evidence that dopamine release evoked

by somatic firing of substantia nigra pars compacta dopamine neurons vs. terminal regulation mechanisms produces unique contributions to behavior. Our previous studies demonstrated that optogenetic stimulation of thalamic terminals in the dorsomedial striatum is sufficient to support operant responding, suggesting that thalamostriatal activity can drive reinforcement learning. However, the ability of thalamostriatal activity to mediate reward and influence other striatum-associated behaviors remains unclear. We used optogenetic stimulation of thalamic terminals in the dorsomedial striatum to assess the potential of thalamostriatal pathway activation to drive reward. We virally expressed channelrhodopsin-2 (ChR2) in the anterior intralaminar thalamus of adult male and female C57BL/6J mice and implanted optic fibers targeting the dorsomedial striatum. In a real time place-preference experiment, mice spent more time in the chamber paired with optogenetic stimulation of thalamostriatal terminals, but did not exhibit conditioned place preference when the stimulation was discontinued. In contrast, mice expressing a control fluorophore (YFP) did not exhibit real-time place preference for light stimulation. We also assessed whether thalamostriatal activation can impact locomotor activity in an open field. Compared with mice expressing YFP, optogenetic stimulation in ChR2-expressing mice enhanced locomotor activity by increasing both velocity and distance traveled. Effects on locomotion were rapidly modulated during repeated light-on versus light-off epochs in a single open field session. Thalamostriatal activation selectively enhanced horizontal locomotion, as we did not observe changes in the performance of other natural behaviors (i.e., rearing and grooming). Together with our previous findings, these results suggest that activation of thalamostriatal neurons can support both reward and reinforcement learning, while also promoting locomotion. Future studies will investigate the potential roles of this circuit in reward and psychomotor stimulation produced by psychoactive drugs.

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Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.27

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant P50 MH096889

Title: Sex dependent influence of early life adversity on reward circuits

Authors: *L. TANIGUCHI[1], M. BIRNIE[2], Y. CHEN[2], T. BARAM[3];
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Abstract: Rationale: Early life adversity (ELA), the exposure to negative experiences during childhood, can have profound impacts on cognitive and emotional processing into adulthood. These outcomes are linked to increased risk for many psychiatric disorders, including depression,

anxiety, and PTSD. The neural basis of these disorders is thought to be caused by disruptions to reward circuitry, but it is not known how ELA directly impacts the development of these circuits. A novel stress neuropeptide corticotropin-releasing hormone (CRH) expressing GABA-ergic projection connecting the basolateral amygdala (BLA) to the nucleus accumbens (NAc) was found to be responsible for reduced reward-seeking behavior in male mice that experienced ELA. The role of this projection in female mice is unknown. It is of particular interest to investigate, given that ELA has opposite effects on rodent male and female reward-seeking behaviors, where in females, reward behaviors are increased. Here we investigate whether the CRH+ GABA-ergic BLA-NAc pathway is also involved in mediating the effects of ELA on reward circuits in female mice. We hypothesize that this pathway is functionally the same and inhibitory, and the hypoactivation of the pathway caused by ELA leads to the increase in reward-seeking behavior. Methods: We used chemogenetic approaches to test the hypothesis that predicts that exciting the projection will overcome the hypoactivation and normalize reward-seeking behaviors in ELA female mice. CRH-IRES-Cre mice raised in either control conditions or ELA conditions were injected bilaterally into the BLA with an excitatory Cre-dependent DREADD, followed by delivery of clozapine N-oxide (CNO) or vehicle, through intraperitoneal injection (IP) or direct infusion into the medial shell NAc. The function of the pathway was tested using reward-related behavioral tasks. Results: Excitation of the cells of origin of the pathway through IP injection of CNO did not yield a significant difference in reward behavior between ELA and control mice. Studies using a direct infusion of CNO into the medial shell NAc to pinpoint the BLA-NAc projection are ongoing. The hypothesis also predicts that inhibiting the pathway in control female mice will generate the ELA phenotype. Future ongoing studies will utilize inhibitory DREADDs to inhibit the pathway in female control mice and recapitulate the increased reward-seeking in ELA mice. Conclusions: A novel pathway mediating the effects of ELA on reward circuits is characterized in males, and we are striving to determine if the neuroanatomy and cell-type specificity of the pathway is the same in females.

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Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 312.28

Topic: G.02. Reward and Appetitive Learning and Memory

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Marie Sklodowska-Curie 844492 MM
EMBO-ALTF-233-2020 AS

Title: Dynamic value representations in amygdala

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Abstract: The amygdala has been predominantly studied in the context of learning and memory. In classical tasks rodents learn the association between an initially neutral stimulus and an unconditioned appetitive or aversive stimulus. While the neuronal activity patterns during learning have been thoroughly characterized, the nature and content of the unconditioned stimulus information in the amygdala remains largely unexplored. Here, we develop a framework to derive a real-time behavioral read out of the perceived value of the appetitive unconditioned stimulus in head-fixed mice and corroborate these findings with a novel foraging task in which animals can choose between two rewards.
We concurrently perform longitudinal Ca^{2+}-imaging during these behaviors using 2-photon microscopy and miniscope imaging in head-restraint and freely moving animals, respectively. We find that amygdala neurons scale their activity in response to appetitive stimuli perceived as more valuable, consistent with the idea that these neurons encode the abstract variable hedonic value. We test this hypothesis explicitly by tracking neurons across different metabolic and anxiety states, as well as reward contexts and find that the activity of BLA neurons indeed follow the perceived value of stimuli.
Our results provide important insights into the principles of the unconditioned stimulus representations in amygdala neuronal populations, which have traditionally been studied mainly in the context of learning.

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Poster

312. Neural Basis of Reward I

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NIH T32DA024635
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A.P. Giannini Fellowship

Title: Opposing amygdala-striatal pathways enable chronic stress to hasten habit formation

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Abstract: When making decisions, we prospectively evaluate all of our potential actions and their consequences before executing a behavior. This allows us to adapt when situations change but it is also cognitively taxing. We also use habits - a more resource-efficient but inflexible strategy in which behaviors are executed without thought of their consequences based on past success. Balance between goal-directed and habitual behavioral control strategies permits adaptive and efficient behavior. However, overreliance on habits is an endophenotype of many psychiatric conditions. Stress is a major contributing factor to these conditions and can tip the balance of behavioral control towards habit. The neural mechanisms that allow this to happen are unclear. Thus, the goal of this project is to uncover the brain processes by which stress hastens habit formation. We focused on amygdala-striatal projections because the dorsomedial striatum (DMS) is critical for controlling goal-directed learning and the amygdala is a known stress hub also implicated in behavioral control. While direct excitatory projections from the basolateral amygdala (BLA) to the DMS have long been known to exist, recent evidence has revealed the central amygdala (CeA) also directly projects to the dorsal striatum. We identified that these inhibitory CeA projections primarily target the DMS. To examine whether and how BLA-DMS and CeA-DMS projections mediate the influence of stress on habit formation, we coupled a task to model chronic stress-induced acceleration of habit learning in mice with pathway-specific fiber photometry calcium imaging or chemogenetic manipulations. We found that BLA-DMS and CeA-DMS have opposing activity patterns during instrumental learning that are oppositely modulated by chronic stress. We also found that these amygdala-striatal projections differentially regulate habit learning. CeA-DMS activity opposes goal-directed learning and permits stress to promote habits. Conversely, BLA-DMS activity opposes the influence of stress on habits and is sufficient to restore normal goal-directed behavioral control. In addition to establishing a function for the newly-discovered CeA-DMS pathway, these data indicate that amygdala-striatal projections have opposing function in goal-directed and habit learning and are differentially regulated by stress to promote habit. This has important implications for conditions influenced by stress and characterized by maladaptive habits. This work was funded by NIH R01DA046679 (KW), NIH T32DA024635 (JG), NIH F32DA056201 (JG), A.P. Giannini Fellowship (JG), and NIH TL4GM118977 (NP).

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Poster

312. Neural Basis of Reward I

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Program #/Poster #: 312.30

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant R01DA053208

Title: Investigating ventral pallidal encoding of expected outcome value

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Abstract: Goal-directed behavior relies on accurate mental representations of outcomes and their expected value. Disruptions in goal-directed decision-making are a core component of several neuropsychiatric conditions, including addiction. An understanding of the neural mechanisms underlying expected value encoding within the brain reward centers is key for developing effective long-term treatments for addiction. The Ventral Pallidum (VP) is a basal forebrain region that is important for motivation and reward processing. Cue-evoked activity in some VP neurons appears to encode the expected value of rewards. Yet, the impact of outcome (reward) devaluation on these neural responses to cues has not been previously assessed. Outcome devaluation, in which the value of the expected outcome is diminished, is a classic test of goal-directed behavior that can be used to test whether these neural responses to cues reflect expected value, or merely other factors that correlate with expected value. In this study, we used *in-vivo* electrophysiology to record from single units in the VP of adult rats responding to Pavlovian reward cues before and after reward devaluation. Adult rats (n= 5, 3 males, 2 females) were trained with an auditory conditioned stimulus (CS+) that signaled the delivery of a 10% sucrose reward and a control cue (CS-) that never predicted reward. They then underwent reward devaluation via sensory specific satiety where they were allowed free access to either sucrose (thereby altering its expected value) or a control substance (maltodextrin). We recorded from the VP of these rats during training and reward devaluation sessions. As expected, we found that many VP neurons (65%) were excited by the CS+, and that these neurons were more responsive to the CS+ than the CS-. Additionally, VP responses to the CS+ were reduced after free consumption of sucrose. Interestingly, free access to maltodextrin also reduced VP responses to the CS+, suggesting that these changes in VP responding were not due to the encoding of the sucrose reward specifically. Future experiments will aim to investigate whether VP neurons respond more selectively to other forms of devaluation. Overall, this study will enhance our understanding of how VP neurons encode changes in the expected value or rewarding outcomes.

Disclosures: A. Sood: None. **J.M. Richard:** None.

Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Program #/Poster #: 312.31

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01DA031695

Title: Optogenetic inhibition of the anterior cingulate cortex impairs task engagement during complex reward-guided decision-making.

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Abstract: In previous studies, we have found that disruption of anterior cingulate cortex (ACC) signaling—both via chemical lesions and after cocaine self-administration—results in decision-making impairments arising from attenuations in attention to the task. However, optogenetic inhibition affords us the ability to conduct within-subject modulation of ACC activity in a temporally precise manner, allowing us to pinpoint how ACC contributes to task performance within and across sessions, and avoid compensatory mechanisms that might be engaged following lesions or cocaine self-administration. For this experiment, we trained rats on a reward-guided decision-making task consisting of four sixty-trial blocks; reward value was manipulated by independently varying the delay to (Blocks 1 and 2) or size of reward (Blocks 3 and 4). Subsequently, we bilaterally injected halorhodopsin into the rat ACC. Every two days, we alternated between using a blue LED (control) or yellow LED to optogenetically inhibit ACC as they performed the previously learned reward-guided decision-making task. LED delivery during the task occurred on 50% of trials, from trial initiation to completion of the response. During inhibition sessions, we found that rats initiated significantly fewer trials, and completed a significantly lower number of sessions—reflecting reduced task engagement. When rats did complete full sessions, free-choice performance was degraded, but forced-choice performance and reaction times were unaffected relative to control days. Finally, we performed a drift-diffusion analysis on the behavioral data; during inhibition, rats accumulated information more slowly (e.g. lower drift rates). Notably, similar results were obtained when we ran the model using our previously obtained lesion data; however, rats with lesions also had higher decision boundaries. Together, these findings support our previous findings that the ACC plays an important role in attention and task engagement that is necessary for optimal decision-making.

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Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: R00DK115895

Title: Nucleus accumbens MSNs activity regulates hedonic feeding

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Abstract: Our research investigating the neural circuitry implicated in hedonic feeding emerges as a response to the ongoing obesity epidemic seen across our nation. Increasing rates of obesity can be partly linked to the heightened availability of calorically-dense, palatable foods that are consumed in the absence of metabolic need. Persistent intake of high fat food has been shown to alter brain reward circuitry, ultimately leading to difficulties in resisting overeating impulses. With this in mind, our study focuses on examining how alterations in the activity of medium spiny neuron (MSN) subtypes in the nucleus accumbens (NAc), a prominent hub of brain reward circuitry, can result in altered high fat intake. Here, we used chemogenetic manipulations of D1 or D2 MSNs in the NAc with the goal of identifying cell-type specific mechanisms regulating hedonic feeding. To investigate the necessity of D1 and D2 MSN activity in high fat intake, we injected an inhibitory DREADD (hM4DGi) or eYFP control AAV bilaterally into the NAc of adult male and female D1-Cre or A2a-Cre mice. Access to high fat was limited to 20 minutes daily for 5 consecutive days, and mice were administered (i.p.) the chemogenetic actuator deschloroclozapine (DCZ) prior to high fat exposure on days 2 through 4. Intake was measured in terms of calories per gram and kilocalories. All experiments were conducted in a blinded manner and replicated to ensure scientific rigor. We find that inhibition of MSN subtypes had opposing effects on high fat intake, consistent with the numerous studies examining how modulation of MSN activity regulates reward-related behavior. Interestingly, we also found a significant basal difference in intake between male and female mice and while inhibition of D1 MSNs similarly impacted intake in both sexes, the effect size was greater in female mice. When DCZ dosage was increased, these trends persisted. Additionally, when utilizing a conditioned place preference assay, we found that inhibition of D1 MSNs altered the preference for the high fat-associated chamber during testing. Future examination of bidirectional modification of D1 and D2 MSNs in the NAc will be crucial for our understanding of how manipulations yield differential effects on high fat intake during acquisition and at subsequent timepoints. Further investigation into basal sex-specific differences of high fat intake behavior should also be prioritized to gain insight into how hedonic feeding is uniquely regulated between sexes.

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Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

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Title: The effects of junk-food diet and subsequent junk-food deprivation on food motivated behaviors

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Abstract: Over-eating and craving drive weight gain and obesity in humans. Activity of the Nucleus Accumbens (NAc) plays a role in feeding behavior, and NAc function is altered by obesogenic diets. For example, blocking calcium-permeable AMPA receptors (CP-AMPARs) in the NAc attenuates enhanced cue-triggered food-seeking in obesity-prone males. Furthermore, after eating a sugary, fatty junk-food diet, NAc CP-AMPAR expression and transmission is increased in obesity-prone, but not obesity-resistant males. Interestingly, increases in NAc CP-AMPAR synaptic transmission require the removal of junk-food and a return to chow (junk-food deprivation) for at least 24 hrs. While we have established that eating junk-food produces distinct effects on NAc glutamate plasticity compared to junk-food deprivation, how this may differentially affect food motivated behaviors remains unclear. Therefore, here we examined 1) the willingness to work for a food cue in a conditioned reinforcement test, 2) motivation for food using progressive ratio testing, and 3) free food consumption with and without food restriction in male obesity-prone rats with a history of junk-food exposure. Rats (n=39) first underwent Pavlovian conditioning in which one cue was always followed by delivery of a banana flavored food pellet, and another was never followed by food. Next, rats were split into three diet groups: chow, junk-food, and junk-food deprivation (10 days followed by return to chow). Following one day of junk-food deprivation, rats underwent a conditioned reinforcement test in which responses in the active nose poke resulted in presentation of the auditory food cue. While we did not find any overt group differences in the willingness to work for presentations of the food cue alone, assessment of relationships between responding for the cue and approach to the food cup during this test are ongoing. Rats from these groups were then trained to lever press for the banana pellets and motivation for food was determined using a progressive ratio task. Break point was reduced in the junk-food group compared to the rats in either the chow or junk-food deprivation groups. Similarly, when allowed to consume pellets freely during a 30 min test, the junk-food group consumed less than the other groups. Thus, motivation for the food pellets appears blunted in the rats still on junk-food. Furthermore, food restriction (4.5 hr) increased pellet intake in both the chow and junk-food deprivation groups, but did not alter food intake in rats on junk-food. These data challenge the idea that eating sugary fatty foods produces compulsive food motivation, while also suggesting that there may be alterations in hunger signals.

Disclosures: T.L. Fetterly: None. **C.R. Ferrario:** None.

Poster

312. Neural Basis of Reward I

Location: SDCC Halls B-H

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: This work was supported by the DICBR of the NIAAA

Title: Elucidating the role of medial septum glutamate neurons in reward seeking behaviors

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Abstract: Reward seeking is fundamental in the onset and maintenance of substance abuse disorders. The medial septum (MS) is classically known as the first region discovered by Olds and colleagues to support electrical intracranial self-stimulation in the rat. Further studies by Heath in the 1970s showed humans will similarly press a button to earn intra-septal electrical stimulation. We have recently shown that mice will lever press to earn optogenetic stimulation of the MS, and in particular MS glutamate (GLU) neurons. In addition, we found that MS^{GLU} neurons in turn project to the VTA to influence DA release in the ventral striatum (Kesner et al., 2021). Further characterization of this novel reward system implicats it in information seeking motivation. Little else is known about how these MS^{GLU} neurons influence motivated behaviors and the current study aims to follow up on these previous findings by using fiber photometric and chemogenetic techniques to examine the role of MS^{GLU} neurons in reward-seeking. We expressed GCaMP7f in MS^{GLU} neurons and recorded this population's activity while mice preformed several operant procedures of increasing complexity. We found that overall MS^{GLU} activity increased while the animals engaged in appetitive and exploratory activity (sniffing and rearing), and markedly decreased during reward consumption. In a complementary set of experiments, we used chemogenetic techniques to modulate MS^{GLU} activity and probe the role of these neurons in exploratory and goal-directed behavior. These studies indicate that chemogenetic excitation of MS^{GLU} neurons tended to increase breakpoint in progressive ratio tests and facilitated learning during lever reversal task. Whereas chemogenetic inhibition of MS^{GLU} neurons reduced rewards earned in a basic fixed-ratio 1 task. These findings are an important step in understanding the role of this understudied population of neurons in an understudied brain region related to reward and motivational processes, and could lead to novel therapeutic interventions for treating psychiatric disorders related to maladaptation in motivated behaviors.

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Poster

312. Neural Basis of Reward I

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: MH108653

Title: Postnatal PCP-induced deficits in learning and cognitive flexibility are attenuated by optogenetic inhibition of ventromedial orbitofrontal cortical glutamate neurons

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Abstract: Introduction: The orbitofrontal cortex (OFC) is essential for decision-making and cognitive flexibility. It updates representations of value and guides decisions to optimize reward payoff. Postnatal phencyclidine (PCP) administration in rats is a neurodevelopmental manipulation that disrupts cognitive function in adulthood similar to those observed in schizophrenia patients. Our aim was to determine if postnatal PCP-treatment resulted in decision-making impairments that could be ameliorated by manipulating the activity of OFC glutamate neurons. **Methods**: PCP (20 mg/kg, s.c.) was administered to male and female Wistar rat pups (n=90) on postnatal days (PND) 7, 9, and 11. When rats reached adulthood (>PND 60) AAV-CaMKIIa-ChR2-eYFP, AAV-CaMKIIa-eNpHR3.0-eYFP, or AAV-CaMKIIa-eYFP was bilaterally injected into the ventromedial OFC (vmOFC) and optic fibers were implanted. After transgene expression, rats were tested in the probabilistic reversal learning (PRL) task once-daily for 20-days while vmOFC glutamate neuron activity was manipulated. Behavioral performance was analyzed using a Q-learning computational model of reinforcement learning. **Results**: Relative to saline-treated rats expressing eYFP, PCP-treated rats exhibited impaired PRL performance; a reduction in reversals ($p<0.05$), target win-stay responses ($p<0.01$) and learning rate ($p<0.01$). PCP-induced impairments in PRL performance were replicated in saline-treated rats when the vmOFC was activated by stimulating ChR2. PRL impairments in postnatal PCP-treated rats were ameliorated when vmOFC glutamate neurons were inhibited by stimulating eNpHR3.0. **Conclusions**: These findings indicate that disrupting early developmental glutamate transmission induces impairments in reversal learning. PCP-induced impairments in PRL may result from disrupted regulation of vmOFC glutamate cell activity as deficits were replicated when vmOFC glutamate neurons were activated and attenuated when vmOFC glutamate neurons were inhibited. This suggests that strategies to normalize vmOFC activity in schizophrenia patients represent a potential therapeutic target for disrupted cognitive flexibility.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.01

Topic: G.04. Emotion

Support: NIMH Intramural Research Program

Title: Amygdala lesions in macaques fail to disrupt autonomic arousal in anticipation of reward

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Abstract: The amygdala, along with the orbitofrontal cortex (OFC), has been implicated in stimulus-reward learning, affect, and modulation of autonomic arousal. For example, the amygdala contributes to the development of neural activity in OFC related to stimulus-reward processing, and removal of the amygdala causes a reduction in both expected and received reward-value coding by OFC neurons. In marmosets, amygdala lesions have been reported to disrupt autonomic responses to the sight of highly palatable foods but not autonomic responses that accompany consumption of that food (Braesicke et al., *Eur J Neurosci* 2005, 21:1733-40). In a Pavlovian conditioning task, humans with amygdala damage were found deficient in their autonomic responses to visual stimuli that predicted aversive outcomes and after receipt of those outcomes (Bechara et al., *J Neurosci* 1999: 19:5473-81). It is not known, however, how selective amygdala removal affects autonomic responses during comparable stimulus-reward learning in macaques. To investigate this issue, we tested rhesus monkeys (Macaca mulatta) with bilateral excitotoxic lesions of the amygdala (n=4) and unoperated controls (n=4) on an appetitive Pavlovian task, using conditioned autonomic responses (pupil size change) as our measure of learning. Monkeys were trained on a task in which Pavlovian conditioning of stimulus-reward associations was superimposed on instrumental conditioning of active visual fixation. On each trial, the monkeys received a small amount of fluid reward (3 drops of 0.1 ml) by maintaining gaze on a central fixation spot for 4 s. On a subset of trials, one of two Pavlovian stimuli (CS+, CS-) was presented on the monitor screen for 1 s during the 4-s fixation period. The CS+ was followed by a large reward (0.5 ml); the CS- led to no reward. If the monkey broke fixation when a CS was presented during the fixation period, the fixation spot was extinguished but the Pavlovian process continued unaffected by the oculomotor behavior. We examined how many sessions the monkeys needed to show pupil responses that differed significantly between CS+ and CS-, and whether the conditioned pupil responses were maintained across 4 consecutive sessions. We found no group differences in either the development of conditioned pupil responses or the ability to maintain those responses across days. Our results indicate that the amygdala is not necessary for the generation of sympathetic autonomic arousal in anticipation of fluid reward, at least as determined by a change in pupil responses. These results argue against the idea that the primate amygdala is essential for generating anticipatory autonomic responses to guide decision making.

Disclosures: J. Hwang: None. **P.L. Noble:** None. **E.A. Murray:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Program #/Poster #: 313.02

Topic: G.05. Mood Disorders

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Medical Research Council MR/V033492/1

Title: Parsing anhedonia, anxiety and rapid antidepressant responses across subcallosal cingulate Area 25 networks in the common marmoset

Authors: *C. M. WOOD[1], L. ALEXANDER[2,1], L. B. MCIVER[1], G. J. COCKCROFT[1], A. C. ROBERTS[1];
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Abstract: To develop more effective therapies for mood and anxiety disorders we must have a better understanding of the dysregulated circuits underlying their symptoms and treatment response. Elevated activity within the subcallosal anterior cingulate cortex, specifically area 25 (scACC-25), is consistently observed in patients and its causal contribution to anhedonia, anxiety and ketamine responsivity has recently been revealed in non-human primates. Here we differentiate the underlying scACC-25 networks that impact these processes. Utilising the excitatory designer receptor exclusively activated by designer drugs (DREADDs), hM3Dq, we target scACC-25 output neurons in the common marmoset using an AAV virus (AAV8-CaMKIIa-HA-hM3Dq). To activate individual scACC-25 pathways temporarily we infused the activator clozapine-N-oxide (CNO, 3uM) into terminals within the nucleus accumbens (NAc) or amygdala via intracerebral cannulae (n=3-5), comparing effects with vehicle infusions. The impact of these manipulations were examined on reward anticipation in an appetitive Pavlovian conditioning paradigm, in which two sounds separately predict access to reward or not (conditioned stimuli, CS+/-), and uncertain threat reactivity in a human intruder test, whereby a multifaceted behavioural response to a novel human is assessed across a 2 minute test period. Using this network information, we then examined the central mechanism of ketamine's sustained anti-anhedonic effects through direct ketamine infusion into the NAc followed by CNO-mediated scACC-25 activation at 3 different timepoints. We observed a double dissociation of these scACC-25 pathways on reward and threat responsivity. Activation of the scACC-25 to NAc pathway blunted anticipation to the CS+, with no effects on threat reactivity. Conversely, activation of the scACC-25 to amygdala pathway heightened threat reactivity whilst leaving reward anticipation intact. Subsequently, we observed that an intra-NAc ketamine infusion prevented scACC-25 induced reward blunting for over a week. Together, these data indicate circuit-specificity with scACC-25 overactivity inducing anhedonia-like and anxiety-like symptomatology through distinct pathways, with the nucleus accumbens a key region for ketamine's ameliorative effects on anhedonia.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Title: Optogenetic inhibition of the central amygdala at discrete time points modulates approach against dynamic threat in rats

Authors: *J. LEE, J.-S. CHOI;
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Abstract: The central amygdala (CeA) is involved in processing both aversive and appetitive stimuli, yet how CeA is recruited to guide optimal choices in a conflict situation is not well-understood. Here we investigated the role of CeA in a conflict situation where both threat and reward exist. Taking advantage of the high temporal resolution of optogenetic stimulation, CeA was inhibited at specific time points during risky foraging against a predator-like robot or the Lobsterbot named after its striking claws, programmed to generate a threat motion against approaching rats seeking sucrose solution. We bilaterally injected a viral vector carrying red-shifted inhibitory rhodopsin and implanted optic fibers over CeA of male Sprague-Dawley rats. During the baseline sessions (5-6 days), rats were trained to approach the sucrose port located near the Lobsterbot, which remained stationary. In the following conflict sessions (3 days), the Lobsterbot initiated striking attacks 6 s after the first lick, disrupting the rats' foraging behavior. Within the conflict session, optogenetic stimulation was delivered at three different time points depending on the group: Pre-attack period (PRE), Attack period (ATTACK), or Both (BOTH). The PRE group received laser stimulation for 6 s from the start of the first lick until the Lobsterbot initiated an attack. The ATTACK group received stimulation for the same amount of time as the PRE group, but the stimulation started simultaneously with the attack. The BOTH group received stimulation from 6 s before the striking attack to 6 s after the attack (total of 12 s). Rats with bilateral optic fibers implanted without a viral vector were used as the control group (CONT). The number of approaches and the number of licks during the conflict session were significantly decreased compared to the baseline sessions. During the conflict session, BOTH showed significantly more approaches than either CONT or ATTACK. Moreover, PRE also showed more approaches than either CONT or ATTACK. Collectively, approach-oriented behavior increased most effectively when inhibitory stimulation was applied during the pre-attack period in which rats anticipate an upcoming threat, suggesting that depending on discrete-time points when the CeA inhibition is applied the approach-oriented responses vary in a conflict situation.

Disclosures: **J. Lee:** None. **J. Choi:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.04

Topic: G.04. Emotion

Support: NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation 29503
NIMH R21MH122965

Title: Acute and long-term effects of positive vs. negative context during psilocybin administration on anxiety circuits and behaviors

Authors: *I. WEBER[1], C. DIAS[1], S. LIM[3], J. BERRY[3], R. HEN[3], G. F. TURI[2];
[2]Systems Neurosci., [1]New York State Psychiatric Inst., New York, NY; [3]Columbia Univ., New York, NY

Abstract: Psychedelics are being shown by medical and scientific methods to be helpful as a mental health therapy across many common diagnostic categories, including depression, PTSD, addiction, and more. As psychedelics may soon be readily available for both recreational and clinical use, systematic and controlled animal research could reveal unknown interactions between psychedelics and various administration contexts, but this research has not yet been done. We hypothesize that the context (whether positively or negatively valenced) in which these compounds are administered may enhance or limit their therapeutic potential. Using mice, we test the hypothesis that psilocybin alters neuronal activation captured by the immediate early gene ARC within regions involved in anxiety and cognition, including the hippocampus, prefrontal cortex, and amygdala, and has a greater anxiolytic effect in a follow-up behavioral battery when administered in a positive, enriched context as opposed to a negative, innately fearful context with 2MT predator odor present. We used the ArcCreERT2 x EYFP mouse line to fluorescently label neuronal populations acutely activated by psilocybin and used the Open Field, Elevated Plus Maze, and Novelty Suppressed Feeding behavioral tasks to assess anxiety-like behavior following treatment. We observed a reduction of the anxiety-like phenotype after psilocybin in the positive context but not after psilocybin in the negative context in the Open Field Task results in one experiment, providing some support for our hypothesis. Additionally, that activation of the ventral CA1 of the hippocampus, an emotional processing center, decreased during psilocybin treatment in the negative context but not in the positive context. We found additional context-dependent alterations in activation of the PFC, with psilocybin-induced decreases in ARC expression only in a negative context. These results provide preliminary evidence that positive versus negative administration context may drive neural and behavioral signatures of psilocybin's effects and outcomes in mice. This newly developed protocol can be used to further our understanding of the effect of psilocybin administration on neural signatures of varying external stimuli, such as innate and learned fearful cues.

Disclosures: I. Weber: None. **C. Dias:** None. **S. Lim:** None. **J. Berry:** None. **R. Hen:** None. **G.F. Turi:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.05

Topic: G.04. Emotion

Support: JASSO

Title: Prefrontal cortical activity in response to affective images: A functional near-infrared spectroscopy pilot study

Authors: **. *TRIPATHI, H. IMAI, M. CLAES, I. ADACHI;
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Abstract: Significant studies have been done to categorise the human emotions in pleasant and unpleasant. However, it is always challenging for researchers and for other people to distinguish partially overlapping emotions in specific categories. Grief and sadness are such emotions which are hard to distinguish. Understanding the underlying behavioral and neural mechanism of grief and differentiating it from other overlapping emotion is pivotal for therapeutic treatment. Therefore, this study attempted to examine the behavioral mechanism and neural correlates of grief perception in the prefrontal cortex (PFC) and its comparison with another overlapping unpleasant emotion, sadness. Twenty-one participants, (12F, 9M, 23-41 years old, 28.95 ± 4.52; mean ± SD) watched pleasant (attachment), unpleasant (sad and grief) and neutral images. After watching the images, participants rated their emotional responses in the three dimensions (Arousal, Pleasure, and Dominance) of the emotional state model, and also categorized the perceived emotion (sad, anger, disgust, happy fear, grief, neutral, surprise). Participants' oxygenated and deoxygenated (oxy and deoxy) hemoglobin (Hb) dynamics in the PFC were measured using near infrared spectroscopy (fNIRS) while they were watching the images. In a preliminary behavioral data analysis, we aimed at finding out whether participants distinguished between pleasant and unpleasant emotion eliciting images. Results of the behavioral analysis showed a significant main effect of valence for pleasant and unpleasant stimuli. Results of the arousal analysis showed significant difference between unpleasant and neutral images, but no difference between pleasant and unpleasant. However, such difference wasn't observed for dominance rating of images. Furthermore, results of the fNIRS analysis showed region specific PFC activity in response to emotion eliciting images. This significant main effect was observed in oxy-Hb in the PFC region, in the Brodmann area 45/46/9. However, such differences were not observed for deoxy-Hb. Given our preliminary results, we can suggest that there is a general effect, which shows participants respond at a behavioral and neurological level to observing different kinds of unpleasant and pleasant stimuli. These preliminary results are a first step toward exploring further differences between overlapping emotions such as grief and sadness. Therefore, analysis of behavioral categorization data and time-series analysis of fNIRS will be conducted for exploring how these two emotions can be perceived and processes differently in humans. This will contribute to the understanding of complex emotions.

Disclosures: *. *Tripathi: None. **H. Imai:** None. **M. Claes:** None. **I. Adachi:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

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Topic: G.04. Emotion

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The Hope for Depression Research Foundation (HDRF)
The Pritzker Neuropsychiatric Research Consortium

Title: High emotional reactivity is associated with activation of a molecularly distinct hippocampal-amygdala circuit modulated by the glucocorticoid receptor

Authors: *V. KUMAR, Q. WEI, S. J. MOORE, F. LI, G. G. MURPHY, S. J. WATSON, H. AKIL;
Michigan Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI

Abstract: Background: Altered emotional reactivity, as clinically observed in mood and affective disorders, is typically characterized by magnitudes of valence, intensity, duration and speed of shifting. The molecular and neural circuitry which regulate the dynamic nature of emotionality is yet to be fully understood. Our previous work showed that glucocorticoid receptor overexpression in the forebrain (GRov) leads to the development of a unique mouse model of high emotional reactivity with increased anxiety behavior and greater shifts in the emotional responses. In the present study, we sought to understand the neural circuitry associated with this lifelong increased emotional lability which gets established early in the development phase. **Methods:** The ventral dentate gyrus (vDG) of wild-type (WT) and GRov mice were selectively stimulated through optogenetics and anxiety responses were measured using elevated plus maze test. Neuronal activity as indexed by the c-Fos and cell type specificity patterns were analyzed through fluorescence in situ hybridization chain reaction (HCR-FISH) methods. **Results**: Our findings showed that the optogenetic stimulation in the vDG of GRov mice leads to a greater range and a prolonged duration of anxiety behavior relative to WT, and this amplified behavioral response was found to be associated with cFos-based increased neuronal activity in specific brain regions, particularly the ventral CA1 and the basal posterior amygdala complex. HCR-FISH studies provided evidence for altered glutamatergic/GABAergic activation balance within these sub-regions. Furthermore, we identified increased activation of molecularly distinct subpopulations: calbindin1^{+} glutamatergic neurons in the vCA1 and DARPP-32/Ppp1r1b^{+} glutamatergic neurons in the posterior basolateral amygdala. **Conclusion:** We propose that a molecularly distinct hippocampal-amygdala circuit is shaped during early life by the stress system, and in particular the glucocorticoid receptors, and tunes the dynamics of emotional responses.

Disclosures: V. Kumar: None. **Q. Wei:** None. **S.J. Moore:** None. **F. Li:** None. **G.G. Murphy:** None. **S.J. Watson:** None. **H. Akil:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

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Program #/Poster #: 313.07

Topic: G.04. Emotion

Title: "Oddly Satisfying" Visual Stimuli Induce Changes in Spontaneous Eye Blink Rate

Authors: *E. BARKLEY-LEVENSON, H. JUDICE;
Hofstra Univ., Hempstead, NY

Abstract: In the contemporary social media landscape, a popular genre of short online videos has emerged: the category of "oddly satisfying" visual stimuli. These videos typically include orderly and repetitive movements, with such diverse stimuli as blocks of soap or kinetic sand being sliced into shapes, chocolates being stamped perfectly onto a conveyor belt, or dirt being cleaned in precise lines from a window. This genre's popularity is visible across social media platforms, with TikTok videos tagged #oddlysatisfying amassing a total of 47.3 billion views. Despite their ubiquity, the underlying psychology and neurochemistry of what makes such a wide variety of experiences so satisfying has not been systematically studied. The concept of satisfaction first appeared in psychological theory as Thorndike's "Law of Effect," suggesting that a behavior leading to a "satisfying state of affairs" will be repeated; this was later refined by Skinner as the idea of positive reinforcement strengthening an action via operant conditioning. Assuming that, consistent with Thorndike's and Skinner's theories, the concept of satisfaction is synonymous with positive reinforcement, we would expect that the experience of satisfaction produced by "oddly satisfying" stimuli is dopaminergic in nature. To test this, thirteen young adult participants completed a within-subjects design, viewing both an "oddly satisfying" video compilation and an "unsatisfying" comparison video, while measuring their spontaneous eye blink rate (SEBR), a known indirect biomarker of central dopamine functioning, in blinks per visible minute (BPVM). SEBR was significantly higher following the satisfying condition (M = 21.09 BPVM, SD = 4.67 BPVM) than the unsatisfying condition (M = 16.48 BPVM, SD = 3.64 BPVM), $t(12) = 3.420$, $p = 0.005$. These preliminary findings suggest that the subjective experience of "satisfyingness" may relate to the experience of positive reinforcement as underpinned by the dopaminergic system.

Disclosures: E. Barkley-Levenson: None. **H. Judice:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

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Program #/Poster #: 313.08

Topic: G.04. Emotion

Support: Supported by Positive Prime Technology P/L

Title: Exploring brain activity from self-guided positive image training

Authors: *A. K. SINGH[1], J. LIU[1], K. SERAFINI[2], A. GAMBHIR[2], C.-T. LIN[1];
[1]Sch. of Computer Sci., Univ. Of Technol. Sydney, Ultimo, Australia; [2]Positive Prime, Cooroy, Australia

Abstract: Our everyday life is surrounded by negative information and activities such as news, poor working culture, stressful jobs, an active demand for high performance, disparities and inequities, etc. These everyday factors are concerning and present an imminent societal challenge to mental health wellbeing. The solution to challenges presented by a dynamic environment is to create positive emotions and feelings that can be considered fundamental to cultivating and building resilience. Several methods have been developed to improve emotional wellbeing and significantly create user resilience. In this work, we have systematically evaluated the brain dynamics of one of such commercially available self-guided imagery training programs for user resilience. Different images indicating neutral, negative, positive, and commercial positive images have been utilized while acquiring electroencephalogram (EEG) and physiological data. Each block consists of two sets of images from each condition, where each condition has a collection of 10 images presented for 1s. After each block, participants were asked to rate their arousal and valence on a five-point Likert scale.The results from the self-rated questionnaire indicate that 96.6% higher degree of valence and 48.1% higher degree of valence with positive images than the negative ones. It was also found that about 2.3% and 2.6% higher degree of valence and arousal, respectively, for positive images from commercially available positive images compared to open positive images dataset. We also looked at spectral changes in the frontal area of the brain associated with stimuli presented among all participants. It was found that there was a significant increase in beta activity from the positive images, which was especially pronounced with positive images used commercially. We also found other physiological changes among participants, such as an increase in pupil size dilation and higher galvanic skin response toward positive images; however, the results were non-significant statistically. These results have an impact on understanding how a user resilience image training program works. It is essential to note that this work represents a pilot study with a small cohort of twelve participants.

Disclosures: A.K. Singh: None. **J. Liu:** None. **K. Serafini:** None. **A. Gambhir:** None. **C. Lin:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Program #/Poster #: 313.09

Topic: G.04. Emotion

Support: NSF DBI2014217
Dorris Scholar

Title: Multi-dimensional characterization of arousal to different odor cues

Authors: *S. TAN, J. JENSEN, S. SINGH, K. AWAKOAIYE, L. STOWERS;
The Scripps Res. Inst., La Jolla, CA

Abstract: Sex, fear, hunger, aggression - the arousal we feel in these different states demands our attention, drives behaviour and is critical for our survival. Abnormally low or high arousal, as can be seen in anxiety/aggression/sexual disorders, imposes a severe burden on medical health. Yet effective treatments for these disorders remain elusive, in part because we know little about how arousal plays a role in different states. Arousal is commonly conceptualized as a single dimension, but are there specific arousal centers in the brain that are activated by different sensory cues to drive different behaviors? To answer this question, we leveraged the power of innate, pheromone driven olfactory responses in mice to embark on a multidimensional, comparative characterization of arousal in different states. We presented different odors to male mice (peanut oil, owl pellet, male and female urine) and elicited different arousal responses in terms of amplitude, duration and persistence as indexed by pupil dilation, respiration, and facial expressions (n = 12, age P90). Our findings suggest that distinct arousal pathways mediate the arousal response to sensory cues, paving the way for specific targeted interventions to treat maladaptive arousal in different states.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Topic: G.04. Emotion

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2021M3A9E4080780
2E30410-20-085

Title: Spontaneous thought dynamics as a signature of positive and negative affectivity

Authors: *E. LEE[1], B. KIM[2], J. HAN[1], S. LEE[3], S. GIM[1], I. CHOI[3], C.-W. WOO[1];
[1]Sungkyunkwan Univ., Suwon-si, Korea, Republic of; [2]Dartmouth Col., Dartmouth Col., Hanover, NH; [3]Seoul Natl. Univ., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Spontaneous thought is a dynamic phenomenon that arises from continuous changes in an individual's internal states. Their dynamic characteristics are likely to reflect individual differences and mental health variables, but few behavioral tasks and analysis methods are available for the quantitative assessment of the spontaneous thought dynamics. Here we conducted three studies (total $N = 392$) with a newly developed free association-based spontaneous thought sampling task named Free Association Semantic Task (FAST). We also implemented a web-based task platform for the task (FAST-web). Using Study 1 data ($n = 117$), which were collected in the laboratory setting using FAST-web, we derived predictive models of positive and negative affectivity based on the trial-by-trial response dynamics using a novel modeling approach named a density map-based predictive modeling. We found that our newly developed predictive models showed significant and robust prediction performances (rs = .42-.60) across multiple independent datasets with varied experimental parameters, including Study 1 re-test data with a different set of seed words ($n = 49$; 8-week interval), Study 2 data collected at home using FAST-web ($n = 213$), and Study 3 data collected within the context of fMRI experiment using verbal report ($n = 62$). Furthermore, using a sub-sample of Study 1 data ($n = 68$), we found that our model responses showed significant correlations with inflammatory marker response (C-reactive protein), suggesting that the spontaneous thought dynamics have a physiologically meaningful impact on the body. Overall, this study suggests that spontaneous thought dynamics can serve as cognitive and affective signatures of individuals.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.11

Topic: G.04. Emotion

Title: Smell and forget: Astroglial mitochondrial cannabinoid receptors link olfaction to cognitive alterations

Authors: *P. GOMEZ SOTRES[1], U. SKUPIO[2], A. CANNICH[1], F. JULIO-KALADZIC[1], D. GISQUET[1], J. S. BAINS[3], G. MARSICANO[1];
[1]INSERM1215, Bordeaux, France; [2]Columbia Univ., New York, NY; [3]Univ. of Calgary, Calgary, AB, Canada

Abstract: In threatening situations, survival mechanisms override most other functions, including cognitive abilities not directly related to the threat itself. Stress can be directly

experienced, but it can also be transmitted between individuals. Thus, stress-related cues are transmitted to non-stressed partners that will display similar stress responses. However, whether such social transmission of stress (STS) can also induce cognitive alterations in individuals who never experienced the threat is currently unknown. Here we show that, through the odor-induced activation of type-1 cannabinoid receptors (CB1) located in astrocytic mitochondria of the olfactory bulb, STS impairs recognition memory in animals that did not experience any direct threat. Exposure to anogenital olfactory cues of a stressed mouse was sufficient to impair novel object recognition (NOR) memory in non-stressed congeners. Mutant mice lacking CB1 receptors in astrocytes or expressing a mutated form of the protein excluding its mitochondrial localization displayed a specific reduction in exploration of the anogenital region of stressed partners. Specific targeted genetic exclusion of mtCB1 from astrocytes of the olfactory bulb also decreased anogenital exploration, and fully abolished STS-induced NOR impairment. Thus, by mimicking specific affective states, the processing of certain odors can lead to cognitive alterations, linking olfactory experience to non-odor related memory functions

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

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Program #/Poster #: 313.12

Topic: G.04. Emotion

Title: Predicting depressed mood according to EEG responses during spoken language processing of a news broadcast in a non-clinical population

Authors: *H. WATANABE, K. FUSEDA, A. MATSUMOTO, Y. NARUSE, A. S. IHARA; Natl. Inst. of Information and Communications Technol., Kobe, Japan

Abstract: Negative information can influence human mental health, and prolonged exposure to negative information, such as during the COVID-19 pandemic, may cause depression. Daily assessments of mood state and restricted access to negative information during periods of depressed mood could prevent depression. Given that affective words modulate event-related potentials (ERP) in depression patients, electroencephalogram (EEG) measurements might be useful for conducting daily mood assessments. However, the degree to which EEG can predict non-clinical depressed mood during daily life activities is unclear. Here, we sought to classify non-clinically depressed individuals while they listened to news broadcasts. We evaluated classification performance using the N1 and P2 components (auditory processing; modulated by attention) and the N400 component (semantic processing) during a news broadcast with negative, neutral, or positive content. The participants were 135 healthy native Japanese speakers (69 women; mean age = 33.2 ± 10.1 years old) who were categorized as having depressed (N = 32) or non-depressed mood (N = 132) using the Beck Depression Inventory-II. We measured

EEG at the Fpz, Cz, and Pz channels while the participants listened to news broadcasts (approximately one-minute duration) with negative, neutral, or positive content (15 news broadcast stimuli in total). To extract EEG responses to individual words in the news stimuli for each participant, we calculated the temporal response function (TRF) weights, which were time-locked to the word onsets, for each news condition and channel. The TRF analysis enabled us to separate the overlapping responses to the adjacent stimuli. As features, we obtained the mean amplitude and peak latency for the N1, P2, and N400 components included in the TRF weights. Finally, we selected six ERP features based on the model coefficients and the statistical analyses. The linear support vector machine was trained to classify participant groups. The performance was evaluated using leave-one-subject-out cross-validation and the area under the receiver operating characteristic curve (AUC). The resulting AUC was 0.730, indicating that non-clinical depressed mood could be detected using the EEG responses recorded during a news broadcast. With the goal of preventing depression through mood assessments during daily life activities, our method might be applied to the development of a system for restricting contact with negative information when individuals are assessed as having depressed mood.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Program #/Poster #: 313.13

Topic: G.04. Emotion

Support: NIH Grant EY000415

Title: Neuronal mechanisms underlying social motivation and approach: lateral habenula, periaqueductal gray, and superior colliculus

Authors: *H. LEE, O. HIKOSAKA;
Natl. Eye Inst., Bethesda, MD

Abstract: Environmental motivation (e.g., social and spatial) is one of the critical starting points to drive and contextually modulate goal-directed behaviors. For example, social motivation could be a key factor to improve social behaviors in autism spectrum disorder patients. How then the environment can motivate animals and initiate their sequential behaviors? For this question, we designed a multiple-step reward contingency task. Monkeys performed diverse instrumental and passive reward tasks in various social (i.e., face) and spatial (i.e., landscape) environmental background images. After a while of learning, monkeys hold their gaze longer toward high-valued environmental images and showed vigorous performance in the task sequentially processed. In the high-valued face images, monkeys showed higher eye contact and face gaze. During the task, we recorded single neurons in the subcortical brain regions. We found that

lateral habenula phasically encodes step-by-step changes in reward predictions and discriminates the effect of each contextual element separately and sequentially based on the intensity of reward prediction error compared to those induced by other elements consisting of the context. Periaqueductal gray showed changes in tonic activities that were consistent with the reward expectation that changed with each step of a trial and were corresponding with the object gaze duration of monkeys. In the additional task, we found that neurons in the superior colliculus, the center of saccade generation, are tonically excited and facilitated in steps that have positive reward prediction errors. This tonic excitation was sustained until the object onset of the next step and monkeys then could make a quicker saccade toward the sequentially presented object based on this previous reward expectation regardless of the reward information of the appeared object. In summary, we found that goal-directed behavior is not only modulated by the information of a single object or single goal but also could be controlled by environmental motivation ahead of the stimuli onset. Here, our data suggest that lateral habenula play a crucial role to encode and integrate various factors of reward prediction changes from an environmental context. Then this environmental reward information would be descended to periaqueductal gray and superior colliculus for the rapid saccade and longer gaze fixation which could be the pivotal source of motor skills to initiate sequential goal-directed behaviors such as eye contact and social approaches.

Disclosures: H. Lee: None. **O. Hikosaka:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Topic: G.04. Emotion

Support: NSF IUCRC BRAIN Award #1650536
NSF REU SIte award #1757949

Title: Brain on Nature: Assaying Neural and Emotional Changes after Exposure to Nature

Authors: *A. DAUBE[1,2], S. SYED[1], S. MOHANTY[1,3], J. L. CONTRERAS-VIDAL[1];
[1]IUCRC BRAIN, Univ. of Houston, Houston, TX; [2]Emory Univ., Atlanta, GA; [3]Carnegie Vanguard High Sch., Houston, TX

Abstract: Stress reduction theory states that spending time in nature can reduce symptoms of stress because of the innate connection to nature. This has been tested in real and virtual nature vs. urban settings during walking or standing/sitting using self-reporting questionnaires; unfortunately, very few studies have assayed the brain activity using quantitative neuroimaging technologies. A systematic review of nature and wellness using neuroimaging resulted in 15 studies. These studies were analyzed based on type of task, neuroimaging modality, and the primary(psychological) and secondary(neurophysiological) outcomes. In the examined studies,

the Profile of Mood States (POMS) test (40.0%) and State-Trait Anxiety Inventory test (26.7%) were the most common psychological questionnaires used. We found that in our search, electroencephalography (EEG) was the most popular neuroimaging modality (81.3%), with a low channel count (2-19 channels). The most common tasks were viewing images of nature (33.3%), nature VR simulation (26.7%), and walking in nature (20.0%). Unfortunately, only the three walking studies provide the closest experimental conditions to real life. Moreover, most (86.7%) of the studies were single sessions, which brings replicability into question. There were small(N<30; 6 studies), medium(30<N <110; 5 studies), and large(N>110;4 studies) sample sizes. The sample populations were all adults, except for one study focused on children. Within these adult populations, only 4 of the studies included clinical patients (anxiety or depression), and there were no studies that included physical ailments. The studies that included functional near-infrared spectroscopy (fNIRS) reported a decrease of O2Hb in the frontal cortex in nature settings, but an increase of O2Hb in urban settings. There was a similar pattern for the EEG studies, where there was a general increase in amplitude of α waves in nature settings but the opposite urban settings. There was a small number of studies that did not show an amplitude increase in α brainwaves, and instead found an amplitude increase in high β brainwaves. The studies that included psychological measurements supported the general pattern of EEG results, as the questionnaires pointed to a decrease in negative emotions and an increase in positive emotions after spending time in nature. These psychological results were consistent across tasks. These studies highlight the positive impact nature has psycho-physiologically and indicate the need for mobile brain-body imaging (MoBI) longitudinal studies with direct contact with nature.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Program #/Poster #: 313.15

Topic: G.04. Emotion

Support: University of Minnesota UROP grant to MS

Title: The Relationship between cerebral asymmetry and measures of psychopathy in a non-clinical sample is moderated by empathic challenge

Authors: *R. L. LLOYD[1], R. J. HJELLE[1], M. SCHUMACHER[2];
[1]Univ. of Minnesota, Univ. of Minnesota, Duluth, Duluth, MN; [2]Wolf Ridge Envrn. Learning Ctr., Finland, MN

Abstract: This study investigated the relationship between psychopathy, assessed by the Levenson Self-Report Psychopathy (LSRP) scale, and cerebral laterality. EEG recordings from frontal cortices (L3 and L4) were taken during both resting conditions and while viewing a video

of an emergency field amputation, used as an empathic challenge. The ratio of alpha power from the two recording site was taken as an index of relative activity in the two hemispheres. Forty students from the University subject pool were recruited as participants. Males (M=52.46; SD=11.05) had significantly higher (F=7.69, p=0.009) LSRP scores than did women (M=44.59; SD=6.85), consistent with the initial report by Levenson et al (1995), with sex accounting for 16.8 percent of the variance in psychopathy scores.While LSRP scores were unrelated to cerebral laterality under resting conditions, there was both a significant linear (R^2=0.113, F=4.22; p=0.048) and quadratic (R^2=0.201; F=4.02; p=0.28) negative relationship between LSRP scores and relative right-hemisphere activity. This relationship was approximately as strong in the female sample as in the combined sample R^2=0.129, linear; R^2=0.191, quadratic), but, with a smaller N, significance was not reached (p=0.078, linear; p=0.097, quadratic). Conversely, the male sample showed only a very weak relationship (R^2=0.053, linear; R^2=0.064, quadratic). Our data suggest that the relationship between psychopathy and cerebral laterality may be sexually dimorphic.Batky et al (2020) failed to find a relationship between psychopathy and hemispheric asymmetry, at rest or during an emotional processing task, in incarcerated male youth, which is consistent with our findings.Many studies suggest that the left hemisphere mediates positive affect and approach, while the right hemisphere mediates negative affect and withdrawal (Davidson, et al, 1990). However, Harmon-Jones et al (2010) present data that left hemisphere activation is also associated with approach to negative stimuli (e.g., anger), distinguishing motivational direction from affective valence.Light et al (2009) distinguished between “empathic concern”, sharing pain or sorrow of another, which is associated with right cortical activation and “empathic happiness”, pleasure in response to someone else’s positive state which is associated with left cortical activity in young children. As in the present study, baseline assessments of laterality were unrelated to empathy, consistent with our findings, suggesting that higher scores on the LSRP are associated with less empathic concern and, thus, less right hemisphere lateralization.

Disclosures: R.L. Lloyd: None. **R.J. Hjelle:** None. **M. Schumacher:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.16

Topic: G.04. Emotion

Title: Examining the predictive role of trait mindfulness on effects of emotionally arousing stimuli using an emotional Stroop paradigm.

Authors: *G. BATCHALLI MARUTHY[1], L. HIMES[2], S. PADHI[1], B. P. RYPMA[1];
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Abstract: Trait mindfulness is the disposition of nonjudgmental present moment awareness. An important aspect of mindfulness is the ability to pay attention to each moment. While

improvements in cognitive measures have been found following mindfulness training, less work has focused on the relationships between trait mindfulness and cognition. The nonjudgmental present-centered awareness that defines mindfulness is posited to allow an individual to completely experience present moment events in a holistic manner while preventing previous emotions from affecting succeeding events. In this study, we assess the effects of emotional valence and arousal on time taken to respond (Reaction Time; RT) to the font color of a word using an emotional Stroop (eStroop) task, in a general undergraduate student population. Following consent, participants answered demographic and mindfulness questionnaires, and completed the emotional Stroop task. Participants practiced the task prior to critical trials to prevent confounds from individual learning differences. The eStroop task involves responding, via button presses, to the font color of a word appearing in either red, blue, green or yellow color on the screen. The words were emotionally valenced (positive, negative or neutral) and arousing (high, low or neutral). We hypothesized that there would be differences in RT across the different valence-arousal conditions. Repeated measures ANOVA revealed significant differences in RT across the different conditions. We also hypothesized that trait mindfulness, as measured by the Mindful Attention and Awareness Scale (MAAS) would predict RT such that higher trait mindfulness will be associated with lower RTs in the different conditions. Pearson bivariate correlations revealed positive relationships between MAAS and RT for the positive valence-high arousal items such that high trait mindful individuals took longer to respond to positive high arousal stimuli. Additionally, reductions in RT across runs was positively associated with MAAS scores. These results indicate that individuals with high trait mindfulness experienced greater emotion-related interference while identifying the ink color of a word. These individuals, however, overcame this interference faster than lower trait mindful individuals. It may be that trait mindfulness sensitizes individuals to the effects of stimuli at the moment. However, the effects of the stimuli over time seem to diminish faster with higher trait mindfulness. This may support the idea that high trait mindful individuals are able to “let go” of the impulses generated by stimulating events.

Disclosures: G. Batchalli Maruthy: None. **L. Himes:** None. **B.P. Rypma:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.17

Topic: G.04. Emotion

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Brain and Behavior Research Foundation #27654
National Science Foundation NSF-GRFP DGE-1650604
FAPESP #2016/17329-3

Title: Control of feeding by a bottom-up brainstem-subthalamic pathway

Authors: *F. REIS[1], S. MAESTA-PEREIRA[2], P. SCHUETTE[1], E. SETHI[1], M. CHAKERIAN[1], D. NGUYEN[1], F. YUAN[1], A. TOROSSIAN[1], J. IKEBARA[3], E. INIGUEZ[1], A. KIHARA[3], A. ADHIKARI[1];
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Abstract: Investigative exploration and foraging leading to food consumption have vital importance, but are not well-understood. Since GABAergic inputs to the lateral and ventrolateral periaqueductal gray (l/vlPAG) control such behaviors, we dissected the role of vgat-expressing GABAergic l/vlPAG cells in exploration, foraging and hunting. Here, we show that vgat l/vlPAG cells encode approach to food and consumption of both live prey and non-prey foods. The activity of these cells is necessary and sufficient for inducing consumption, and vgat l/vlPAG activation produces exploratory foraging and compulsive eating without altering defensive behaviors. Moreover, l/vlPAG vgat cells are bidirectionally interconnected to several feeding, exploration and investigation nodes, including the zona incerta. Remarkably, the vgat l/vlPAG projection to the zona incerta bidirectionally controls approach towards food leading to consumption. These data indicate the PAG is not only a final downstream target of top-down exploration and foraging-related inputs, but that it also influences these behaviors through a bottom-up pathway.

Disclosures: F. Reis: None. **S. Maesta-Pereira:** None. **P. Schuette:** None. **E. Sethi:** None. **M. Chakerian:** None. **D. Nguyen:** None. **F. Yuan:** None. **A. Torossian:** None. **J. Ikebara:** None. **E. Iniguez:** None. **A. Kihara:** None. **A. Adhikari:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.18

Topic: G.04. Emotion

Support: JSPS KAKENHI Grant Number JP20K14251
Utokyo Center for Integrative Science of Human Behavior (CiSHuB)

Title: Synergistic effects of disgust and anger on amygdala activation while recalling memories of interpersonal stress: an fMRI study

Authors: *S. OZAWA[1], H. NAKATANI[2], C. M. MIYAUCHI[3], K. HIRAKI[1], K. OKANOYA[1];
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Abstract: Occurrence of an unpleasant interpersonal event in daily life may cause an individual to experience unpleasant emotions and recall memories regarding it. These emotions—manifesting in daily social interactions—are often complex and mixed. In the laboratory, autobiographical recall is frequently used to induce emotions; however, it often involves recalling memories associated with a specific discrete emotion (e.g., sadness). To examine the neural activity of emotions similar to real-life experiences, we examined neural activity while recalling memories of stressful interpersonal events in daily life, without specifying a discrete emotion. Of the 23 university students recruited, 21 were analyzed and asked to freely recall memories and given a series of questions concerning their recalled memories through a monitor, while their neural activity was measured with functional magnetic resonance imaging. Amygdala activity increased while receiving the instructions, followed by a decrease in activity. This indicates that the participants' arousal and vigilance initially increased in response to a novel stimulus, and then decreased by habituation. Disgust and anger, which are known to frequently occur as negative interpersonal feelings, were most prominently produced with strong associations with each other. More importantly, amygdala activation—while responding to questions regarding the recalled memories—was positively correlated with disgust or anger only when not controlling for anger or disgust, respectively. These results indicate that responding to questions facilitated the generation of a mixed emotional response as compared to when adopting free recall alone. Furthermore, disgust and anger as a mixed emotion can synergistically activate amygdala.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.19

Topic: G.04. Emotion

Support: NIH R01MH119511

Title: How does mood influence computations of cost and reward in effort-based decision making?

Authors: *J. C. CHIU, I. M. BERWIAN, Y. NIV;
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Abstract: Effort-based decision making is the process of choosing how much effort one is willing to exert to obtain a reward. Effort-based decision making is often studied in clinical populations in an attempt to uncover potential underlying mechanisms of symptoms, such as anhedonia in depression and negative symptoms in schizophrenia.
However, there exists a knowledge gap on how mood influences effort-based decision making.

The literature on emotions indicate two conflicting models: an affect-as-information model suggests individuals behave in ways that are congruent with their feelings (from this approach, when we are sad, we want to act in ways that are congruent with the sadness); on the other hand, the feeling-is-for-doing model posits that emotions elicit motivational goals that guide our behaviour (from this approach, when we are sad, we want to act in ways that reduces the sadness).
To date, this debate is largely still unanswered. Thus, we aimed to investigate this question of how mood influences effort-based decision making by dissociating the different components of the decision-making process and how reward and costs are computed by individuals under different emotional states.
Here, we present the results of a study where participants' moods were manipulated during an effort-based decision making task. Using a combination of valued-based and drift-diffusion models, we assessed the influence of mood on the calculations of: the perceived anticipatory cost of effort; the actual effort expenditure and ability to execute; the valuation of the expected reward; and the experience of the reward.

Disclosures: **J.C. Chiu:** None. **I.M. Berwian:** None. **Y. Niv:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Ministry of Science and Technology (MOST 108-2410-H-010-005-MY3)
Ministry of Science and Technology (MOST 108-2410-H-009-020-MY3

Title: Acute aerobic exercise on emotional regulation in marathon runners: a pilot functional magnetic resonance imaging study

Authors: C.-H. CHIANG[1], Y. CHENG[2], C. CHEN[3], *Y.-C. CHEN[4];
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Abstract: Aerobic exercise, in relation to high physical activity level, has been shown to have beneficial effects on anxiety. However, the underlying neural mechanism remains elusive especially on experienced marathon runners. Therefore, we examined the effect after a 1-h-run on positive and negative emotional processing in 10 experienced marathon runners using repeated explicit and implicit (backward masked) perception of emotional faces and functional magnetic resonance imaging (fMRI), also proceeded to assess their state anxiety and emotional

state by the use of the State-Trait Anxiety Inventory (STAI-S) and the Positive and Negative Affect Scales, (PANAS), respectively. Using a within-subject crossover design, this fMRI study examined how exercise (treadmill running versus rest) mediated amygdala reactivity to perception of emotional faces in young runners (N = 10). After running, Subjects did not show significant STAI-S reduction (P = .643). The PANAS scores were entered into a 2 (time: pre-/post-exercise) × 2 (scale: positive affect scale/negative affect scale) repeated measures analysis of variance (ANOVA). There were no significant main effects of time (P = .077) and no significant interactions for time × scale (P = .419). Whole brain analysis showed that compared to rest, insula, middle frontal gyrus, superior frontal gyrus less activated when participants perceived explicit happiness face after running (uncorrected p<.001). precentral gyrus (BA 6), Medial frontal gyrus, ACC less activated and dorsolateral prefrontal cortex more activated when participants perceived explicit fearful face after running. Moreover, ROI results showed that as compared to controls, the amygdala reactivity in running condition was significantly lower to happiness, but higher to implicit threat (p<.05). A seemingly contradictory result is the observation of decreased activation in the insula and amygdala when perceived happiness faces in running condition compared to resting control condition whereas increased activation in the amygdala when perceived fearful faces. This dissociation, ascribed to their failure to compromise getting fatigue, might contribute to anxiety after long duration acute aerobic exercise.

Disclosures: **C. Chiang:** None. **Y. Cheng:** None. **C. Chen:** None. **Y. Chen:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.21

Topic: G.04. Emotion

Title: Identifying anxiety-related genes in the hippocampus of Hatano rats

Authors: *K. SATO[1], A. ISHII[2], T. HATAKEYAMA[1,3], S. CHIBA[4], S. MORIYA[5], R. OHTA[6], M. KAWAGUCHI[1,2];
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Abstract: The prevalence of psychiatric disorders is increasing in the world and there is concern that this trend is the cause of soaring mortality by suicide especially in youth. One of these disorders is anxiety disorder, so it is important to determine factors to attenuate anxiety disorder symptoms. Animals also suffer from anxiety disorders, which can cause behavioral abnormalities. Hatano high avoidance animals (HAA) and low avoidance animals (LAA) are inbred strains of Sprague-Dawley (SD) rats that are selected based on their performance in an

active avoidance test. HAA shows a higher level of anxiety-like behavior than LAA in the elevated plus maze test at 5 weeks of age. Based on these findings, the present study focuses on the hippocampal function which is associated with anxiety-like behavior. Thus, we investigated the expression of the hippocampal *c-fos* gene in HAA and LAA during an elevated plus maze test (Experiment 1). In addition, we used microarray analysis to identify other expressed genes and then assessed the expression levels of these genes using RT-qPCR (Experiment 2). In Experiment 1, HAA and LAA were introduced to the laboratory conditions at 3 weeks of age, and then a 10-minute elevated plus maze test was performed at 5 weeks of age. Twenty minutes after completion of this test, the hippocampal formation was collected. The mRNA expression levels of the hippocampal *c-fos* gene were measured by RT-qPCR. During the elevated plus maze test, the *c-fos* mRNA expression levels in LAA were higher than those in HAA. In Experiment 2, HAA and LAA were again introduced to the laboratory conditions at 3 weeks of age, and the hippocampal formations were sampled at 5 weeks of age for microarray analysis and RT-qPCR. The microarray analysis revealed differences between HAA and LAA in mRNA expression levels. Nineteen genes were related to ligands and receptors in the nervous system. RNA was extracted from the remaining hippocampus, and the expression levels of the 19 genes identified by microarray analysis were measured by RT-qPCR, revealing differential expression between HAA and LAA for 12 of the genes. The differentially expressed genes identified by microarray analysis and validated by RT-qPCR included genes reported to be involved in stress-related behaviors. However, it is not clear yet whether these differentially expressed genes are really associated with anxiety, so further studies are needed to confirm if they are involved with anxiety in the hippocampus. This work was supported by Meiji University International Collaborative Research Promotion Project Grant Number MU-OSRI-ICRPP2017-204.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.22

Topic: G.04. Emotion

Title: Neural correlates of emotional contagion in prairie voles

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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by significant deficits in social interaction, social communication, and repetitive behaviors. Moreover, social interaction deficits encompass social recognition, empathy, and social learning challenges. However, the neural circuits of social cognition implicated in ASD are yet to be

uncovered. Here, we use the monogamous prairie voles (Microtus ochrogaster), known to present hallmarks of prosocial behaviors similar to humans. We developed a behavioral paradigm to assess social learning, inspired by the social transmission of fear and consoling paradigms that have been used in rats and voles. We assess prairie vole's behavioral responses toward a stressed cagemate or sibling. We are measuring the amount of freezing and consoling during observation of freezing behavior in the other partner and the amount of freezing during a memory test. By combining our behavioral paradigm with well-established assays, we assess c-fos expression of social learning in stressed cage mates in areas thought to be implicated in the negative valence network. We foresee increased expression in the anterior insular cortex, anterior cingulate, and amygdala, which are known to be implicated in a wide range of conditions and behavior such as maternal love, cognitive and motivational control of behavior, fear, and social decision-making. By establishing neural correlates of social and emotional contagion, we can elucidate an intrinsic map of potential target areas for future drug studies

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.23

Topic: G.04. Emotion

Title: Cognitive emotion regulation use predicts COVID-related stress and resiliency

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Abstract: The COVID-19 pandemic has served as an unprecedented and universal stressor that has altered all aspects of daily life. While stress exposure is widely thought to have detrimental effects on psychological functioning and well-being, there is also evidence that the tendency to use emotion regulation strategies can mitigate these stress effects. For example, recent work suggests that the use of cognitive reappraisal can reduce the negative impact of stress on cognition. Here, we sought to understand whether the tendency to use emotion regulation strategies may render individuals more resilient to COVID-related stress.
We conducted a large-scale, smartphone app-based longitudinal study to track how emotion regulation tendency predicted COVID-related stress and resilience. Participants completed the *COVID Stress Assessment* (CSA), a self-report survey that we developed to identify general and domain-specific stressors as well as how unpredictable, uncontrollable and overwhelming each were perceived to be over the previous week. Participants also completed the Emotion Regulation Questionnaire (ERQ), which measures the relative use of cognitive reappraisal vs. expressive suppression. We focused our analysis on a subset of participants (n=148, 82% female; mean age = 42.02, SD=14.65) that completed both of these scales between April 2020-2021. Data were analyzed longitudinally using linear mixed-effects models in R.

Perceived stress decreased over time and that perceived controllability, predictability, and coping for weekly stressors all predicted lower overall COVID-related stress, suggesting these served as protective factors that buffer stress responses. Due to their highly-correlated nature, we next collapsed all perceived controllability, predictability, and coping data to calculate an aggregate value that represents how resilient participants were to COVID-related stress. We used this value to examine how cognitive reappraisal and expressive suppression tendency predicted stress resilience. We observed a statistically significant effect of ERQ-CR tendency on our resilience measure, such that greater use of cognitive reappraisal predicted higher resilience to COVID-related perceived stress over time. In contrast, we saw a higher tendency to use of expressive suppression predicted lower resilience to COVID-related stress.
Our findings provide a longitudinal account of stress resilience and emotion regulation use in the wake of the COVID-19 pandemic and open future avenues of research into how regulation strategies can be optimized to promote stress resilience among the challenges of the pandemic.

Disclosures: **A. Secmen:** None. **A. Mellis:** None. **P. Glimcher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); The data collection system used in this study was produced by Datacubed Health. PG is an owner and officer of Datacubed Health.. **C.M. Raio:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.24

Topic: G.04. Emotion

Support: NRF-2020R1A4A1019623

Title: Differentiated brain responses to negative emotions evoked by positive and negative stimuli pairs

Authors: J. BAE[1], S. LEE[2], Y. LEE[2], J.-H. KIM[3], J.-H. KIM[2], *Y. SON[2];
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[3]Dept. of Psychiatry, Gachon Univ. Colllege of Medicine, Gil Med. Ctr., Incheon, Korea, Republic of

Abstract: Although the study of emotions in the human brain has been going on for decades, there is still much debate about the specific domains in the brain of each emotion. Most functional emotion studies usually used the visual stimuli, such as emotional face or situation images, according to the open database to evoke the specific target emotion. These stimuli used the neutral or non-emotional images as control to evoke the specific target emotion. To find the distinct brain regions for the negative emotions, the novel approach for visual stimulus is needed. To differentiate brain regions for negative emotion, a “Pos-Neg” paired stimulus for functional magnetic resonance imaging (fMRI) was devised. Negative emotion stimuli were composed of

images and sounds with situations related to negative emotions such as anger, anxiety, and sadness, and preceded positive emotion stimuli were composed of ones related to the corresponding situations. Total of 49 healthy volunteers (16 male and 33 female, ages from 19 to 50 years old with an average of 29.17), were participated and fMRI data were acquired. After the fMRI task, emotion evaluation scores for each stimulus were collected. The fMRI images were processed using statistical parametric mapping (SPM) 12. The four emotion evaluation scores, of which each was convolved with hemodynamic response function (HRF), were used as regressors. As a second analysis, one-way ANOVA was used. At significance threshold of $p_{uncorr}<0.0001$ (cluster level $p_{FWE}<0.05$), the left superior frontal area (BA8/9; x = -14, y = 42, z = 54) and the right superior temporal area (BA42; x = -24, y = 50, z = 10) were positively activated for anger and anxiety, respectively, and no regions were found for sadness. At significance threshold of $p_{uncorr}<0.001$ (cluster level $p_{uncorr}<0.05$), the right temporal middle area (rostroventral BA39; x = 60, y = -60, z = 16) was negatively activated for sadness. Using a "Pos-Neg" paired stimulus, we could find distinct brain regions for each negative emotions. Because a pair of positive and negative stimuli were designed to have the same components but opposite emotional elements, negative emotions were effectively elicited. It is interesting that sadness was negatively activated, while anger and anxiety were positively activated. Our results could be used as distinct biomarkers in psychological diseases related to the negative emotion.

Disclosures: **J. Bae:** None. **S. Lee:** None. **Y. Lee:** None. **J. Kim:** None. **J. Kim:** None. **Y. Son:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

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Program #/Poster #: 313.25

Topic: G.04. Emotion

Title: Positive influence of negative affective word stimuli during retention period on the performance of visual working memory

Authors: *M. PRIYA, S. SONI, A. YN, P. TAYADE, S. KAUR, R. SHARMA, S. MUTHUKRISHNAN;
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Abstract: Working memory (WM) is an essential cognitive resource enabling action of current goals. Literature suggests that various kinds of affective stimuli affect the WM performance. However, the effect of affective word stimuli on visual WM is unclear. The current study aimed to investigate the effect of affective word stimuli during varying retention period (short - 6s; long - 16 s) on the retrieval (categorization) of visual objects. Sixty-six healthy adult participants (N = 66; 29 males, 37 females; mean age 24.9±2.49 yrs) participated in the study and performed a visual WM task designed using PsychoPy (v2021.2.3). Stimuli used in the study consisted of 96 neutral images (valence = 4-6) belonging to four categories (human, inanimate, plant and animal)

from International Affective Picture System (IAPS) and Nencki Affective Picture System (NAPS). Low perceptual features of the images were equalized using SHINE toolbox in MATLAB. Affective words (585 words for male; 578 words for female; 4-6 characters; valence 1-4: negative, 4-5: neutral, 6-9: positive) from Affective Norms for English Words (ANEW) were presented during retention period of the task. Repeated measures ANOVA was performed to analyze the effect of within subject factors (visual object category, affective word category, retention period length) on the accuracy and reaction time of visual object categorization. Significant effects were observed for the object category [$F(1.817,118.096) = 69.627$, $p<0.001$, $\eta p^2 = 0.517$] and affective words during retention [$F(2.832,184.102)=13.018$, $p<0.001$, $\eta p^2 =0.167$] on the accuracy of visual object categorization. Post-hoc pairwise comparisons revealed higher accuracy of object recognition with negative words compared to positive and neutral word stimuli. Significant effects were observed for the object category [$F(2.440,158.625)=5.417$, $p<0.05$, $\eta p^2 =0.077$] and retention period length [$F(1.000,65.000)=61.100$, $p<0.001$, $\eta p^2 =0.485$] on the reaction time. Less reaction time was observed for trials with long as compared to short retention period. The current study design is a novel approach to understand the influence of emotional words as interfering stimulus in a visual WM task. Better accuracy due to negative emotional words could imply that negative words are ineffective distractors and have positive effect on goal-oriented behavior. Faster response in trials with long compared to short retention period could be attributed to the better rehearsal and maintenance of the information during long retention period and ease of the task for healthy adult participants.

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Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

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Program #/Poster #: 313.26

Topic: G.04. Emotion

Support: NIDA Grant R21-DA029189 (TLW)
Hanlon Foundation

Title: Pharmacological MRS study of dACC creatine and subjective states: Creatine's role in aversive somatic sensations in women

Authors: *T. L. WHITE[1], M. A. GONSALVES[2], H. JOYCE[3];
[1]Behavioral and Social Sciences, Sch. of Publ. Health; Carney Inst. for Brain Sci., [2]Neurosci. Grad. Program, [3]Undergrad Program in Cognitive Neurosci., Brown Univ., Providence, RI

Abstract: Creatine (tCr) is a neurometabolite whose role in affective states remains poorly understood. While many studies find a significant difference between the level of tCr in cortex of persons living with mood disorders compared to healthy controls, directionality of this difference

is inconsistent and often reflects gender differences. Given documented dysregulation of brain tCr in females with mood disorders and the effectiveness of tCr supplementation as an adjunctive treatment for depression in females, we hypothesize that pharmacological treatments that increase neocortical tCr should alleviate aversive somatic symptoms and negative subjective states in females, compared to placebo conditions. Using a pharmacological challenge approach, healthy young adult volunteers underwent MRI imaging after consumption of *d*-amphetamine (AMP, 20 mg oral) and placebo (PBO) on two test sessions (MRS sample $N = 20$, 55% female). MRS acquisition was localized to the right dorsal anterior cingulate cortex (dACC) using a PRESS acquisition, with tCr evaluated in response to drug and PBO within-subjects. Self-report measures of mood, subjective states and drug effects were evaluated at half-hour intervals on each test session and analyzed using principal components analysis (PCA), yielding three factors of subjective drug effects: positive subjective states (Factor 1), aversive somatic sensations (Factor 2), and negative subjective states (Factor 3). In females, correlation analysis indicated a significant inverse relationship of AMP-induced change in dACC tCr and aversive somatic sensations ($r = -.721$, $p = .006$, 1-tailed, $N = 11$) and a negative trend for drug-induced change in tCr and negative subjective states ($r = -.456$, $p = .079$, 1-tailed, $N = 11$). These findings suggest a mechanistic role of neocortical tCr in visceral processing and aversive somatic sensations in healthy young adult females. These data argue against use of creatine referencing and instead argue for use of water referencing of MRS metabolites, particularly in studies of drug effects and emotion in healthy samples. Future research should investigate gender differences for the role of tCr in aversive somatic symptoms in healthy individuals and in individuals living with mood disorders.

Disclosures: **T.L. White:** None. **M.A. Gonsalves:** None. **H. Joyce:** None.

Poster

313. Positive and Negative Affect: Neuroanatomy and Neuromodulation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 313.27

Topic: G.04. Emotion

Support: NIH Grant GR107695

Title: Persistent arousal states and hypermotivated exploratory behaviors in a Shank 2 mouse model

Authors: *P. N. NEGRON-MORENO, Y.-H. JIANG;
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Abstract: Transitions between brain states are essential for survival and adaptation in response to changes in external factors such as sensory input or emotional burden. Organisms strategically use brain states and external information to appropriately execute differential behavioral states depending on specific contexts. However, the mechanisms by which neural networks mediate

brain state transitions and regulate behavioral states are largely unknown. Previous work from our lab has suggested alterations in brain state transitions such as arousal and emotional states, as well as reward and risk-taking behaviors in a *Shank2* complete knockout mouse model with a deletion of exon 24 (*Shank2*Δe24-/-). SHANK2 is an important scaffolding protein located at the postsynaptic density of excitatory neurons, specifically involved in synaptic regulation through the formation and stabilization of synapses during development. The *SHANK2* gene has been strongly implicated in neuropsychiatric disorders from genomic studies, including mood disorders and autism spectrum disorder which exhibit a potential brain state transition deficit or inflexibility. Therefore, in this study, we sought to test the hypothesis that *Shank2*Δe24-/- mice exhibit sustained arousal states after repeated exposures to the same context. We analyzed the time spent interacting and exploring an object across multiple days using an object habituation test. Preliminary results suggest that although most mice, regardless of genotype, extensively explore the object on the first day of exposure, the *Shank2*Δe24-/- mice display a significant increase in exploration time ($p<0.0001$). Interestingly, *Shank2*Δe24-/- mice also exhibit increased exploration time ($p<0.0001$) and visit the object more frequently ($p<0.0001$) after multiple days of exposure to the same object. Additionally, *Shank2*Δe24-/- mice spent more time investigating the center, where the object was located, after multiple exposures to the same context ($p<0.0001$). These data suggest that *Shank2*Δe24-/- mice display a persistent state of arousal that might drive hypermotivation to explore an object after repeated exposures. Future work will use neural recording and optogenetic techniques to identify the neural circuitry modulating behavioral regulation of arousal state transitions during task performance.

Disclosures: P.N. Negron-Moreno: None. **Y. Jiang:** None.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NWO Vidi Grant

Title: The role of the nucleus accumbens shell in alcohol use despite negative consequences

Authors: *A. J. MCDONALD[1], P. NEMAT[2], T. VAN 'T HULLENAAR[2], J. HAGEMANN[2], S. VELLERE[3], O. BUSLENKO[2], I. ALONSO-LOZARES[1], D. SCHETTERS[1], Y. VAN MOURIK[1], M. VAN DER ROEST[1], T. J. DE VRIES[1], N. J. MARCHANT[1];
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Abstract: Alcohol use is widespread across most societies. While most people can control their alcohol use, a vulnerable sub-population develops alcohol use disorder (AUD), characterized by

continued alcohol use despite negative consequences. We used a rat model of alcohol use despite negative consequences, to identify neurobiological activity that may underlie addiction-like behavioral vulnerability. It was previously shown that this approach reliably identifies two sub-populations. One group substantially decreases alcohol use in the face of punishment (punishment-sensitive, controlled use) and another group continues alcohol use despite negative consequences (punishment-resistant, addiction-like behavior). In this study, we aim to identify the role of the nucleus accumbens shell (NAcSh) in alcohol use despite negative consequences. We trained Long-Evans outbred rats (n=92, m/f) to self-administer alcohol, and then introduced punishment with response-contingent footshock. Interestingly, we found that more female rats developed punishment-resistant alcohol use compared to male rats. In one group of rats (n=24) we used immunohistochemical detection of c-Fos to identify brain activity associated with punishment-resistant alcohol use. We found that lower c-Fos expression in NAcSh was associated with punishment-resistant alcohol use, compared to punishment-sensitive use, and rats tested without punishment. To test for a causal role of NAcSh activity in punishment-resistant alcohol use, in another group of rats (n=68) we used chemogenetic inhibition (hM4Di) of NAcSh throughout different phases of the experiment. We found that chemogenetic NAcSh inhibition does not affect unpunished alcohol self-administration. In punished sessions, however, NAcSh inhibition showed a small but significant increase in punished alcohol-seeking only in punishment-resistant rats at higher shock intensities. These results imply that NAcSh may be involved in alcohol use despite negative consequences in vulnerable individuals. Understanding the contribution of NAcSh, and associated neural circuits, in alcohol use despite negative consequences will provide us with a greater understanding of the neurobiological underpinnings of AUD.

Disclosures: A.J. McDonald: None. **P. Nemat:** None. **T. van 't Hullenaar:** None. **J. Hagemann:** None. **S. Vellere:** None. **O. Buslenko:** None. **I. Alonso-Lozares:** None. **D. Schetters:** None. **Y. van Mourik:** None. **M. van der Roest:** None. **T.J. De Vries:** None. **N.J. Marchant:** None.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-IRP, NIH, Z1A-DA000611

Title: Exploring the neurochemical actions of atypical dopamine uptake inhibitors: highlighting a potential therapeutic avenue for psychostimulant use disorder

Authors: *M. HERSEY[1], A. CHEN[1], A. H. NEWMAN[2], G. TANDA[1];
[1]Medication Develop. Program, [2]NIDA-IRP, NIH, NIDA IRP, Baltimore, MD

Abstract: Psychostimulant use disorder (PSUD) is an increasingly prevalent health condition with no FDA approved pharmacological treatments. Potential therapeutic options currently being explored include atypical dopamine (DA) uptake inhibitors (DUIs), that produce behavioral and neurochemical effects inconsistent with those elicited by typical abused psychostimulants which also interact with the uptake of dopamine. Modafinil (MOD), an agent approved for treatment of sleep disorders, is a low affinity DUI with a distinctly different pharmacological profile from psychostimulants like cocaine. MOD has been suggested as a potential PSUD medication but with limited efficacy. To improve efficacy, analogs of MOD with different pharmacological profiles, JJC8-088 and JJC8-091, have been synthesized. Nucleus accumbens shell (NAS) DA transmission plays a role in PSUD, and here we employed fast scan cyclic voltammetry (FSCV) for investigation of evoked DA release/clearance in the NAS of rodents. We began by exploring sex differences in response to DUIs in C57BL/6 mice. Cocaine (10 mg/kg; i.p.) increased evoked NAS DA release, and this effect was more pronounced in female mice than in male mice. The MOD-analog JJC8-091 (10 mg/kg; i.p.), produced little if any increase in evoked NAS DA release in male and female mice. Both agents did cause slowing of DA clearance in mice. Next, we found that pretreatments with the MOD-analogs, JJC8-088 (3-10 mg/kg, i.p.) and JJC8-091 (10-32 mg/kg, i.p.), produced divergent effects on NAS DA dynamics elicited by i.v. cocaine administration (0.1, 0.3, and 1.0 mg/kg) in male Sprague-Dawley rats. JJC8-088, which in previous studies showed a typical, cocaine-like DUI profile, enhanced cocaine-induced increases in evoked DA release suggesting an additive effect of these compounds. In contrast, JJC8-091, an atypical DUI that elicits limited, if any, cocaine-like stimulant effects, produced mild reductions in cocaine-induced stimulation of evoked DA release suggesting a sub-additive effect when these compounds are co-administered. Both JJC8-088 and JJC8-091 produced modest slowing of DA clearance both alone and in combination with cocaine. Taken with previous behavioral work, which has shown that JJC8-091 attenuates the reinforcing effects of cocaine, our findings suggest that this agent blunts the neurochemical effects of cocaine, further elucidating the mechanistic actions underlying the potential efficacy of atypical DUIs for the treatment of PSUD.

Disclosures: **M. Hersey:** None. **A. Chen:** None. **A.H. Newman:** None. **G. Tanda:** None.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Tactogen

Title: Locomotor stimulant effects and persistent serotonin depletions following [1-Benzofuran-5-yl)-N-methylpropan-2-amine (5-MAPB) treatment in Sprague-Dawley rats

Authors: C. B. JOHNSON[1], R. L. BURROUGHS[1], M. J. BAGGOTT[2], C. J. DAVIDSON[3], S. A. PERRINE[3], L. E. BAKER[1];
[1]Psychology, Western Michigan Univ., Kalamazoo, MI; [2]Tactogen, Palo Alto, CA; [3]Psychiatry and Behavioral Neurosciences, Wayne State Univ. Sch. of Med., Detroit, MI

Abstract: The emergence of novel psychoactive substances (NPS) on the illicit drug market is cause for public health concern. Benzofuran molecules with structural similarities to the entactogen, 3,4-methylenedioxymethamphetamine (MDMA), have been examined in recent preclinical studies of NPS for both abuse liability as well as medication development. Microdialysis studies in mice indicate the benzofuran [1-Benzofuran-5-yl)-*N*-methylpropan-2-amine (5-MAPB) may pose a higher risk for acute toxicity compared to equimolar amounts of MDMA. 5-MAPB has been marketed as a less neurotoxic analogue of MDMA, but no studies have addressed whether 5-MAPB can cause the long lasting serotonergic changes seen with high or repeated MDMA dosing. The current study employed locomotor activity screening followed by brain tissue analyses to evaluate the effects of repeated 5-MAPB dosing in rats. Forty-eight male Sprague Dawley rats were randomly assigned to one of three treatment conditions (0, 1.2, or 6 mg/kg 5-MAPB, n=16). Intraperitoneal injections were administered every two hours for three injections over the course of a single day to mimic binge-like use. Locomotor activity was monitored in an open field for one hour prior to injections and for two hours after each successive injection. Rats were euthanized 24 hours or two weeks after the last injection (n=8 per group), and brain tissues were rapidly dissected and immediately frozen for later analysis. Prefrontal cortex, striatum, and hippocampus were analyzed for monoamine content via High-Performance Liquid Chromatography (HPLC) with electrochemical detection. Acute injections of 6 mg/kg 5-MAPB produced a statistically significant increase in distance traveled, time in center, and stereotypy counts following the first injection, though these effects were reduced after subsequent injections. Increases in stereotypy counts, time in center, but not distance traveled were significantly higher following 1.2 mg/kg 5-MAPB compared to saline-treated rats, and time in center showed a cumulative increase with repeated injections of this dose. Neurochemical analyses indicated a statistically significant reduction in 5-HT and 5-HIAA in all brain regions assessed 24 hours and two weeks after 6 mg/kg 5-MAPB, with no statistically significant differences in monoamine levels between 1.2 mg/kg and saline-treated rats. There were also non-significant trends for reductions in striatal dopamine at both time intervals after 6 mg/kg 5-MAPB. These results show that 5-MAPB can dose-dependently produce persistent changes in 5-HT and 5-HIAA that appear analogous to those produced by MDMA.

Disclosures: C.B. Johnson: 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.; Tactogen. **R.L. Burroughs:** 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.; Tactogen. **M.J. Baggott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Tactogen. **C.J. Davidson:** None. **S.A. Perrine:** None. **L.E. Baker:** 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.; Tactogen.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Tactogen

Title: Evaluation of benzofuran and benzothiophene molecules for MDMA-like discriminative stimulus effects in Sprague-Dawley rats

Authors: C. B. JOHNSON[1], R. L. BURROUGHS[1], K. A. STECK[1], M. J. BAGGOTT[2], *L. E. BAKER[1];
[1]Psychology, Western Michigan Univ., Kalamazoo, MI; [2]Tactogen, Palo Alto, CA

Abstract: Illicit novel psychoactive substances (NPS) present a global health concern. Benzofurans, the third most prominent group of NPS on the market, are structurally and pharmacologically similar to 3, 4-Methylenedioxymethamphetamine (MDMA). The cardiovascular and neurobehavioral risks of benzofurans may also be similar to those of MDMA, particularly when misused at higher doses. Sulfur-containing analogues of benzofurans, benzothiophenes, are less studied but have recently been proposed as potential therapeutic agents. Characterizing the neurobehavioral effects of NPS is crucial to understanding their abuse liability and/or potential medicinal uses. Drug discrimination is a widely accepted preclinical behavioral assay utilized to discern the *in vivo* pharmacological activities of psychoactive substances. Previous studies utilizing this assay demonstrated full substitution and higher potency of select benzofurans compared to MDMA. The present study evaluated several novel benzothiophene [5-EAPBT, 5-MAPBT, 6-MAPBT] and benzofuran molecules, [(R)-BK-5-MAPB, (S)-BK-5-MAPB, (R)-BK-6-MBPB, (S)-BK-6-MBPB] in rodent drug discrimination studies. Sixteen male Sprague-Dawley rats were trained to discriminate MDMA from saline in a standard food-reinforced operant drug discrimination procedure and the aforementioned compounds were assessed for substitution at a range of doses. With the exception of (R)-BK-5-MAPB, and (R)-BK-6-MBPB, the novel molecules produced dose-dependent increases in MDMA lever responses and fully substituted at the highest dose assessed. These findings support previous reports that benzofurans share similar psychoactive effects to MDMA and they extend these findings to benzothiophenes. Further assessment of these compounds for abuse liability is warranted.

Disclosures: C.B. Johnson: 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.; Tactogen. **R.L. Burroughs:** 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.; Tactogen. **K.A.**

Steck: None. **M.J. Baggott:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Tactogen. **L.E. Baker:** 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.; Tactogen.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.05

Topic: G.09. Drugs of Abuse and Addiction

Support: ZIA DA000611-01

Title: Antagonism at oxytocin receptors blunts oxytocin induced enhancement of methylphenidate stimulated increases in accumbens dopamine levels

Authors: *G. TANDA[1], M. HERSEY[1], A. BACON[1], L. BAILEY[1], M. R. LEE[2], L. LEGGIO[1]; [1]NIH, NIDA IRP, Baltimore, MD; [2]NCATS, Bethesda, MD

Abstract: Antagonism at oxytocin receptors blunts oxytocin-induced enhancement of methylphenidate stimulated increases in accumbens dopamine levels Gianluigi Tanda, Melinda Hersey, Amanda Bacon, Lydia Bailey, Mary Lee, Lorenzo LeggioMedication Development Program, NIDA IRP, Baltimore, MD
While the illicit, non-medical use and misuse of stimulants like methylphenidate (MP) has increased in the past years, there are still no FDA approved treatments for psychostimulant use disorder (PSUD). Recently, preclinical and clinical studies have proposed oxytocin (OT) as a potential therapeutic avenue for PSUD. We have shown that OT pretreatments blunt the reinforcing effects of MP in self-administration studies. Interestingly, OT did not blunt the dopaminergic effects of MP, but, unexpectedly, it potentiated MP-induced stimulation of dopamine (DA) extracellular levels in the nucleus accumbens shell (NAS) in rats, as measured by microdialysis. In this study, we use fast scan cyclic voltammetry (FSCV) to assess the ability of OT to directly interfere with MP-induced changes in phasic NAS DA dynamics, i.e. release and clearance rate, in male Sprague-Dawley rats. We also use microdialysis to investigate the involvement of OT receptors in enhancement of MP-induced stimulation of rat NAS DA levels, by administration of the OT receptor antagonist atosiban. Administration of OT alone did not significantly change evoked NAS DA dynamics measured by FSCV. FSCV tests also showed that OT did not potentiate MP-induced increases in phasic DA release, nor did OT alter MP-induced reduction in DA clearance rate, suggesting no direct interactions of OT with the MP-induced inhibition of DA uptake. In agreement with previous microdialysis results, administration of OT alone did not elicit significant changes on extracellular NAS DA levels measured by microdialysis, and OT pretreatments (2 mg/kg, i.p.) potentiated MP-induced (0.1, 0.3, 1.0 mg/kg, i.v.) efflux of extracellular DA levels. The latter effect was abolished when rats

were pretreated with an OT receptor antagonist, atosiban (2 mg/kg, i.p.), suggesting that OT receptors mediate its effect on MP. Overall, our results suggest that OT receptors play a role in OT potentiation of MP-induced stimulation of extracellular NAS DA levels, likely driven by modulation of DA receptor signaling pathways, without affecting the MP blockade of the DA transporter.This work was supported by the National Institute on Drug Abuse, Intramural Research Program, NIH

Disclosures: G. Tanda: None. **M. Hersey:** None. **A. Bacon:** None. **L. Bailey:** None. **M.R. Lee:** None. **L. Leggio:** None.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Designing and implementing a new awake fMRI set up in rodents to study the effects of drugs of abuse in BOLD fMRI.

Authors: *S. SHUCHI[1], L. M. COLON-PEREZ[2];
[1]UNTHSC, [2]Univ. of North Texas Hlth. Sci. Ctr., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Abstract: fMRI is a significant noninvasive technique used in many studies spanning a large number of species from small rodents to non-human primates to small rodents, to indirectly assess brain activity. It works by detecting the changes in blood oxygenation due to the neurovascular coupling between the vasculature and neuronal activity. fMRI is traditionally used to study the brain's responses to tasks. More recently it has been more extensively explored to studying spontaneous brain activity known as RSFC (resting state functional connectivity). RSFC assesses the temporal correlation of spontaneous BOLD signal, which is the basis of fMRI, among spatially dispersed brain regions with the notion that regions with interconnected activity develop functional networks. fMRI BOLD scans in human subjects are almost exclusively performed in conscious subjects; however, animals, including rats, are usually anesthetized. Anesthesia is used to reduce head movement, distress, and anxiety in the animals. The most common anesthetic in animal studies is isoflurane, which is vasodilator and has detrimental effects on fMRI signal. In turn, isoflurane may affect the analysis of the results by intervening with normal brain function and neurovascular coupling, which would hinder our ability to determine accurate biomarkers of drug use in preclinical models of substance abuse. Of particular interest is the effect of illicit drugs in the brain. These drugs include cocaine, heroin, LSD, MDMA among others. Because most drugs of abuse are illicit in nature, there are no studies on these drugs naïve humans. Therefore, preclinical studies on brain function are done in animals to acquaint the public and lead clinical researchers in their endeavor to understand the human condition; however, they can only be relatable if the same imaging modalities, and under

similar physiological conditions is accomplished by both. One main difference between human and animals' studies is the use of anesthesia. Therefore, it is ideal to study the effects of drugs of abuse on animals when they are fully awake. With this work, we will develop a pipeline (hardware, and habituation) to study the effect of drug of abuse on the RSFC of awake rats using fMRI using an MR Solution 7T scanner and a custom-made 3D Printed Restraint Kit. We will show data validated in control rats and rats under the influence of a single acute injection of cocaine.

Disclosures: S. Shuchi: None. **L.M. Colon-Perez:** None.

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Dorsal striatum responses to reward in psychiatric inpatients with substance use

Authors: *J. MYERSON[1], M. MONTELONGO, Jr[1], M. PATRIQUIN[1,2], R. SALAS[1,2,4,3], H. OH[1,2];
[1]The Menninger Clin., The Menninger Clin., Houston, TX; [2]Menninger Dept. of Psychiatry and Behavioral Sci., [3]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [4]Michael E. DeBakey VA Med. Ctr., Houston, TX

Abstract: Introduction: Substance use is a major public health problem that impacts millions every year and is often comorbid with other mental health disorders (e.g., depression, anxiety, suicidal behaviors). We examined alterations of brain responses to reward within the left striatum between psychiatric inpatients with substance use (ADD), non-substance use (psychiatric controls, PC), and healthy controls (HC). In addition, this study investigated whether the severity of depression and anxiety is associated with dysfunctional reward responses. Methods: Participants were 130 psychiatric adult inpatients admitted to The Menninger Clinic (Houston, TX) divided into either ADD or PC groups based on total WHO-ASSIST scores (ADD: a score of 4+ in any of the 1- drug subscales, 11+ in alcohol; PC: 0-3 in all categories, 0-10 for alcohol). Demographic characteristics and psychiatric diagnoses (excluding substance or alcohol use disorders) were matched between the ADD and PC groups. HC group (n = 65) was matched for age and sex to the ADD. The PHQ-9 and the GAD-7 were utilized as a self-report measure for depression and anxiety symptoms, respectively. Participants were scanned in a 3T Siemens Trio MR scanner located at the Center for Advanced MRI at Baylor College of Medicine. A structural image (1 mm isotropic voxels, TE = 2.66 ms, TR = 1200 ms) and 4 functional images (3.4x3.4x4 mm voxels, TE = 40 ms, TR = 2000 ms) were acquired. The first two functional runs included 45 normal events (a 1s duration yellow light cue followed by sweet juice delivery 6s later). The last two functional runs included 24 normal events and 12 catch events (delayed juice delivery by 4s). The juice reward experiment data were analyzed using AFNI. Results: Our findings revealed

that relative to the HC group, the ADD group exhibited lower responses to juice delivery in the left striatum ($p < 0.05$). We found correlations between the WHO-ASSIST total score from the ADD group and brain responses to juice delivery ($R^2 = 0.016$, $p < 0.01$). We also found correlations between brain responses to juice delivery and severity of anxiety ($R^2 = 0.042$, $p < 0.05$) and depression ($R^2 = 0.016$, $p < 0.01$) from the ADD and PC groups. Conclusions: Our findings suggest that the brain response to reward stimulus in the left striatum is decreased in the ADD group. Future research examining reward processing and substance abuse should compare how different substances (i.e., cocaine, alcohol, tobacco, etc.) alter the brain's neural pathways.

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Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 314.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA027768
U01AA025932
NIDA R01DA046457

Title: Drug Reinforcement Impairs Cognitive Flexibility via Inhibiting Striatal Cholinergic Neurons

Authors: *H. GANGAL[1], X. XIE[1], Y. CHENG[1], X. WANG[1], J. LU[1], X. ZHUANG[1], A. ESSOH[1], Y. HUANG[1], L. N. SMITH[1], R. J. SMITH[2], J. WANG[1];
[1]Dept. of Neurosci. & Exptl. Therapeut., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX; [2]Dept Psychological and Brain Sci., Texas A&M Univ., College Station, TX

Abstract: Reinforcing behaviors are associated with deficits in cognitive flexibility, wherein a subject is required to extinguish an old behavioral strategy and explore a new one if the old strategy is no longer rewarding. The neural circuitry underlying this flexibility deficit is not known. Cholinergic interneurons (CINs) in the dorsomedial striatum (DMS) are known to play a critical role in behavioral flexibility. In this study, we used chronic cocaine and alcohol exposure as reinforcement models to investigate the role of DMS CINs in the reversal of instrumental learning. We found that chronic cocaine or alcohol exposure increased GABAergic inputs to DMS CINs and reduced their spontaneous firing activity. Morphological and physiological studies demonstrate that the major GABAergic input to the DMS CIN originates from the direct-pathway medium spiny neurons (dMSNs) and this input was potentiated by chronic cocaine or alcohol exposure. To artificially mimic the inhibition of DMS CINs, we employed chemogenetic and time-locked optogenetic approaches to downregulate DMS CIN activity, which led to deficits in behavioral flexibility. The dMSN to SNr (Substantia Nigra Reticulata) pathway is

known to mediate reinforcing behaviors. Functionally, we test that dMSNs also send collateral inhibitory connections to the CINs. Therefore, we propose that, on one hand dMSN to SNr pathway causes reinforcement, at the same time dMSN to CIN pathway leads to inflexibility, thus providing the neural substrate for deficits in flexibility caused by reinforcing behaviors.

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Poster

314. Addictive Drugs: General Neural Mechanisms

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Program #/Poster #: 314.09

Topic: G.09. Drugs of Abuse and Addiction

Support: K99
NIH DA046522
P20GM121310

Title: Identifying and activating neural ensembles in the nucleus accumbens linked to cocaine and chocolate place preference

Authors: *K. SANDUM[1,2], L. FLOM[1,3], J. CROUSE[4], L. VACCARO[1], T. HERRERA[1], C. LITIF[1,5], S. HODGINS[6], A. BOBADILLA[1,7];
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Abstract: Neuronal ensembles in the nucleus accumbens core (NAcore), an integration center of rewards and motivation, play a role in substance abuse disorder and relapse. Seeking ensembles are sparse networks of neurons activated during reward-seeking behaviors that are thought to drive relapse behavior. The purpose of this study is to map NAcore ensembles involved in seeking cocaine and compare them to those involved in seeking natural rewards (chocolate). We also hope to determine if activating ensembles is sufficient to provoke drug and natural reward-seeking behavior. To test the first hypothesis, we conditioned cohorts of 11-12 genetically modified $Fos^{iCreERT2}$/Ai14 male and female mice with chocolate or cocaine and quantified the ensembles activated during reward seeking using the conditioned place preference model. Both cocaine- and chocolate-seeking behavior activated about one percent of the neurons in the NAcore. Next, mice were conditioned to receive both chocolate and cocaine in different compartments and the opportunity to seek either during test day in absence of reward. The mice showed a preference for cocaine. A Gq-DREADD virus allowing specific activation of ensemble cells was injected into the NAcore of male and female $Fos^{iCreERT2}$ mice and the neuronal ensembles were activated after conditioning with cocaine or chocolate. This led to a recreation of cocaine-seeking behavior even in animals that otherwise showed no seeking behavior.

Characterizing these reward-specific ensembles will allow us to better understand the neuronal circuits driving reward seeking and relapse

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Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.10

Topic: G.09. Drugs of Abuse and Addiction

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Texas A&M University Presidential Excellence Fund X-Grant

Title: Crf regulates striatal circuitry in alcohol use disorder

Authors: *A. ESSOH[1], Z. HUANG[1], H. GANGAL[1,2], X. WANG[1], L. UGARTEMENDIA[3], N. J. JUSTICE[3], J. WANG[1,2];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Sch. of Med., Bryan, TX; [2]Inst. for Neurosci., Texas A&M Univ., College Station, TX; [3]Ctr. for Metabolic and Degenerative Dis., Univ. of Texas Hlth. Sci. Ctr., Houston, TX

Abstract: Alcohol use disorder (AUD) is a chronic relapsing disease and a leading cause of preventable death in the United States. The behavioral characteristics of AUD, such as compulsive alcohol-seeking and consumption following periods of withdrawal, have been observed after salient environmental stress. Stress causes the release of corticotropin-releasing factor (CRF) from the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST). Furthermore, the dorsomedial striatum (DMS) contains cholinergic interneurons (CINs), which we recently found to regulate flexible drinking behavior. Despite the body of work surrounding CRF release in the CeA and withdrawal from addictive substances, it remains unknown how stress-induced CRF release in the striatum influences relapse in AUD. Using mouse genetics, we found CRF receptor 1 (CRFR1)-, but not CRF-positive neurons in the DMS. Furthermore, slice electrophysiology recordings reveal that exogenous CRF potentiates the spontaneous firing of DMS CINs. To investigate the source of these CRF inputs onto DMS CINs, we infused AAVretro-DIO-GFP into the DMS of CRF-Cre;tdT rats and discovered that a high percentage of CeA and BNST CRF-positive neurons project to the DMS. This result supports the hypothesis that CRF inputs to the DMS that potentially influence AUD behaviors are mediated, in part, by the CeA and BNST. We are currently investigating whether these inputs contribute to CRF-related changes in the reinstatement of alcohol-seeking behavior. Together,

these studies will provide insight into the striatal mechanisms underlying stress-induced relapse of AUD.

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Poster

314. Addictive Drugs: General Neural Mechanisms

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.11

Topic: G.09. Drugs of Abuse and Addiction

Support: Swedish Research Council (Grant Nos. 2013-07434)

Title: Role of the histone methyltransferase ezh2 in punishment-resistant alcohol self-administration in rats

Authors: *E. BARBIER[1], E. DOMI[2], L. HÖGLUND[3], L. XU[3], S. TOIVAINEN[3], K. CHANTHONGDEE[3], M. A. HEILIG[3];
[1]Linköping Univ., Linköping, Sweden; [2]Dept. of Clin. and Exptl. Medicine,, Linkoping, Sweden; [3]Linkoping Univ., Linkoping, Sweden

Abstract: ROLE OF THE HISTONE METHYLTRANSFERASE EZH2 IN PUNISHMENT-RESISTANTALCOHOL SELF-ADMINISTRATION IN RATS Estelle Barbier, Esi Domi, Leon Höglund, Li Xu, Sanne Toivainen, Kanat Chanthongdee, Heilig Markus.Center for Social and Affective Neuroscience, Department of Biomedical and Clinical Sciences, Linköping University, S-581 85 Linköping, Sweden.Alcohol use disorder (AUD) is a major health problem accounting for 5.3% of global disease burden. It is a complex psychiatric disease characterized by persistent neuroadaptations in key brain structures, including the amygdala. A core feature of AUD is continued alcohol use despite adverse consequences, or “compulsive use”. In humans, only a subset of individuals transitions from recreational to compulsive alcohol use. Compulsive alcohol intake can be modeled in rat using a contingent footshock-punished alcohol self-administration procedure where lever pressing to obtain alcohol is paired with an electric footshock. This behavior was associated with increased activity of neurons expressing protein kinase C delta (PKCδ) in the central amygdala (CeA). Activity-dependent tagging, followed by chemogenetic inhibition of neurons activated during punishment-resistant self-administration, suppressed alcohol taking, suggesting a functional role of this neuronal population. In the present study, we aim to identify the molecular mechanisms that promote the activation of the CeA PKCδ neuronal ensemble during compulsive alcohol intake. Using the NanoString® technology, we found that compulsive alcohol intake was associated with increased expression of the Enhancer of Zeste 2 (EZH2) in the CeA. EZH2 is a histone 3 lysine 27 (H3K27) methyltransferase and is a catalytic component of the polycomb repressive complex 2 (PRC2). Intraventricular microinfusions of tazemetostat, a potent and

selective *Ezh2* inhibitor, decreased resistance to punishment in “compulsive” rats and showed no effect in “non-compulsive” rats. In line with these results, a shRNA-mediated knockdown of *Ezh2* in the CeA also resulted in decreased resistance to punishment in “compulsive” rats suggesting a functional role of Ezh2 in compulsive alcohol intake. Together our findings highlight the contribution of epigenetic mechanisms and more particularly EZH2, in mediating compulsive alcohol intake.

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Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 314.12

Title: WITHDRAWN

Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 314.13

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant: R01AA025784
NIH Grant: R01AG072897
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Brain & Behavior Research Foundation grant: #24989

Title: Decreased SK channel-mediated medium afterhyperpolarization enhances secondary motor cortex excitability during cocaine withdrawal

Authors: *D. HUANG, V. G. GOMEZ, Y.-Y. MA;
Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Chronic relapse in cocaine use disorder necessitates motor actions. Although it has been recognized that relapse involves complex behaviors, we remain uncertain about the role of the motor cortex in addiction, despite its role in mediating the acquisition and execution of skilled movements. The present study aims to examine the neuronal excitability of pyramidal neurons in the primary motor cortex (M1) and secondary motor cortex (M2) during withdrawal day (WD) 1 or 45 (i.e., maintained by drug abstinence), to determine its role in the enhanced

vulnerability toward relapse during drug withdrawal. Using an intravenous self-administration (IVSA) model of cocaine addiction, Sprague-Dawley (SD) rats with saline experience or cocaine experience (abbreviated as S-IVSA or C-IVSA respectively), 1-hour daily sessions for 5 days, were used to investigate the neuronal excitability of pyramidal neurons in M1 and M2, on WD 1 and WD 45. Our data indicate an incubation of cocaine-seeking after prolonged withdrawal. Relative to WD 1, C-IVSA SD rats on WD 45 showed increased cocaine-seeking (demonstrated by increased presses on the lever previously paired with an intravenous infusion of cocaine). Compared to S-IVSA and WD 1 C-IVSA SD rats, whole-cell patch clamp recordings on motor cortex-containing slices showed M2 pyramidal neuron excitability increased (indicated by higher number of action potentials generated by depolarizing current pulses) after 45 days of cocaine withdrawal. Further analysis showed a decrease in medium afterhyperpolarization (mAHP) but not fast afterhyperpolarization amplitude in C-IVSA vs. S-IVSA SD rats on WD 45. Bath application of a small conductance (SK) calcium-activated potassium channel activator, 1-EBIO (100 μM and 300 μM) was able to concomitantly increase mAHP amplitude and decreased M2 pyramidal neuron excitability in C-IVSA but not S-IVSA trained SD rats on WD 45. In addition, *in-vivo* bilateral microinjection of 1-EBIO (59 mM, 1μL per injection site) vs. vehicle pharmacologically attenuated cocaine-seeking behavior. We conclude that (1) during C-IVSA withdrawal, decreased SK channel-mediated mAHP amplitude increases the excitability of pyramidal neurons in M2, and (2) SK channel in M2 may be a novel target for preventing cocaine relapse.

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Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 314.14

Topic: G.09. Drugs of Abuse and Addiction

Support: R00DA045795
P30DA033934

Title: Reprogramming ZFP189 transcription factor function regulates drug specific behavioral responses and transcription

Authors: *J. A. PICONE, G. M. SILVA, K. KIM, N. L. TRUBY, X. CUI, P. J. HAMILTON; Virginia Commonwealth Univ. Neurosci. Grad. Program, Virginia Commonwealth Univ. Neurosci. Grad. Program, Richmond, VA

Abstract: Understanding the molecular substrates of the stages of drug addiction may allow for the design of pharmacotherapies that block or reverse key events of the progression of drug addiction. *Zfp189* is a CREB-target gene which itself encodes an unstudied nucleus accumbens (NAc) neuronal transcription factor that has been demonstrated to regulate transcriptional

networks in neuropsychiatric disorders. Preliminary data reveals that using the CRISPR/dCas9-mediated CREB delivery to the *Zfp189* CRE site increases *Zfp189* mRNA levels in the NAc and decreases reward associations for mild doses of cocaine. To further examine the downstream relationship between ZFP189 and physiological response to saline, cocaine, and morphine, three reprogrammed synthetic ZFP189 variants were used to study behavioral responses. The three ZFP189 variants used were: $ZFP189^{WT}$ (the endogenous gene with transcriptionally inhibitory KRAB domain), $ZFP189^{DN}$ (maintains binding domain but lacks KRAB domain), and $ZFP189^{VPR}$ (KRAB domain was replaced with the powerful transcriptional activator VP64-p65-Rta (VPR)). An *in vitro* luciferase assay revealed that $ZFP189^{WT}$ acts as repressor and $ZFP189^{VPR}$ was a powerful activator. Next, mice received one of these ZFP variants to the NAc via viral-mediated gene transfer. We then performed a drug locomotor sensitization assay with saline, cocaine, or morphine. In response to cocaine treatment, mice with $ZFP189^{VPR}$ intra-NAc moved significantly more than the $ZFP189^{WT}$ group. Even following dissipation of viral expression, the $ZFP189^{VPR}$ group maintains heightened cocaine locomotor sensitization. More interestingly, this increased locomotion appears to be unique to cocaine, as there is no difference in locomotor activity between the ZFP189 variant groups in response to saline or morphine administration. Bulk RNA sequencing of manipulated NAc tissues from these mice revealed that the differences in behavioral response to cocaine across the variant groups coincided with transcriptional changes. Specifically, our synthetic $ZFP189^{VPR}$ was only able to regulate NAc transcription in mice that had been treated with cocaine. These results suggest ZFP189 specifically drives cocaine-induced transcription and behaviors. Combined with the CRISPR/dCas9 results, we propose that CREB's activation of *Zfp189* and ZFP189's downstream function in NAc represents a cocaine-specific molecular cascade. Forward directions include examining the downstream targets of ZFP189 and its NAc cell-specific function.

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Poster

314. Addictive Drugs: General Neural Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant MH119049
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Hellman Fellows Foundation

Title: Using ultrasonic vocalizations to interrogate rat subjective states: Drug intoxication and chemogenetic manipulations of VTA dopamine neurons

Authors: K. A. LAWSON, S. V. MAHLER;
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Abstract: Rats emit ultrasonic vocalizations (USVs) to convey emotional arousal, and/or as a form of social communication about salient rewards and threats in the environment. In adult rats, these USVs fit into two broad categories; “22kHz” calls that are produced when the rat is in a painful or dangerous situation, and “50kHz” calls, which are emitted during experience or anticipation of rewards like play, mating, or certain drugs of abuse. Relatively recent advances in USV detection hardware and analysis software have made it possible to examine more nuanced aspects of rat USVs, for example showing that “50kHz” calls occur at a range of frequencies and patterns—with up to 14 subtypes (Wright et al 2010). It is not presently clear whether these complex signals contain information that could be useful for interrogating nuances of rats’ subjective states, such as those elicited by drugs of abuse. Here, we examined locomotion and USVs emitted by male and female Long Evans TH::Cre rats after acute injections of the drugs of abuse amphetamine, heroin, and ketamine. Furthermore, to investigate the roles of VTA dopamine neurons in USVs and locomotion, we expressed inhibitory (hM4Di), excitatory (hM3Dq), or no DREADDs selectively in VTA dopamine neurons using Cre-dependent viral vectors. We then tested each drug of abuse twice in each rat, following counterbalanced injections of vehicle or CNO. To examine locomotion, we used Ethovision software to measure distance traveled over the course of each 1hr session. To identify and quantify USVs, we recorded them using Avisoft hardware and software, then validated and employed Deepsqueak (Coffey et al 2019) software to precisely identify the acoustic features of identified USVs on each test day. This generated large datasets which allowed us to employ high dimensionality reducing analyses (as in our prior work, Lawson et al 2021) to determine how the characteristics of USVs are altered by these pharmacological and chemogenetic manipulations. We found numerous intriguing and sometimes paradoxical changes in locomotion and USV production after DREADD and drug manipulations. We hope this study will facilitate further data-driven investigations into the meaning of rat USVs, and their usefulness for interrogating the otherwise inaccessible subjective states of rats during drug intoxication, circuit manipulations, or other common behavioral neuroscience manipulations.

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Poster

314. Addictive Drugs: General Neural Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH National Institute on Drug Abuse DP1 DA039658 to CDF
TRDRP T31R1767 to CDF

Title: Cellular functions mediated by purinergic signaling in the medial habenula

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Abstract: Cellular functions mediated by purinergic signaling in the medial habenula

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Tobacco smoking remains a leading cause of preventable death worldwide, and nicotine is considered to be the primary reinforcer underlying tobacco dependence. In these studies, we sought to assess the role of purinergic signaling on neuronal activity in the medial habenula. First, we examined ATP release in the medial habenula by utilizing the GRABATP sensor to monitor real-time ATP release. To understand the effect of this ATP release related neuronal activity, brain slices from male and female wildtype mice were sectioned, and neurons in the medial habenula were examined with patch clamp electrophysiology. Initial studies characterized the baseline firing rates and the effects of a receptor agonist on cholinergic habenular neurons. Next, since the medial habenula has been highly implicated in mediating symptomology associated with nicotine withdrawal, we then examined the effects of purinergic receptor signaling on neuronal firing during a state of nicotine withdrawal in both sexes. We found that purinergic signaling modifies neuronal activity of the medial habenula, with differential effects occurring during nicotine withdrawal. Together, these data contribute to our understanding of the factors mitigating withdrawal effects that may underlie the nicotine dependence state.
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Poster

314. Addictive Drugs: General Neural Mechanisms

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Program #/Poster #: 314.17

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA049837

Title: The effect of early life stress on incubation of morphine craving

Authors: *A. HEHN[1], A. CUARENTA[2], J. FLOWERS[2], M. DUPUIS[2], C. DECKERS[2], A. INGRAM[2], M. E. WIMMER[2], D. BANGASSER[2];

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Abstract: Our society continually struggles with substance use disorder (SUD), in part due to its co-morbid nature with other psychiatric disorders. There are many factors at play that contribute to SUD behaviors, including stress. While early life trauma increases SUD, mild adversity such as early life stress (ELS) can be protective, promoting later resilience to stress. In our lab, we utilize the limited bedding and nesting (LBN) model, which limits a dam's access to nesting materials during the pups first week of life. Prior research from our lab found that LBN reduces low dosage morphine self-administration in adult males, but not females. We also found that it decreases impulsive choice and risk-taking behaviors. With this information in mind, we decided to investigate if these changes could be extended to cue-driven drug-seeking behavior. It is possible that LBN alters the processing of cues that ultimately direct behavior. Here, we extended prior behavioral studies to determine whether LBN alters the incubation of morphine craving - a cue driven behavior. Incubation of craving refers to the notion that a prolonged abstinence period can result in an intense drug desire when an organism is placed back into the same environment where drugs were taken previously. In our study, adult male and female rats (control or LBN) were placed into operant chambers and allowed to press levers on a fixed ratio (FR1) schedule for morphine infusions (0.75 mg/kg/infusion) - a higher dose that is not affected by LBN across sex. Presses on the "active lever" resulted in an infusion accompanied by a 5-s light cue and a subsequent 20-s timeout period during which the house light was off and lever presses were recorded but had no corresponding drug infusion. These sessions began at the start of the animals' dark cycle and ended 12 hours later for 10 consecutive days. Following the 10 days on FR1, the rats were tested for behavioral signs of drug seeking during Day 1 and Day 30 of abstinence using the presentation of drug-associated cues while morphine was no longer available. Our results demonstrate that LBN had no influence on incubation of craving. Rats exposed to LBN lever pressed at the same rate as control rats on Day 1 and Day 30 of abstinence. Both LBN and control rats show the incubation effect, pressing more on Day 30 than on Day 1 with no sex differences. These results suggest that while LBN reduces morphine taking, it does not affect cue-driven incubation of morphine craving. Understanding the precise mechanisms by which LBN affects addiction-related behavior will allow us to leverage this model to develop more targeted therapeutics.

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Poster

314. Addictive Drugs: General Neural Mechanisms

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Program #/Poster #: 314.18

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA grant U01 DA051993

Title: Transcriptional and epigenetic mechanisms in the medial prefrontal cortex associated with individual differences in vulnerability to morphine self-administration in rats

Authors: S. X. LIU[1,2], Z. L. MATTI[1], P. MUELKEN[4], A. RAMAKRISHNAN[5], M. G. LESAGE[4,1,3], J. R. SMETHELLS[4,3], L. SHEN[5], P. V. TRAN[2], A. C. HARRIS[4,1,3], *J. C. GEWIRTZ[1];
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Abstract: Understanding mechanisms underlying individual differences in opioid addiction vulnerability is essential for developing more effective, personalized strategies for prevention and treatment. In this study, we used RNA-seq to assess how gene regulation in the rat medial prefrontal cortex (mPFC) varies as a function of Withdrawal-Induced Anhedonia (WIA) after acute morphine administration. Our previous study showed that WIA predicts the magnitude of various measures of subsequent morphine self-administration (MSA). We also evaluated changes in gene regulation as a function of the magnitude of behavioral economic Demand and Reinstatement, two measures of MSA. Our initial experiments indicate that all three paradigms revealed a substantial number of genes whose expression correlated with the magnitude of behavior. One emerging theme was the involvement of cholinergic-pathway genes in WIA. Specifically, genes encoding cholinergic receptor subunits α4 and α5 (*Chrna4* and *Chrna5*) and the presynaptic acetylcholinesterase membrane anchor precursor PRiMA (*Prima1*) were among the top-ranked genes whose activity varied as a function of WIA magnitude. In addition, TCF7L2, a transcription factor implicated in nicotine addiction, was the highest ranked upstream regulator implicated in WIA magnitude. Our data also indicate that expression of a substantial number of genes covaries with both WIA and Demand or Reinstatement magnitude. For example, 56 of the 203 genes whose expression significantly covaried with the magnitude of Demand also covaried with WIA intensity. This points towards common genomic machinery underlying individual differences in acute morphine withdrawal and long-term MSA. Our initial assessment of alteration in epigenetic regulation via ATAC-seq showed that 15% ~ 28% of the dysregulated genes also exhibited significant changes in chromatin accessibility. These epigenetic changes were significantly involved in gene networks associated with reward signaling and synaptic plasticity, including endocannabinoid, opioid, and dopamine feedback pathways, as well as synaptic long-term potentiation and depression. While our ATAC-seq data partially indicated long-term epigenetic regulatory changes associated with MSA vulnerability and gene dysregulation, further work on other epigenetic marks (e.g., histone modifications) with these models is planned to provide a more comprehensive understanding of mechanisms underlying opioid addiction vulnerability.

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Poster

314. Addictive Drugs: General Neural Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 314.19

Topic: G.09. Drugs of Abuse and Addiction

Title: Examining the effects of oxytocin administration on dopamine release following chronic cocaine administration

Authors: *S. BERRETTA[1], P. NALAN[2], M. LOVE[3], R. PACE[4], D. B. LESTER[5];
[1]Univ. of Memphis, Univ. of Memphis, Memphis, TN; [2]Psychology, Univ. of Memphis, Bartlett, TN; [3]Univ. of Memphis, Memphis, TN; [4]Psychology, Univ. of Memphis, Moscow, TN; [5]Psychology Dept., Univ. of Memphis, Memphis, TN

Abstract: Oxytocin is being researched both clinically and preclinically as a possible treatment option for psychostimulant use disorder. Behavioral studies in rodents have shown that oxytocin administration can reduce effects of psychostimulants. Furthermore, a previous study from our lab showed that subchronic oxytocin administration decreased the dopaminergic response to a psychostimulant. These previous findings were observed in psychostimulant-naïve mice. The present study aimed to expand on our previous study by examining the effects of oxytocin administration on dopamine functioning in C57BL/6J mice that have been exposed to chronic cocaine (20 mg/kg i.p. daily for 8 days). After chronic cocaine exposure, one group of mice received the oxytocin treatment (1 mg/kg i.p. for 6 days) while another group of mice were administered saline. Altogether, there were three experimental groups: cocaine-oxytocin, cocaine-saline, and saline-saline. The day following the final injection, in vivo fixed potential amperometry was used to quantify VTA stimulation-evoked dopamine efflux in the nucleus accumbens (NAc) of anesthetized mice before and after a challenge injection of cocaine (20 mg/kg i.p.). Dopamine efflux was broken down into measurements of dopamine release and synaptic half-life, an indicator of reuptake rates and dopamine transporter (DAT) functioning. No differences were observed in baseline dopamine release or half-life; however, significant differences were observed in the percent change in dopamine half-life following the in-test cocaine challenge. Cocaine-oxytocin and cocaine-saline mice displayed a decreased percent change in dopamine half-life following cocaine compared to saline-saline mice. Thus, chronic exposure plus a withdrawal period led to a decreased dopaminergic response to cocaine, and oxytocin treatment during the withdrawal period did not alter this dopaminergic drug response. These findings do not fit with our previous findings showing that oxytocin treatment reduces the dopaminergic response to a psychostimulant, meaning that oxytocin does not have the same dopaminergic effect in drug-naïve and cocaine-exposed mice. More studies are needed to determine the neurochemical mechanisms and therapeutic potentials of oxytocin, especially in disease-state animal models.

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Poster

314. Addictive Drugs: General Neural Mechanisms

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Program #/Poster #: 314.20

Topic: G.09. Drugs of Abuse and Addiction

Title: Oxytocin receptor activation in the nucleus accumbens alters phasic dopamine release

Authors: P. NALAN[1], R. PACE[2], S. BERRETTA[3], M. LOVE[4], ***D. LESTER**[4];
[1]Psychology, Univ. of Memphis, Bartlett, TN; [2]Psychology, Univ. of Memphis, Moscow, TN;
[3]Psychology, [4]Univ. of Memphis, Memphis, TN

Abstract: The neuropeptide oxytocin alters the salience of rewards and is being investigated as a treatment for substance use disorder; however, the effects of oxytocin on reward-related neural circuits are not understood. The detection of rewarding stimuli is regulated in part by the mesolimbic dopamine system, with dopaminergic cell bodies in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc). The current study aimed to determine the effect of oxytocin receptor activation in the NAc on mesolimbic dopamine release. Fixed potential amperometry was used to measure stimulation-evoked dopamine release in the NAc of anesthetized C57BL/6J mice before and after an intra-NAc infusion of oxytocin (0.6 ug in 0.5 ul volume over a 1 min period). Oxytocin infused into the NAc significantly reduced dopamine release (-24%) relative to the vehicle infusion of phosphate buffered saline (PBS, -4%); however, the oxytocin infusion did not significantly alter the synaptic half-life of dopamine (-2%) relative to PBS (-4%). No differences were observed between males and females. These results suggest that oxytocin receptor activation in the NAc inhibits dopamine release but does not alter reuptake rates (i.e., dopamine transporter functioning). Such findings aid in understanding the neural mechanisms of oxytocin on the reward system and further provide implications for the utility of targeting the oxytocin system in pharmaceutical treatments for substance use disorder.

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Poster

315. Mechanisms for Addictive Substance Use and Abuse

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA U01DA051972

Title: Transcriptional initiation profiling of primary brain cells to study gene regulatory mechanisms associated with substance use disorders

Authors: *A. KUMAR, P. MONTILLA PEREZ, C. BENNER, F. TELESE;
Med., UCSD, La Jolla, CA

Abstract: Genetic factors influence individual vulnerability to substance use disorders (SUDs). Genetic variants in noncoding regulatory elements are thought to direct the function of key transcription factors. Transcription factors respond to a variety of signaling pathways to regulate the expression of target genes implicated in various cellular functions underlying addiction-related behavior. However, it is still unknown how noncoding regulatory elements respond to distinct stimuli in different cell types of the brain. Understanding these transcriptional mechanisms will shed light on the molecular mechanisms underlying addiction. To elucidate components of transcriptional regulatory networks associated with SUDs, we rely on capped-small (cs)RNAseq. CsRNA-seq detects transcription start sites (TSSs) of both stable and unstable RNA with single-nucleotide resolution. As such, csRNA-seq quantifies the activity of regulatory elements genome-wide with higher sensitivity and spatial resolution than previous epigenomic assays. Using csRNA-seq, we profiled TSSs in response to dopamine and depolarization medium in primary neurons and in response to interleukin-1 beta in primary astrocytes. The bioinformatic analysis identified TSSs that were differentially transcribed when compared with untreated cells, and we have begun to identify the transcription factor networks that regulate them. Furthermore, we hope to facilitate the annotation and functional characterization of GWAS risk variants with regulatory functions. In conclusion, this approach will gain novel insights into the role of gene regulatory elements involved in SUD and impacted by genetic variants in various cell types.

Disclosures: **A. Kumar:** A. Employment/Salary (full or part-time):; UCSD. **P. Montilla Perez:** A. Employment/Salary (full or part-time):; UCSD. **C. Benner:** A. Employment/Salary (full or part-time):; UCSD. **F. Telese:** A. Employment/Salary (full or part-time):; UCSD.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIMH Grant R01-30006291
NIDA R01D038613
UMSOM Substance use in pregnancy
UM MPowering the state
UMSOM dean's challenge ACCEL-MED

Title: Transcriptomic adaptations in reward brain regions in perinatal fentanyl exposed mice

Authors: *J. OLUSAKIN[1], G. KUMAR[2], C. HAGA[3], M. BASU[4], M. D. TURNER[7], J. B. ALIPIO[8], C. A. CALARCO[5], A. KELLER[5], S. AMENT[9], M. LOBO[6];
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MD; [4]Inst. for genome sciences, [5]Univ. of Maryland Sch. of Med., [6]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [7]UMB Sch. of Med., UMB Sch. of Med., Baltimore, MD; [8]Univ. of Maryland Baltimore, Univ. of Maryland Baltimore, Baltimore, MD; [9]Institue for Genome Sci., Univ. of Maryland school of Med., Baltimore, MD

Abstract: Since the early 2000s, there has been an increase in opioid consumption within the US, including among pregnant women. Use of the synthetic opioid fentanyl has increased ~300% within the last decade. Preclinical mice models of perinatal fentanyl exposure show reduced birth weights and increased mortality rates - findings parallel to observations in humans. Little is known about the molecular mechanisms underlying fentanyl use particularly during brain development. In this study, we show that perinatal fentanyl exposure alters a wide range of shared and unique transcriptional programs in juvenile mice. 10μg/ml fentanyl was administered in drinking water of pregnant C57BL/6 mice dams from embryonic day 0 (E0) through gestational periods until weaning at postnatal day 21 (P21). RNAs were extracted from tissue punches collected from 3 reward brain areas of juvenile aged mice (~P35) and processed for bulk RNA-sequencing. Brain regions analyzed include the nucleus accumbens (NAc), ventral tegmental area (VTA) and prelimbic (PrL) region of the prefrontal cortex. These experiments were performed in both sexes as we previously observed sex differences in some lasting mood related behaviors. Using a fold change cut-off of $\pm$ 0.3 and $p < 0.05$, we found that the VTA had the most differentially expressed gene substrate dysregulated in perinatal fentanyl exposure. The NAc had the most sex-specific differentially expressed genes. Gene ontology analysis reveal sex-specific biological functions within reward brain regions. Specifically, we observed neuronal guidance processes downregulated in the NAc of male juvenile mice while female mice had processes involved in neurotransmitter transport downregulated. We observed mitochondrial respiration to be upregulated in the male NAc and downregulated in the female VTA. Immune response processes were specifically observed to be upregulated in female VTA. Co-expression analysis reveal modular gene sets enriched in synaptic, cell adhesion and mitochondrial processes. Finally using enriched modular biological processes, we identified putative upstream transcriptional regulators that could be mediating these regional reward gene expression changes in perinatal fentanyl use. Collectively, this studyuncovers unique gene expression adaptations across reward brain regions relevant for lasting behaviors observed in perinatal fentanyl exposed mice.

Disclosures: J. Olusakin: None. **G. Kumar:** None. **C. Haga:** None. **M. Basu:** None. **M.D. Turner:** None. **J.B. Alipio:** None. **C.A. Calarco:** None. **A. Keller:** None. **S. Ament:** None. **M. Lobo:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.03

Topic: G.09. Drugs of Abuse and Addiction

Title: E-cigarette cue-reactivity in sign-trackers and goal-trackers

Authors: *P. KROM, A. DOUGLAS, M. BLANK, M. V. CHERKASOVA;
Psychology, West Virginia Univ., Morgantown, WV

Abstract: Cue-reactivity is an important predictor of addiction course and relapse. However, cue-reactivity is only observed after an addiction develops. As such, it is unclear to what degree cue-reactivity represents a state stemming from the addiction process versus a trait-like propensity towards developing cue-reward associations. Work in animal models has pointed to important individual variation in trait-like propensity to attribute incentive salience to reward-predictive cues that is associated with addiction-relevant behavioral and neurobiological features. These individual differences manifest as sign-tracking (ST) and goal-tracking (GT) behaviors during Pavlovian conditioning. Little research has attempted to translate ST and GT phenotypes to humans or relate them to cue-reactivity. The current study is first to examine electronic cigarette (ECIG) cue-reactivity in human sign-trackers versus goal-trackers. Individuals who were current ECIG users and have not used other tobacco products were classified as ST or GT based on and Pavlovian conditioning paradigm accompanied by eye-tracking and were exposed to two different cue types: ECIG cues (e.g., devices, vape clouds) and neutral cues (e.g., water). We compared ECIG cue-reactivity, assessed as cue-induced cravings, and EEG responses between sign-trackers and goal-trackers. Our preliminary data in 7 participants showed that all 3 sign-trackers and one out of 4 goal-trackers showed a cue-induced increase in cravings. Data collection is in progress and results from a more complete dataset will be presented. The findings will increase understanding of individual differences in addiction and cue-reactivity. This may have implications for treatment and prevention of addictive disorders.

Disclosures: P. Krom: None. **A. Douglas:** None. **M. Blank:** None. **M.V. Cherkasova:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.04

Topic: G.09. Drugs of Abuse and Addiction

Title: The molecular determinants of cannabis use disorder; leveraging co-occuring psychiatric GWAS to reveal novel genes and clinically actionable pathways.

Authors: *L. GRECO, W. R. REAY, C. V. DAYAS, M. J. CAIRNS;
Univ. of Newcastle, Callaghan, Australia

Abstract: Substance use disorders (SUDs) are highly heritable and remain a significant public health issue globally. Despite extensive research into the molecular underpinnings of cannabis use disorder (CUD) and the tremendous potential of genetics, there remains no effective treatment options available. Although most psychiatric disorders often only effect a small percentage of the population, the life-time co-occurrence of CUD in people with these

psychiatric disorders is considerably higher than the general population, which is indicative of a shared underlying biology. To localise the specific genetic overlap amongst these co-ocurring disorders, we conducted a gene-based and gene-set pairwise meta-analyses between CUD and five psychiatric disorder phenotypes; ADHD, Bipolar disorder (BIP), Major depressive disorder (MDD), OCD and schizophrenia (SZ) in over 2.8 million individuals. We investigated the involvement of 14,969 gene sets in four categories including Biological Process (BP), Cellular Component (CC), Molecular Function (MF) and Human Phenotype Ontology (HPO) and genes that passed multiple testing correction for the individual and pair-wise analyses were annotated to Allen Brain structures for adults and across several development stages (pre-natal-adolescent). These bivariate meta-analyses identified six associations not observed in the individual GWAS that were common hits in 3 or more paired disorders (MED19, SLC38A3, TMEM161B, FBXL17, CTNND1 and HYAL3). We then implemented a pharmagenic enrichment score (PES) approach for genetically informed drug repurposing on an independent cohort in the Australian Schizophrenia Research Bank (ASRB) and identified several pathways as potential therapeutic targets for high-risk individuals. Despite the high heritability and co-occurance of CUD in many psychiatric disorders, current Genome Wide Association studies (GWAS) for CUD have only been able to identify minimal genes and pathways involved in this disorder. Understanding the molecular determinants of CUD has the potential to identify new therapeutic targets, which may lead to improved clinical outcomes.

Disclosures: L. Greco: None. **W.R. Reay:** None. **C.V. Dayas:** None. **M.J. Cairns:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Institutional Support

Title: Executive function in young adults by alcohol and other drug (AOD) recovery status

Authors: A. LOWERY[1], D. FOUNDS[2], *E. HOLLIDAY[1];
[2]Computer Sci., [1]Kennesaw State Univ., Kennesaw, GA

Abstract: Substance use initiation peaks during emerging adulthood (ages 18-25), and initiation during this time associates with prefrontal cortex (PFC) hypoactivity, causing executive impairment. Collegiate recovery programs (CRPs) can facilitate student success while maintaining recovery, but existing literature does not allow for generalizable conclusions. In addition, emerging adults report low treatment-seeking motivation and poorer outcomes compared to adolescents and older adults. The CRP "Brain Games" study seeks to describe emerging adults' experience of substance use disorder (SUD) and subsequent recovery using (1) externally validated measures of executive function including the delayed discounting task,

number-letter task, stop signal task, and Sternberg working memory task, and (2) several self-reported surveys including a demographics questionnaire, the Perceived Stress Scale (PSS), a mindfulness questionnaire (MAAS), and the Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ). In addition, these measures will reveal individual differences contributing to variation in cognitive performance in students utilizing CRPs. Participants provided informed consent and completed the surveys and "brain games" remotely. Removal of incomplete and duplicate observations resulted in a data set containing 20 participants. Statistical analysis, conducted in R, included exploratory factor analysis (EFA), using the principal axes (PA) method due to the small sample size and Promax variable rotations to replicate EFA results retrievable from SPSS, and one-way analyses of variance. Ultimately, the results of this study help identify diverse neuropsychological correlates of emerging adulthood recovery and allow for targeted recovery efforts that support the stable and long-term recovery uniquely observed in emerging adults.

Disclosures: A. Lowery: None. **D. Founds:** None. **E. Holliday:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.06

Topic: G.09. Drugs of Abuse and Addiction

Support: Estonian Research Council grant PRG1473

Title: Delta-9-tetrahydrocannabinol alters the gene expression and enzymatic activity of DNA methyltransferases and Ten-eleven translocation enzymes in human peripheral blood mononuclear cells

Authors: *K. SOMELAR-DURACZ, K. ANIER, A. KALDA;
Pharmacol., Univ. of Tartu, Tartu, Estonia

Abstract: Exposure to cannabinoids produces long-term alterations in brain processes that are involved in addiction. The psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC) elicits its effects primarily through endocannabinoid type 1 (CB1) receptor in the central nervous system. Additionally, THC has been shown to indirectly affect dopamine release from the shell of *nucleus accumbens*, a key area related to reward, motivation and drug-seeking behavior. Several studies have indicated that epigenetic modifications are involved in the molecular processes underlying the development of addiction. Our earlier work has investigated the effect of cocaine on DNA methyltransferases (DNMTs) and ten-eleven translocation enzymes (TETs), which are epigenetic modifiers involved in DNA methylation and demethylation, respectively. Previously, we have identified a role for DNMTs in behavioral sensitization to cocaine. However, little information is known about the effects of THC on DNMTs and TETs. In the current study, we used an *in vitro* model of human peripheral blood

monoculear cells (PBMCs) to study the effect of THC on epigenetic editors. PBMCs serve as useful material to study the mode of action of THC, since PBMCs express both CB1 and CB2 receptor as well as dopamine receptors. Additionally, PBMCs are easily obtainable material of human origin in comparison to brain tissue. PBMCs from healthy male donors (n=8, aged 27-40 y) were collected and cultured *in vitro*. Daily incubations with tetrahydrocannabinol (200ng/ml) or vehicle were carried out for 1 hour in five consecutive days. PBMCs were collected 24 h after each incubation. qPCR was used to measure the mRNA levels of *DNMTs* and *TETs*. Enzyme activity of DNMTs and TETs was assessed with an ELISA-based assay. Our qPCR data demonstrated a time dependent effect of THC on the mRNA levels of *DNMT1, DNMT3a, DNMT3b* as well as on *TET1, TET2* and *TET3*. Moreover, THC incubations carried out for 1 h on two, three and four consecutive days lowered the enzyme activity of DNMTs compared to the control group, which returned to normal levels after 1h long incubations on 5 consecutive days. Interestingly, the activity of TETs were lowered after 1h incubation on one day and increased after 1h long incubations on two and three days, while the enzyme activity returned to control levels when incubated for 1 h on 4 and 5 consecutive days. Our results indicate that THC alters the gene expression and enzymatic activity of epigenetic modifiers DNMTs and TETs and can induce tolerance effect on the enzymatic activity of DNMTs and TETs.

Disclosures: K. Somelar-Duracz: None. **K. Anier:** None. **A. Kalda:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.07

Topic: G.09. Drugs of Abuse and Addiction

Support: 1R43DA050399-01A1

Title: Automated pupillometry for assessing withdrawal in patients with opioid use disorder

Authors: *C. J. EVONKO, K. C. VIGILANTE, N. B. LELI, R. S. POND, Jr., E. G. VAN LEUVEN, D. A. MACQUEEN;
Psychology, Univ. of North Carolina Wilmington, Wilmington, NC

Abstract: Opioid misuse has contributed to countless overdose deaths over the last few decades. The economic cost of fatal opioid overdoses was over $550 billion as of 2017. The most effective treatment of opioid use disorder (OUD) is medication assisted treatment (MAT) with agonist drugs such as methadone and buprenorphine. While MAT is a popular and effective option, patient retention is an issue due to the challenges of stabilizing patients on an appropriate MAT dose during their induction onto the drug. Dosing that is not optimal can result in patient dropout or potential overdose. Accurate and objective quantification of opioid withdrawal can be beneficial in optimizing dose titration during induction. The goal of the present study was to validate the use of cellphone based pupillometry for the assessment of withdrawal in OUD.

Forty-Six patients were recruited from the patient population at Coastal Horizons Substance abuse Center that is currently engaged in MAT for opioid dependence. Patients were required to come in daily for fourteen consecutive days to report on any daily drug use, their perceived craving and withdrawal, and to complete an automated eye examination prior to receiving their daily dose of MAT. Pupillometry measures and self-reported craving and withdrawal measures were compared against the time since last dosing to evaluate the sensitivity of measures to withdrawal. A multilevel modeling approach was conducted to account for the nested data. There was a significant cross level interaction of MAT moderating the association between percent change in pupil size and time since last dosing. The percent change metric was significantly associated with time since dose, though only amongst those receiving methadone. To evaluate the effects of opioid dose, doses of both drugs were converted to morphine milligram equivalents (MME). As with drug, mixed model analyses revealed a significant cross level interaction of MME moderating the association between percent change and time since last dose. The association between pupillometry and time since dose was significant only for those receiving high doses. Given that participants on methadone were receiving significantly higher MME doses as compared with those on buprenorphine, low dose concentrations likely accounted for the failure to detect an association between withdrawal and pupillometry amongst those receiving buprenorphine. Neither self-reported craving nor withdrawal was significantly associated with dose timing. These findings suggest that pupillometry measures are superior to commonly used self-report measures for assessing withdrawal in the context of MAT for OUD.

Disclosures: C.J. Evonko: None. **K.C. Vigilante:** None. **N.B. Leli:** None. **R.S. Pond:** None. **E.G. Van Leuven:** None. **D.A. MacQueen:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH-NIDA Grant R00DA045749

Title: Effects of repetitive Transcranial Magnetic Stimulation to Prefrontal Cortex on State Anxiety in People Who Smoke Cigarettes

Authors: *M. APOSTOL[1], N. PETERSEN[2];
[1]UCLA, [2]UCLA, Los Angeles, CA

Abstract: Tobacco use disorder and cigarette smoking are leading causes of preventable disease and death worldwide. A barrier to smoking cessation is the severity of withdrawal symptoms, which past research has found are related to anxiety; however, the exact nature of the relationship is unclear (Garey et al., 2020). Noninvasive brain stimulation is a promising treatment for anxiety and tobacco use disorders alike. Past work has found that repetitive

Transcranial Magnetic Stimulation (rTMS) to the dorsolateral prefrontal cortex (dlPFC) may be an effective treatment for reducing both withdrawal and anxiety symptoms (Paes et al., 2011; Zangen et al., 2021). The current study was designed to determine if rTMS to dlPFC reduced cigarette withdrawal symptoms, and whether this reduction was related to state anxiety - feelings of anxiety in the present moment. The three predictions were that 1) there would be a positive relationship between withdrawal and anxiety symptoms, 2) rTMS to dlPFC would lead to a significant reduction in withdrawal symptoms, and 3) a significant proportion of this reduction would be accounted for by changes in state anxiety. Participants (N = 29) were adults ages 18 - 45 that reported daily use of 5 or more cigarettes. After an in-person screening, participants received rTMS to dlPFC (experimental site) and the visual cortex (v5; control site) in a randomized, within-subjects design. They completed fMRI scans, the Shiffman-Jarvik Withdrawal Scale (SJWS), and the State-Trait Anxiety Inventory (STAI) Form Y1 before and after each rTMS treatment. There was a significant positive relationship between withdrawal and anxiety, $r = 0.53$, $p < .001$, $R^2 = 0.28$. Two preliminary paired samples t-tests were conducted to test the second prediction, which found a significant decrease in SJWS scores after dlPFC stimulation (pre: $M = 3.63$, $SD = 1.22$; post: $M = 3.39$, $SD = 0.98$)[$t(21) = 1.76$, $p = .047$], and no significant decrease after rTMS to v5 (pre: $M = 3.46$, $SD = 1.25$; post: $M = 3.39$, $SD = 1.01$)[$t(21) = 0.40$, $p = .347$]. However, when a linear mixed-effects model was calculated to determine the effects of rTMS site on changes in withdrawal and anxiety, no significant effects were detected, $p = .275$. A power analysis ($\alpha = .05$, $1 - \beta = 0.8$, Cohen's $d = 0.24$) indicated that data should be collected from 108 participants. Although more data is needed, these preliminary results are evidence that rTMS to dlPFC reduces the severity of cigarette withdrawal symptoms. These findings will help advance our understanding of how state anxiety is related to withdrawal symptoms, with the hope of promoting long-term smoking cessation. This study is preregistered at: https://osf.io/82s6p.

Disclosures: **M. Apostol:** None. **N. Petersen:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.09

Topic: G.09. Drugs of Abuse and Addiction

Title: Single Exposure Conditioned Place Preference to Ketamine in Mice

Authors: H. JONES, C. KREMER, ***J. E. GRISEL**;
Bucknell Univ., Lewisburg, PA

Abstract: The veterinary anesthetic ketamine has shown promising therapeutic effects as an antidepressant, though its mechanism of action in reducing depressive symptoms is still debated. Ketamine is also recreationally used, and abused, so developing a better understanding of both the therapeutic and recreational effects is needed. Previous research suggests that females are

more sensitive to ketamine than males, but limited research is available to understand possible sex differences in the reinforcing and rewarding effects of ketamine. Experiment 1 investigated the initial subjective rewarding effects of ketamine in adult, naïve male and female C57BL/6J and DBA/2J mice using a single exposure conditioned place preference paradigm. On Day 1, animals are given an intraperitoneal injection of ketamine (10 mg/kg or 20 mg/kg) or saline and placed into one side of a three-chambered apparatus, with a unique floor texture. On the Day 3, animals that received ketamine on Day 1 are given saline, and vice versa, and placed on the alternate side of the apparatus with a different floor texture (the protocol is fully counterbalanced). Animals are undisturbed on Days 2 and 4. On Day 5, following saline injection, mice are given free access to the two conditioning chambers as well as a neutral central region (Grisel et al. 2014). Females of both strains were more likely than their male counterparts to find the drug reinforcing. Experiment 2 evaluated the contribution of the opioid peptide b-endorphin to the initial subjective rewarding effects of ketamine by comparing b-endorphin deficient (βE -/-; B6.129S2-Pomctm1Low/J) mice with control (C57BL/6J) mice in the single exposure conditioned place preference paradigm used in Experiment 1. Compared to C57BL/6J controls, mice deficient in b-endorphin were insensitive to the rewarding effects of ketamine, as well as the drug's locomotor effects. Taken together, these data suggest that the effects of ketamine are dependent on sex and b-endorphin, suggesting that individual differences in sensitivity may modulate the reinforcing and therapeutic effects of ketamine.

Disclosures: H. Jones: None. **C. Kremer:** None. **J.E. Grisel:** None.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.10

Topic: G.09. Drugs of Abuse and Addiction

Support: R43DA050399-01A1

Title: Identifying opioid use and withdrawal with biometric data obtained from a wrist-worn wearable device

Authors: *D. A. MACQUEEN[1], J. C. P. B. SHAW[2], W. D. WASHINGTON[1], M. M. ENGELHARD[3], J. C. EVONKO[1], T. M. SUGDEN[2], D. REESER[2];
[1]Psychology, Univ. of North Carolina Wilmington, Wilmington, NC; [2]OpiAID, Wilmington, NC; [3]Biostatistics and Bioinformatics, Duke Univ., Durham, NC

Abstract: Opioid misuse has reached epidemic levels in the United States, claiming the lives of more than 75,000 people in 2021. Opioid agonist therapies are the gold standard treatment for opioid use disorder (OUD) and have been confirmed by meta-analysis to reduce associated mortality. Poor retention of patients during treatment induction (initial four weeks) limits the impact of this approach. Dose titration during induction is critical to reducing opioid withdrawal

and/or overdose and thus, patient retention. The present study sought to develop algorithms to detect acute instances of opioid use and periods of significant withdrawal using biometric data derived from a wrist-worn wearable device (Empatica E4). Fourty-two OUD patients currently enrolled in an opioid agonist therapy program were recruited to wear the device for 14 days during which they received a treatment dose (methadone or buprenorphine). Drug, dose, and timing of administration were documented, and patients were asked to report on opioid craving, withdrawal, and past 24-hour substance use daily. Time-series features summarizing raw accelerometer, photoplethysmograph, and electrodermal readings were extracted from raw sensor data collected by the wearable device. XGBoost binary classification models were trained to (a) distinguish between treatment sessions and an equal number of non-treatment sessions of the same length selected at random; and (b) distinguish between periods immediately prior to treatment with high (Clinical Opiate Withdrawal Scale > 5) versus low (≤5) self-reported withdrawal. Models were trained and evaluated using 5-fold cross validation, with all data from a given participant assigned to the same fold. Performance was quantified as the average area under the receiver operating characteristic curve across all folds (AUC). The resulting models achieved excellent performance when detecting acute opioid use (AUC=0.970) and high-withdrawal periods (AUC=0.996). These findings demonstrate that instances of acute opioid use and periods of opioid withdrawal can be identified with the biometric information obtained from sensors available in modern wrist-worn wearables. Though additional study is necessary to demonstrate that high levels of prediction generalize to the broader OUD patient population, these algorithms hold promising potential to improve OUD treatment outcomes. Remote detection of opioid use and withdrawal is likely to have beneficial applications in guiding induction to opioid agonist therapy, managing a tapered approach to opioid abstinence, and/or for the remote patient monitoring of outpatients, particularly in rural settings.

Disclosures: **D.A. MacQueen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OpiAID. **J.C.P.B. Shaw:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OpiAID. **W.D. Washington:** None. **M.M. Engelhard:** None. **J.C. Evonko:** None. **T.M. Sugden:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OpiAID. **D. Reeser:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); OpiAID.

Poster

315. Mechanisms for Addictive Substance Use and Abuse

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 315.11

Topic: F.04. Neuroimmunology

Title: Building a method for assessing toluene concentrations in biospecimens using gas chromatography with flame ionization detection

Authors: *D. W. SVENSON[1], M. M. NADDAF[2], S. E. BOWEN[1];
[1]Wayne State Univ., Wayne State Univ., Detroit, MI; [2]Col. of Osteo. Med., Michigan State Univ., East Lansing, MI

Abstract: Inhalant use is a prevalent and pernicious form of substance misuse that disproportionately impacts certain demographics, such as young adolescents. Many inhalants, such as toluene, produce behavioral and pharmacological effects similar to those of ethanol. However, the neurobehavioral (e.g., motoric coordination) dose-response effects for toluene have not been fully characterized to the extent of ethanol. The present experiment aimed to build a method for the detection of toluene in blood and organ tissue using gas chromatography with flame ionization detection (GC-FID). Male Swiss-Webster mice (N's = 6-12) were exposed to one of five behaviorally active concentrations of toluene (0, 500, 1000, 2000, or 4000 parts per million [ppm]). Mice were immediately euthanized post-exposure to excise the heart, lungs, liver, kidneys, and brain, as well as to obtain blood samples. Biospecimens were immediately placed into glass chromatography vials with 2 mL of methanol (MeOH) and sealed. Six standards (0, 5, 25, 50, 75, and 100 ppm) were created from a toluene/methanol (400 ppm) stock solution. The improved GC-FID method was built following data collection using a ramp-up program in the GC oven, starting at 50ºC and increasing over 5 min to 175ºC in combination with a detector temperature of 275ºC. Toluene concentrations in brain, lungs, and heart demonstrated a linear progression that corresponded to the concentration of toluene administered (average r = .971, p's < .05). This contrasted with the liver and kidneys, which showed non-linear changes in bioavailability. To improve replicability and peak resolution, a second method was developed using the same set of standards but with two additional unknowns (X and Y ppm) for the purpose of validating the method. Three standards were run non-consecutively, yielding linear plots (R^2's = .996 - .999). The standards used to assess the random values yielded a regression equation (Y = 0.060X + 0.063 (R^2 = .995)) that successfully predicted unknown values with limited error (0.22 - 2.60% variation). The method developed in this experiment demonstrated great precision. However, further experimentation and validation is required. Future experiments with this method will be used to correlate blood toluene dose-response curves with toluene-induced neurobehavioral effects (e.g., cerebellar ataxia).

Disclosures: D.W. Svenson: None. **M.M. Naddaf:** None. **S.E. Bowen:** None.

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 316.01

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in VTA GABA activity and affective behavior during withdrawal

Authors: *D. J. KALAMARIDES, A. SINGH, S. L. WOLFMAN, J. A. DANI;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Negative affect during opioid withdrawal contributes to high relapse rates and women report more negative affect than men. Ventral tegmental area (VTA) inhibitory activity contributes to both reward and aversive behaviors, however, VTA GABA dynamics and associated negative states have not been fully characterized during extended withdrawal, particularly with little consideration for sex as a biological variable. To model protracted withdrawal in rodents, mice underwent repeated morphine exposures followed by up to one week of abstinence. Affect related behavior and synaptic slice physiology was performed either one day or one week after the final morphine exposure. Mice of both sexes exhibited negative states, but results varied by assay. Male opioid withdrawn mice had decreased latency to immobility in the tail suspension test, while female opioid withdrawn mice had decreased grooming during the splash test. Sex specific effects were also observed during acute slice recording in VTA dopamine neurons. Neurons from morphine treated male mice had canonically increased GABA release probability, but inputs to dopamine neurons from morphine treated female mice did not differ from drug naïve controls. Underlying mechanisms of negative affect may differ by sex despite both males and females exhibiting some form of negative states during withdrawal. These results further our understanding of mesolimbic circuitry changes caused by opioids and highlight the need for nuanced behavioral modeling and inclusion of female subjects in future studies to balance the lopsided field.

Disclosures: D.J. Kalamarides: None. **A. Singh:** None. **S.L. Wolfman:** None. **J.A. Dani:** None.

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 316.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA049531 (EMB)
Tufts University Russo Family Award (EMB, FMV, EY)
Tufts Initiative on Substance Use and Addiction Award (EMB & EY)

Title: Neural mechanisms of feeding dysregulation following prenatal opioid exposure in male and female rats

Authors: *K. R. GILDAWIE[1], S. B. ISGATE[1], K. E. BUDGE[1], L. JACKSON[1], F. M. VASSOLER[1], E. YEN[2], E. M. BYRNES[1];
[1]Tufts Univ. Sch. of Vet. Med., North Grafton, MA; [2]Dept. of Pediatrics, Tufts Univ. Sch. of Med., Boston, MA

Abstract: Opioid use disorder among women of reproductive age has escalated dramatically in recent years, increasing the likelihood of fetal drug exposure and subsequent development of neonatal opioid withdrawal syndrome (NOWS). While medication-assisted treatment (MAT),

e.g. buprenorphine or methadone, provides treatment for opioid use disorder, over 50% of infants born to mothers on MAT experience hyperphagia, an early withdrawal sign that is associated with more severe withdrawal course and varies by sex. It is therefore critical to investigate the potential sex-dependent mechanisms underlying opioid-induced feeding dysregulation. To do this, adult Sprague Dawley females were implanted with osmotic minipumps filled with methadone (10 mg/kg/day) or buprenorphine (1 mg/kg/day) dissolved in dH_2O or saline control (2.5 µl/hour for 28 days) and mated one week later. On postnatal day (PND)1, pups were culled, weighed, and cross-fostered with drug-naïve dams. On PND2, PND7 and PND12, pups were tested for hyperphagia via assessment of postnatal feeding behavior. Following feeding sessions with the donor dam, brain tissue was harvested and cryosectioned. Recent human work implicates alterations in the expression of salivary dopamine receptor type 2 (*DRD2*) and neuropeptide Y2 receptor (*NPY2R*) in NOWS-related hyperphagia. Moreover, microglia are neuroimmune cells that play a key role in the development and response to opioids through the toll-like receptor 4 pathway, which is partially regulated by the glycoprotein cluster of differentiation (CD)200. Therefore, RNAscope in situ hybridization was performed to quantify the expression of *DRD2, NPY2R,* and *CD200* in the ventral striatum (reward) and arcuate nucleus of the hypothalamus (feeding). Remaining pups were weaned at PND21 and in adulthood (PND70) began sucrose self-administration to assess the motivational strength of palatable food in MAT-exposed animals compared to saline vehicles. Training occurred on a fixed ratio (FR)1 schedule for 10 days followed by 3 days of FR5. Animals were then transitioned to six days of progressive ratio (PR) testing to measure the breaking point of food-motivated behavior. Our findings suggest that maternal MAT results in reduced offspring body weight at PND1, as well as sex-dependent developmental and long-term feeding dysregulation - where males experienced increased neonatal feeding and enhanced food-motivated behavior - that may be driven by changes in the expression of *DRD2, NPY2R,* and *CD200* in the brain. These data provide insight into the mechanisms driving MAT-induced changes in neural and glial modulation of reward- and feeding-related behaviors.

Disclosures: K.R. Gildawie: None. **S.B. Isgate:** None. **K.E. Budge:** None. **L. Jackson:** None. **F.M. Vassoler:** None. **E. Yen:** None. **E.M. Byrnes:** None.

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA 000523-14

Title: Pharmacological effects of non-fentanyl synthetic opioids that are appearing in recreational drug markets

Authors: *M. H. BAUMANN, G. C. GLATFELTER, L. CHEN, M. TSAI, D. WALTHER, L. SHI;
IRP, NIDA, NIH, Baltimore, MD

Abstract: Illicitly manufactured fentanyl is driving the current opioid crisis, but a number of non-fentanyl synthetic opioids are emerging on recreational drug markets worldwide. Little information is available about the pharmacology of most novel opioids when they first appear on the street. U-47700, brorphine, and isotonitazene are examples of non-fentanyl synthetic opioids associated with human overdose fatalities. We investigated the pharmacology of U-47700, brorphine, and isotonitazene using *in vitro* and *in vivo* methods. *In vitro* opioid receptor binding assays were carried out in rat brain membranes, whereas inhibition of forskolin stimulated cAMP was measured in cells transfected with mu-opioid receptors (MOR). *In vivo* experiments were carried out in male Sprague-Dawley rats to examine the effects of s.c. drug administration on hot plate latency, catalepsy scores, and body temperature. Non-fentanyl opioids displayed selective affinity for MOR, with Ki values of 15.4 nM for U-47700, 32.8 nM for brorphine, and 14.9 nM for isotonitazene. The compounds also induced robust inhibition of cAMP accumulation, and all acted as full agonists at MOR, with EC_{50} values of 4.46 nM for U-47700, 2.06 nM for brorphine, and 0.05 nM for isotonitazene. When administered to rats, all compounds produced dose-dependent antinociception, catalepsy, and hypothermia. Findings from the hot plate test revealed that ED_{50} values for U-47700 (0.404 mg/kg), brorphine (0.086 mg/kg), and isotonitazene (0.007 mg/kg) were much more potent than morphine (4.164 mg/kg). *In vivo* potency estimates for the drugs were positively correlated with *in vitro* EC_{50} values at MOR but not Ki values. Our results demonstrate that non-fentanyl synthetic opioids can be much more potent than morphine *in vivo*, posing serious risks for unsuspecting users. Importantly, *in vitro* functional potencies at MOR predict *in vivo* effects of the drugs better than affinity values at the receptor.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant F31DA056194
Whitehall Foundation Grant ID# 2017-12-54
NARSAD Young Investigator Grant 27197

Title: Opioid withdrawal potentiates synaptic output from the ventral pallidum to the lateral habenula

Authors: *J. TOOLEY, M. C. CREED;
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Abstract: The aversive state of withdrawal is a main reason people with opioid use disorder continue or relapse on opioids. In opioid use disorder, the goal is no longer to achieve euphoria, but to avoid the dysphoric symptoms of withdrawal. The ventral pallidum (VP) has previously been implicated in driving relapse behavior after withdrawal. Recent studies from our group and others have shown that in reward seeking paradigms, activity of a glutamatergic subpopulation of VP neurons (VP_{Glu}) constrains reward seeking and direct activation of these neurons is aversive. VP_{Glu} neurons send a dense projection to the lateral habenula (LHb), a region that encodes aversive stimuli and has also been causally implicated in the emergence of negative affective states in drug withdrawal. However, it is completely unknown how self-administration and withdrawal from prescription opioids alters the output and function of VP_{Glu} neurons. With neurochemical, genetic, and electrophysiology approaches, we discovered that withdrawal from oxycodone increases the excitability of glutamatergic VP neurons and potentiates their output to the LHb. We performed fluorescent in situ hybridization experiments to establish whether mu opioid receptors are expressed on glutamatergic VP neurons and we found that these neurons are enriched in mu opioid receptors. Using patch-clamp electrophysiology, we recorded from VP_{Glu} neurons or LHb neurons while optogenetically activating VP_{Glu} neurons to determine the effect of mu opioid receptor activation on transmission at this synapse. Application of mu opioid agonists decreased excitability of VP_{Glu} neurons and modulated VP_{Glu} output to the LHb. We also investigated the effect of withdrawal from oxycodone self-administration on these neurons. After withdrawal, VP_{Glu} neurons are more excitable relative to oxycodone-naïve neurons. Finally, protracted withdrawal increased the frequency of evoked quantal events from VP_{Glu} neurons to the LHb while the amplitude of these events remained the same. Together, our results suggest that withdrawal from oxycodone self-administration increases the excitability of VP_{Glu} neurons and potentiates their synaptic output to the LHb. This may be a neural mechanism underpinning the noxious state of opioid withdrawal that dives relapse.

Disclosures: J. Tooley: None. **M.C. Creed:** None.

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.05

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1S10OD018124-01A1 (TA)

Title: Differential c-Fos expression in rodents with acute heroin exposure

Authors: *B. EVANS, S. BALLARD, K. NEWMASTER, Y. KIM, P. GRIGSON;
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Abstract: While we are familiar with brain areas and pathways that are implicated in opioid use disorder (OUD), we do not have a full understanding of the neural circuits activated upon drug exposure. In order to identify areas of the brain most activated by opioids, we ran a study using transgenic c-Fos-GFP mice and naïve wild-type Sprague-Dawley rats acutely injected with saline interperitoneally (ip) or heroin (10 mg/kg, ip) and examined brain-wide activity patterns using quantitative high-resolution mapping methods. Two-photon tomography was used on the mouse brains and light sheet microscopy on the rat brains. In the mice, we examined c-Fos expression in the Nucleus Accumbens (NAc), Lateral Hypothalamus (LH), Orbitofrontal Cortex (OFC), Cingulate Cortex (CC), Basolateral Amygdala (BLA), Habenula, Nucleus Tractus Solitarus (NTS), Parabrachial Nucleus (PBN), Locus Coeruleus (LC), and Periaqueductal Gray (PAG). These regions are known to be highly activated by drug and most evidenced an increase in c-Fos expression with acute heroin exposure. For the rats, we looked at c-Fos activation patterns in the cortex following the acute administration of saline (ip) or heroin (10 mg/kg, ip), in rats pretreated subcutaneously (sc) with saline or the glucagon-like protein-1 receptor agonist (GLP-1RA), liraglutide. There is some evidence in literature that GLP-1RAs can treat different substance use disorders in rodents and our lab has found that GLP-1RAs can reduce OUD behaviors in rats. Results showed a trending increase in c-Fos activation in mice with heroin exposure compared to mice who received saline. Also, there was increased c-Fos signaling in the cortex of the rat following the acute administration of heroin and this activation pattern was reversed by pretreatment with the GLP-1RA, liraglutide. Taken together, the data show the impact of the acute administration of heroin on brain, and reversal of these activation patterns in both mice and rats following treatment with the known satiety agent and GLP-1RA, liraglutide. As such, GLP-1RAs may mitigate not only relapse in drug-experienced subjects, but acquisition of drug-taking as well.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.06

Topic: G.09. Drugs of Abuse and Addiction

Support: UNTHSC Seed Grant

Title: Effects of Stress and Experimenter Related Experience on Brain Connectivity using fMRI and Network Analysis

Authors: *K. BRUNETTI[1], L. M. COLON-PEREZ[2];
[1]UNTHSC, UNTHSC, Fort Worth, TX; [2]Pharmacol. and Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Neuroimaging, particularly functional Magnetic Resonance Imaging (fMRI), is becoming an essential tool in translational studies in neuroscience. A theme of interest is the effects of pharmacological agents on the brain, particularly opioids such as morphine, and how it modifies in vivo functional brain circuits. A simple approach to determine the pharmacokinetics and acute effects of drugs are to deliver drugs intraperitoneally (IP) by the experimenter. Although this approach is used extensively in conjunction with neuroimaging, one must be mindful of the potential confounding effects of the experience of rodents to the experimenter and the IP injection itself. In behavioral neuroscience, a standard approach is to habituate animals to the experimental techniques and experimenter. Habituation diminishes the physiological or emotional response to a frequently repeated stimulus. In prior studies without habituation, we have observed changes in brain connectome indices following a single saline IP injection, one-hour vs. 24 hours post-injection. We observed a 57% decrease in brain network clustering parameters, a 20% reduction in network distance, and a 22% reduction in the small-world organization. To this end, we want to determine whether a habituation period stabilizes brain connectivity markers utilizing fMRI neuroimaging in rats administered morphine. Our study will habituate rodents by exposing them to the experimenter and doing mock injections over seven days. After the habituation period, the rats will receive an IP injection of morphine or saline one hour before a BOLD fMRI scan. We have two control cohorts. The first cohort is a negative control, which will be naive animals that will be anesthetized and scanned. The second cohort is a positive control, which will not be habituated and will receive an IP injection of either morphine or saline one-hour before MR imaging, with no habituation. BOLD fMRI will be acquired with a spin-echo EPI sequence for a total acquisition time of 20 mins. We will present functional connectome data using 150 regions of interest (ROI) based on a Paxinos-Watson atlas-guided segmentation. The networks are characterized by graph theory indices. Following the fMRI scan, animals will have blood withdrawn via the tail vein for markers of stress that will inform us of the effects of a single IP injection on brain connectivity fMRI markers. We will show that habituating rodents leads to a decrease in stress induced by the injection and more stable brain markers of connectivity derived from fMRI and a better understanding of the effects of opioids on brain connectivity.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.07

Topic: G.09. Drugs of Abuse and Addiction

Support: Start up fund from University of Kansas
NIH-DA050908

Title: Insulin-like growth factor 1 and its receptor in prefrontal cortex regulates heroin-induced behavioral and synaptic plasticity

Authors: *S. YUE, Y. WANG, G. LI, A. SINGH, Z. WANG;
Pharmacol. and Toxicology, The Univ. of Kansas, Lawrence, KS

Abstract: Insulin-like growth factor 1 and its receptor in prefrontal cortex regulates heroin-induced behavioral and synaptic plasticity Shuwen Yue[a], Yunwanbin Wang[a], Archana Singh[a], Zi-Jun Wang[aa] Department of Pharmacology & Toxicology, University of Kansas, Lawrence, Kansas, USAOpioid use disorder (OUD) is a chronic, relapsing mental illness characterized by compulsive drug seeking and vulnerability to relapse. However, the understanding of the neurobiology of OUD is still unclear. Clinical studies show that the neuronal responses to stimuli in the prefrontal cortex (PFC) from individuals with OUD are disrupted. Consistently, preclinical data also report opioid-induced synaptic dysfunction in the PFC. Given the critical role of PFC in regulating opioid-related behaviors, it is vital to investigate the molecular mechanisms underlying opioid-induced PFC dysfunction and its role in shaping opioid-induced behavioral plasticity. Increasing studies have shown that insulin-like growth factor 1 (IGF1) and IGF1 receptor (IGF1R) regulate synaptic transmission, but the involvement of IGF1/IGF1R in opioid addiction-induced synaptic deficits remains unknown. Here we used a mouse heroin self-administration (SA) model to investigate the role of IGF1/IGF1R on heroin-induced behavioral and synaptic plasticity. We first found that IGF1 in PFC was decreased after prolonged abstinence from heroin SA. Moreover, intra-PFC IGF1 administration attenuated while IGF1R selective knockdown in PFC pyramidal neurons potentiated heroin-seeking behavior. Furthermore, we used whole-cell patch-clamp method to detect changes in synaptic plasticity. Our data showed that intra-PFC IGF1 administration restored heroin abstinence-induced decrease in α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor- and N-methyl-D-aspartate (NMDA) receptor-mediated evoked excitatory postsynaptic currents (eEPSCs). In addition, IGF1 also recovered the elevated AMPA/NMDA ratio in response to heroin-associated cues in mice underwent heroin abstinence. These data indicate that IGF1/IGF1R system in the PFC play a key role in regulating heroin-induced behavioral and synaptic plasticity, which will provide a novel therapeutic target for the development of OUD treatment strategies.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.08

Title: WITHDRAWN

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 316.09

Topic: G.09. Drugs of Abuse and Addiction

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Title: Imaging opioid biosensors across primary neurons, acute brain slices, and in vivo photometry

Authors: *C. H. KIM[1], A. K. MUTHUSAMY[2], H. A. LESTER[1];
[1]Biol. and Biol. Engin., [2]Chem. and Chem. Engin., Caltech, Pasadena, CA

Abstract: The ongoing opioid crisis poses an increasing health burden due to opioid use disorder (OUD) and opioid-related mortality by overdose. Elucidating molecular mechanisms of tolerance and dependence and creating tools to inform personalized drug dosing can reduce the burdens of OUD. For both cases, the Lester lab studies the pharmacokinetics of drugs of abuse using genetically encoded fluorescent biosensors to detect the concentration of these drugs in several biological preparations. These biosensors consist of a periplasmic binding protein, a mutated OpuBC (substrate binding protein for choline transporter), and a circularly permuted GFP.
We have previously reported methods of mutating OpuBC to recognize a variety of drug classes and now report a biosensor selective for S-methadone. HeLa cells were transfected with this biosensor and spinning disk confocal microscopy confirmed the subcellular localization of biosensor targeted to the plasma membrane (PM), endoplasmic reticulum (ER), and the Golgi apparatus. We then bath perfused HeLa cells with S-methadone and observed [S-methadone] in the ER that nearly matched that of the PM and an accumulation in the Golgi by a factor of 2.9 to 4.4x across the relevant pH range. Prior work has shown that opioids act as pharmacological chaperones in the ER and elicit signaling from acidic compartments, including the Golgi apparatus. We show the first evidence of an opioid drug accessing these organelles where endogenous peptides cannot.
To measure whole animal pharmacokinetics, we validated a fentanyl biosensor in vivo using an AAV9-hSyn construct. In primary mouse neuron cultures, we tested a multiplicity of infection (MOI) ranging 10,000 to 500,000. The biosensor could be imaged using a MOI as low as 10,000, and the optimal MOI was ~100,000. We performed stereotaxic surgery to infect the VTA of mice. After two weeks, we sliced the region and imaged under artificial cerebrospinal fluid while perfusing 1 μM fentanyl. We observed a dynamic range of $\Delta F/F0$ ~1.0, within a factor of two of the primary culture experiments. Finally, we implanted a fiber optic implant to measure the real-time fentanyl pharmacokinetics in a behaving mouse after an intraperitoneal injection of

fentanyl. A null biosensor negative control showed no response. Histology confirmed the fiber placement and biosensor expression.
Our work enables real-time pharmacokinetics in animals to study how dosage affects behavior. Creating biosensor color variants will enable correlation of the activity of neurons using other biosensors. Studying subcellular to whole animal pharmacokinetics will provide a means to test hypotheses of mechanisms underlying addiction.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P20GM103506

Title: Gene expression of ΔFosB, EFR3A, SYT4, CX3CR1, and FLT1 in the hippocampus of postmortem human brain from opioid overdose

Authors: M. ZRZAVY[1], L. HERRERA[2], M. VANHORN[2], C. TYLEK[3], ***L. JABBOUR**[2]; [1]Fordham Univ., New York, NY; [2]Franklin Pierce Univ., Rindge, NH; [3]AbbVie, Worcester, MA

Abstract: FosB is a transcription factor present in normal cells, while its truncated form, ΔFosB, was reported to be highly expressed following chronic drug exposure. ΔFosB is unique in that it accumulates in response to repeated stimulation due to its unusual protein stability. Animal studies have established the role of ΔFosB in addiction, however, little is known about it in humans with opioid use disorder. In collaboration with the New Hampshire Medical Examiner, a unique human postmortem tissue collection was obtained following opioid overdoses. We dissected hippocampi from both control and opioid cases. Using immunohistochemistry, we assessed the presence of the FosB and ΔFosB proteins. Qualitative analysis revealed a consistent nuclear appearance of ΔFosB protein in the dentate gyrus and cornu ammonis regions of the hippocampus, while FosB was in contrast, barely visible or absent in most cases tested. Therefore, as seen in the rodent model, ΔFosB is the dominant form of the *fosB* gene expressed in the opioid-exposed human brain and may have a similar role in humans. That is: ΔFosB gradually converts acute drug responses into relatively stable adaptations that contribute to the long-term neural and behavioral plasticity that underlies addiction. Additionally, we used a comprehensive multiplex gene expression analysis assay (Nanostring®), to evaluate gene expression in the hippocampus (control n=4; opioid n=5). We normalized against housekeeping genes and we normalized for batch, sex, and age differences. Following these stringent normalization steps, we identified 9 significantly differentially expressed genes (with a p-value below 0.05). Out of the 9 biologically significant genes, *EFR3A, SYT4, CX3CR1, and FLT1* presented with distinct means between control and opioid samples, which is also significant.

Transcription factor E2F3a mediates cocaine's effects on gene expression and addiction-related behaviors (Cates, Nestler, 2019), while SYT4 is implicated in synaptic transmission. CX3CR1, a receptor expressed on microglia, may protect against microglial neurotoxicity, while FLT1, which is a receptor implicated in angiogenesis, is modulated by morphine. Our findings provide new insights into gene expression in cases of opioid overdose. Work is underway to better understand the role of these genes in the context of opioid use disorder so that strategies can be applied to the development of therapeutics for the treatment of opioid abuse.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.11

Topic: G.09. Drugs of Abuse and Addiction

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Title: In vivo photopharmacology with light-activated opioid drugs

Authors: *S. P. MCCLAIN[1], X. MA[1], D. A. JOHNSON[1], C. A. JOHNSON[1], A. E. LAYDEN[1], J. C. YUNG[1], S. T. LUBEJKO[1], G. LIVRIZZI[1], X. J. HE[1], J. ZHOU[1], E. C. BERG[1], E. VENTRIGLIA[2], J. BONAVENTURA[3], M. R. LEVINSTEIN[2], J. L. GOMEZ[2], M. MICHAELIDES[2], M. R. BANGHART[1];
[1]Neurobio., UC San Diego, La Jolla, CA; [2]Intramural Res. Program, Natl. Inst. on Drug Abuse, Baltimore, MD; [3]Univ. Barcelona, Barcelona, Spain

Abstract: Light-sensitive drugs afford precise control over the time and location of drug delivery; advantages that can drive mechanistic studies and reduce side effects in a clinical setting. Here we report photoactivatable or "caged" variants of two clinically used opioid drugs: the agonist "PhOX" (photoactivatable oxymorphone) and the antagonist "PhNX" (photoactivatable naloxone). We find that both molecules can be administered systemically and photoactivated by delivering UV light through fiber optic cables in the brain. We demonstrate that these compounds are inactive at mu opioid receptors in cultured cells, that photoactivation engages endogenous opioid receptors in hippocampal brain slices, and that PhOX and PhNX accumulate in the mouse brain at levels similar to their parent drugs. We further demonstrate *in vivo* photoactivation in reward and pain processing circuits by measuring the impact of local illumination on mouse behavior. PhOX photoactivation in the ventral tegmental area (VTA) produces locomotor activation and conditioned place preference, whereas photorelease in the

periaqueductal grey (PAG) produces analgesia. In fiber photometry recordings with the dopamine sensor dLight1.3b in the nucleus accumbens (NAc), we found that VTA PhOX photoactivation produces a time-locked increase in dopamine release. Using PET imaging with 18F-fluorodeoxyglucose in mice, we demonstrate localized brain activity after PhOX photoactivation in the VTA. Our study demonstrates the feasibility of *in vivo* photopharmacology using caged drugs and the compatibility of combining UV uncaging with common fluorescent probes to study the neural mechanisms of drug action in the brain.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.12

Topic: G.09. Drugs of Abuse and Addiction

Support: College Undergraduate Research Awards Office from Brigham Young University

Title: Changes to morphine sensitivity in the ventral tegmental area after morphine conditioning

Authors: *C. W. HAFEN[1], C. J. TREANOR[1], T. W. WOODHOUSE[1], P. D. FLORA[2], G. C. JONES[2], D. Z. OTTESON[2], S. BAKER[1], E. R. YOUNG[1], J. T. BREINHOLT[2], W. BAIRD[2], H. A. WADSWORTH[1], S. C. STEFFENSEN[2], J. T. YORGASON[1];
[1]Dept. of Cell Biol. and Physiol., [2]Dept. of Psychology, Brigham Young Univ., Provo, UT

Abstract: Dopamine circuit function in the nucleus accumbens (NAc) and ventral tegmental area (VTA) is implicated in the reinforcing effects of drugs of abuse including opioids. Opioid receptor desensitization occurs during acute and prolonged exposure to opioids such as morphine, which may have long lasting effects on dopamine circuit function. Fast scan cyclic voltammetry (FSCV) was performed in mice that have undergone morphine behavioral conditioning. In NAc brain slices, morphine has no apparent direct effects on dopamine release. In contrast, morphine bath application increases VTA dopamine release, which is reversed by naloxone. Interestingly, morphine induced increases in VTA dopamine release were greater in morphine vs saline conditioned mice, supporting a model of circuit sensitization. Surprisingly, blocking voltage gated potassium channels resulted in a switch in morphine effects, where morphine reduced VTA dopamine release. Morphine treated mice had reduced sensitivity to morphine effects on dopamine release in the presence of potassium channel blockers, suggesting that potassium channels are underlying the increases in sensitivity observed in morphine conditioned mice. Experimental protocols were approved by the Brigham Young University

Institutional Animal Care and Use Committee according to the National Institutes of Health Guide for the care and use of laboratory animals. Research was funded by Brigham Young University. There are no conflicts of interests to disclose.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 316.13

Topic: G.09. Drugs of Abuse and Addiction

Support: NS027881
DA034748

Title: Differential impact of chronic morphine on the electrophysiological properties of hypocretin/orexin (H/O) neurons is reversible after four weeks of spontaneous withdrawal

Authors: *E. BERRY[1], E. N. HUHULEA[1], P. SHEU[1], M. ISHIBASHI[2], R. MCGREGOR[3], J. M. SIEGEL[3], C. S. LEONARD[1];
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Abstract: H/O neurons of the hypothalamus exhibit structural plasticity in response to chronic administration of both cocaine and opioids. Both drugs of abuse lead to a significant increase in the number of detectable orexin-A positive neurons, and opioid administration also results in a 20% decrease in average soma size (James et al. 2019, Thannickal et al. 2018). To determine if this structural plasticity is accompanied by changes in the intrinsic electrical properties of H/O neurons, we prepared acute brain slices from male H/O-EGFP mice (9-18 W) after 2 W of daily s.c. saline (S) or 50mg/kg morphine sulfate (M). With investigators blind to treatment, we used whole-cell recording to examine the passive and active electrical properties of the two previously identified subtypes of H/O neurons (D- and H-type), which are distinguishable by membrane responses manifest by A-type K^+ currents in H-cells (Schöne et al. 2011). We found that after 2 W morphine treatment, H-type neurons exhibited an increase in rheobase (mean ± sem; S: 22.9 ± 3.2 pA, M: 33.3 ± 3.3 pA, $p< 0.05$, t-test), a smaller spike amplitude (S: 91.1 ± 2.1 mV, M: 83.4 ± 1.7 mV, $p < 0.01$, t-test), a longer spike half-width (S: 1.03 ± 0.03 ms, M: 1.13 ± 0.04 ms, $p < 0.01$, t-test) and a reduced ability to repetitively fire (neurons able to fire continuously post saline: 75%, post morphine: 33.33%, $p < 0.01$, X^2 test). In contrast, these properties of D-type neurons from the same animals were unaffected by morphine treatment.
Since the decrease in H/O soma size induced by 2 W of morphine treatment was reversed by 4 W

of withdrawal (Thannickal et al. 2018), we evaluated the intrinsic properties of H/O-EGFP neurons after a 4 W delay/withdrawal period following 2 W of saline or morphine exposure. We found that H-type neurons from morphine-treated mice no longer showed a difference from saline-treated mice in rheobase (S: 14.4 ± 2.7 pA, M: 14.2 ± 2.5 pA, p=0.96), spike amplitude (S: 89.0 ± 1.5 mV, M: 86.4 ± 1.6 mV, p=0.75) or spike half-width (S: 1.17 ± 0.06 ms, M: 1.17 ± 0.03 ms, p=0.96). Their ability to repetitively fire was also restored to baseline levels (neurons able to fire continuously, S: 81.81%, M: 71.4%, p=0.32).
Collectively, these data show that chronic morphine exposure induces a selective decrease in excitability of H-, but not D-type, H/O neurons and that this decrease in excitability is reversible after 14 days of morphine abstinence. This indicates that chronic morphine exposure induces a functional plasticity that parallels the previously described structural plasticity of H/O neurons. Moreover, it suggests that H- and D-type neurons are functionally distinct in their participation in reward pathways and addiction.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Topic: G.09. Drugs of Abuse and Addiction

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NIH R01 DA042779

Title: Chronic Morphine Facilitates Opioid Signaling in a Behaviorally Relevant Thalamo-Striatal Circuit

Authors: *E. JAECKEL, K. RAMSEY, W. BIRDSONG;
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Abstract: The dorsomedial striatum (DMS) plays a critical role in producing voluntary movement. Opioids such as morphine stimulate locomotor activity in animal models and repeated administration induces locomotor sensitization, whereby the locomotor-stimulating effects of opioids are enhanced. While this phenomenon is well-defined, the underlying mechanisms are not fully understood. Because the DMS is implicated in motor activity, understanding opioid signaling within this region may help elucidate the mechanisms driving locomotor sensitization. Morphine effects are mediated through activity at the mu-opioid receptor (MOR). The DMS receives strong glutamatergic innervation from medial thalamic projection neurons, and MORs are present at these thalamo-striatal terminals. Cellular tolerance to morphine has been observed when examining MORs present on cell bodies. Relatively less is known about chronic morphine effects at presynaptic MORs on axon terminals. In this study, we

investigated how chronic morphine exposure affects responses to a subsequent morphine challenge in a medial thalamic-DMS synapse and whether MOR signaling within these inputs is required for locomotor sensitization. Using patch-clamp electrophysiology and optogenetics in mouse brain slices, morphine inhibition of glutamate release was measured at thalamo-striatal terminals in drug-naïve and chronically morphine-treated mice. Morphine inhibition of glutamate release was enhanced in slices from chronically treated mice compared to drug-naïve, indicating that chronic morphine induces facilitation of opioid signaling at these terminals. This effect was sex-specific as facilitation was only observed in male mice. Using a locomotor assay in transgenic mice, we found this thalamic-DMS pathway to be important for the development of locomotor sensitization. This study demonstrated a neural pathway in which chronic morphine exposure resulted in facilitation of, rather than tolerance to, morphine signaling. Moreover, this pathway may be an important driver of morphine-mediated hyperlocomotion, a behavioral effect to which sensitization develops. Understanding how chronic opioid exposure differentially alters opioid signaling based on brain region and subcellular locations, and how these effects relate to opioid-sensitive behaviors, will provide a better understanding of opioid function overall.

Disclosures: E. Jaeckel: None. **K. Ramsey:** None. **W. Birdsong:** None.

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

Location: SDCC Halls B-H

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Program #/Poster #: 316.15

Topic: G.09. Drugs of Abuse and Addiction

Support: CIHR FDN# 148407

Title: Biomimetic optogenetics: Reconstructing opiate-induced firing patterns reveals predicted reward

Authors: *L. EL-FAYOMI[1], H. STEENLAND[2], M. BERGAMINI[3], D. VAN DER KOOY[1]; [1]Mol. Genet., Univ. of Toronto, Toronto, ON, Canada; [2]Neurotek Innovative Technol. Inc., Toronto, ON, Canada; [3]Albert Einstein Col. of Med., New York City, NY

Abstract: Functional brain mapping studies typically involve tonic optogenetic stimulation (20 Hz, for instance), although this rarely reflects natural firing patterns generated by target populations. Underscoring the importance of these temporally patterned action potentials, we demonstrate that reward and aversion can be elicited from a single ventral tegmental area (VTA) cell type using different optogenetic stimulation modes. Activating GABAergic VTA neurons using laser pulse sequences that mimic morphine-induced activity elicits reward behavior in adult male and female GAD65-cre mice (n=18), while stimulating the same population using continuous light is aversive. These findings are consistent with non-optical studies implicating VTA GABA neurons in morphine reward processing under previously opiate naïve conditions. Furthermore, both tonic stimulation (n=5) and non-tonic patterns reflecting opiate-free contexts

(n=4) produce no motivational effects. Mathematical modelling of VTA GABA neuron activity patterns indicates that at baseline, firing can be described by a mixed Pareto-Log Normal model, whereas that Log Normal quality is lost once morphine is administered systemically. Our results suggest that firing patterns should be considered when using optogenetics for neurobehavioral research.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 316.16

Topic: G.09. Drugs of Abuse and Addiction

Title: Assessment of pharmacological determinants behind oliceridine's therapeutic profile

Authors: *E. N. VENTRIGLIA[1], A. RIZZO[4,5], J. L. GOMEZ[1], O. C. SOLÍS[1], S. LAM[1], A. NEWMAN[2], Y. SHAHAM[3], J. BONAVENTURA[4,5], M. MICHAELIDES[1];
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Abstract: Oliceridine (Olinvyk®) is the first selective mu opioid receptor (μOR) G protein-biased agonist approved by the FDA in 2020 for in-patient i.v. pain management. Oliceridine purportedly demonstrates a greater therapeutic index compared to morphine, a profile which has been attributed to its biased agonism. However, the extent to which biased agonism is involved in the adverse effects of μOR agonists has been challenged by recent studies.
To better understand the *in vivo* pharmacological actions of oliceridine, we performed a comprehensive assessment of oliceridine's *in vitro* and *in vivo* pharmacology in cell culture and in rodents. We performed radioligand binding and signaling experiments in rat brain membranes as well as transfected HEK293T cells and, in agreement with the literature, we found that oliceridine is a potent and selective μOR agonist that displays weak efficacy to activate μOR *in vitro* and *ex vivo*. To better gauge its *in vivo* properties, we generated [^{3}H]oliceridine. After validating it using *in vitro* assays, we assessed its *in vivo* biodistribution and found that it exhibits low brain uptake. Subsequent screening assays showed that oliceridine is a substrate for the P-glycoprotein (P-gp) efflux pump. Using P-gp knock-out mice and rats, and procedures such as the hot plate, pulse oximetry, and intravenous self-administration, we determined that oliceridine's *in vivo* potency as an analgesic and its adverse effect profiles (respiratory depression, abuse liability) are strongly determined by its P-gp activity. Finally, analysis of brain metabolic activity using positron emission tomography (PET) and [^{18}F]fluorodeoxyglucose

(FDG) in rats exposed to equipotent doses of morphine (10 mg/kg, SC) or oliceridine (1 mg/kg, SC) showed that each drug produced widespread decreases in brain metabolic activity. Together, these data suggest that oliceridine's greater therapeutic index over other "non-biased" opioids such as morphine is determined to a large extent by its high potency and fast brain clearance due to P-gp.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Topic: G.09. Drugs of Abuse and Addiction

Support: NEI EY029227

Title: A retinal contribution to opioid-induced sleep/wake dysregulation

Authors: *N. BERGUM, J. VIGH;
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Abstract: Melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) mediate the entrainment of mammalian sleep/wake rhythms to environmental light. Interestingly, recent studies from our lab revealed that mouse ipRGCs express μ-opioid receptors (MORs), the primary molecular target for opioid analgesics. Furthermore, MOR agonists can directly inhibit ipRGC firing, which could prevent ipRGCs from regulating sleep/wake rhythms in response to light. In humans, opioid metabolites can be detected within eye following opioid use. However, it remains unclear whether opioids accumulate in the mouse retina following systemic exposure. To confirm that morphine reaches the mouse retina following systemic delivery, we collected retina, hypothalamus and serum from adult male mice at different time points after 6 days of twice-daily 20 mg/kg morphine treatment. Morphine levels in serum, hypothalamus and retina were measured using tandem liquid chromatography-mass spectrometry. Importantly, results from this study show that systemically administered morphine accumulates in the mouse retina, but not in the serum or hypothalamus. Additionally, we implanted mini-telemetry devices into adult male mice to assess how chronic morphine alters their sleep/wake behavior. To establish the role of ipRGCs in opioid-induced perturbations in sleep/wake behavior, we performed these experiments in wildtype (n=11) mice along with mice lacking MORs exclusively in ipRGCs (McKO, n=8) as well as with mice lacking MORs globally (MKO, n=7). While MKO mice predictably did not acquire morphine-induced locomotor sensitization, McKO exhibited decreased morphine-induced locomotor sensitization compared to wild type. These findings corroborate previous studies regarding the necessity of MORs for morphine-induced locomotor

sensitization, while also implicating MORs expressed by ipRGCs as a potential mediator of opioid-related sleep/wake dysregulation. Taken together, these results support the idea that opioid that accumulation in the eye could persistently activate MORs on ipRGCs, altering the ability of ipRGCs to transmit light information to the brain's sleep-wake circuitry. This alteration in photic input to the brain could underlie some of the sleep/wake problems associated with long-term opioid use.

Disclosures: **N. Bergum:** None. **J. Vigh:** None.

Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA039895
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Title: Role of neuromedin s-expressing ventral tegmental area neurons in morphine behavior

Authors: *C. RIVERA QUILES, M. ALDAY, O. DODSON, M. S. MAZEI-ROBISON; Michigan State Univ., East Lansing, MI

Abstract: Opioid dependence and addiction constitute a major health and economic burden, but our limited understanding of the underlying neurobiology limits better interventions. Alteration in the activity and output of dopamine (DA) neurons in the ventral tegmental area (VTA) is known to contribute to drug effects, but the mechanisms underlying these changes remain relatively unexplored. We used TRAP to identify gene expression changes in VTA DA neurons following chronic morphine and found that Neuromedin S (NMS) is enriched in VTA DA neurons, and its expression is robustly increased by morphine. However, whether all VTA DA neurons express NMS, and their potential functional impact has yet to be determined. We hypothesize that NMS neurons represent a novel subset of VTA neurons that contribute to morphine-elicited behavior. Specifically in these studies, we hypothesize that activating and inhibiting VTA-NMS neurons will promote and inhibit morphine behaviors, respectively. To test this, adult male and female NMS-Cre mice and wild-type littermates were used. Cre-dependent viral vectors were stereotaxically injected into the VTA to allow for DREADD-mediated activation (Gq) or inhibition (Gi) of VTA-NMS neurons. Behavioral analyses were completed two weeks after surgery. Locomotor activity was assessed following saline (d1), saline + Clozapine-N-oxide (CNO, 0.3mg/kg, ip, d2-d3), and morphine (15mg/kg) + CNO (d4-d8). A morphine + CNO challenge was done 1 week following d8. Repeated-measures two-way ANOVA was used to determine significant differences ($p<0.05$) in locomotor behavior. Using DREADDs, we found that activation or inhibition of VTA-NMS neurons did not affect general

locomotor activity or elicit CNO-conditioned place preference or aversion. We find that both male and female Gq mice exhibit increased morphine-induced locomotor activity. Additionally, locomotor response to a challenge morphine + CNO injection was significantly increased in these mice compared to controls. Gi mice show a trend for the opposite effect, with decreased morphine-elicited locomotor activity and decreased response to challenge. Thus, VTA-NMS neuron manipulation alters morphine-elicited behaviors including locomotion and sensitization. Future studies will determine whether VTA-NMS neuronal activity modulates morphine conditioned place preference. Our current data suggest that VTA-NMS neurons represent a subset of neurons that may be functionally relevant for morphine responses.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Title: Differential patterns of synaptic plasticity in the nucleus accumbens caused by continuous and interrupted morphine exposure

Authors: *E. LEFEVRE, E. GAUTHIER, L. BYSTROM, J. SCHEUNEMANN, P. ROTHWELL;
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Abstract: Opioid exposure and withdrawal both cause adaptations in brain circuits that may contribute to abuse liability. These adaptations vary in magnitude and direction following different patterns of opioid exposure, but few studies have systematically manipulated the pattern of opioid administration while measuring neurobiological impact. In this study, we compared cellular and synaptic adaptations in the nucleus accumbens shell caused by morphine exposure that was either continuous, or interrupted by daily bouts of naloxone-precipitated withdrawal. At the behavioral level, continuous morphine administration caused psychomotor tolerance, which was reversed when the continuity of morphine action was interrupted. Using ex vivo slice electrophysiology in female and male mice, we investigated how these patterns of morphine administration altered intrinsic excitability and synaptic plasticity of medium spiny neurons (MSNs) expressing the D1 or D2 dopamine receptor. We found that morphine-evoked adaptations at excitatory synapses were predominately conserved between patterns of

administration, but there were divergent effects on inhibitory synapses and the subsequent balance between excitatory and inhibitory synaptic input. Overall, our data suggest that continuous morphine administration produces adaptations that dampen the output of D1-MSNs, which are canonically thought to promote reward-related behaviors. Interruption of otherwise continuous morphine exposure does not dampen D1-MSN functional output to the same extent, which may enhance behavioral responses to subsequent opioid exposure. Our findings support the hypothesis that maintaining continuity of opioid administration could be an effective therapeutic strategy to minimize the vulnerability to opioid use disorders.

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Poster

316. Opioids, Neurophysiology, Circuits, and Withdrawal

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Program #/Poster #: 316.20

Topic: G.09. Drugs of Abuse and Addiction

Support: 1ZIADA000566-10

Title: The narrow time window for naloxone to reverse fentanyl-induced brain hypoxia in a rat model

Authors: *E. A. KIYATKIN[1], C. CURAY[2], M. IRWIN[2];
[1]NIDA-IRP, NIH, DHHS, NIH, Natl. Inst. On Drug Abuse, Baltimore, MD; [2]NIDA-IRP, Baltimore, MD

Abstract: Opioid drugs are known to induce respiratory depression that results in brain hypoxia, coma, and possible death during overdose. Naloxone, an opioid antagonist, is still the best tool to prevent adverse effects of fentanyl, but this treatment is often less successful than that for other opioids. While low naloxone doses are commonly viewed as the primary factor limiting its effectiveness, timing between drug exposure and start of naloxone treatment may be another critical factor. Here, we used oxygen sensors coupled with amperometry to examine how naloxone affects fentanyl-induced oxygen changes in brain and periphery when administered before and after fentanyl injections. Intravenous fentanyl at a modest (20 μg/kg) and high (60 μg/kg) doses induced strong and prolonged oxygen decreases in the subcutaneous space and biphasic changes in the brain, with strong but relatively transient decreases followed by more prolonged weaker increases. When administered before (-10 min) fentanyl, intravenous naloxone (0.2 mg/kg) fully blocked the hypoxic effects of low-dose fentanyl in both brain and periphery for at least 100 minutes. However, when injected 10 minutes after fentanyl, when brain oxygen levels already increased, naloxone had no effects on oxygen levels in both locations. However, in the periphery naloxone strongly attenuated hypoxic effects of high-dose fentanyl, but in the brain, it induced only a transient oxygen increase associated with behavioral awakening.

Therefore, the timing to initiate naloxone treatment after appearance of fentanyl overdose is a critical variable determining its treatment effectiveness.

Disclosures: E.A. Kiyatkin: None. **C. Curay:** None. **M. Irwin:** None.

Poster

317. Mechanisms of Attention: Non-Human Primates

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Topic: H.01. Attention

Support: NIH
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Title: Neural representations of attention following exogenous and endogenous cues in monkey parietal cortex and thalamus

Authors: *R. BOSHRA[1], M. K. ERADATH[2], K. DOUGHERTY[3], S. KASTNER[4];
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Abstract: Covert spatial attention enables prioritized processing of stimuli in cued locations. The neural mechanisms that instate the allocation of attention to a specific spot in the visual field following exogenous and endogenous cues are not clearly understood. In this study, we recorded local field potentials and single-unit spiking activity from macaque lateral intraparietal area (LIP) and its projection zone in pulvinar (Pul), a thalamic nucleus implicated in attention function and control, using linear arrays as the animals performed a modified version of the Egly-Driver task. Informative cues were either endogenous (unique color cues at fixation memorized by the animals to correspond to one of four target locations) or exogenous (salient cues presented at a target location), and preceded a low-contrast target after a variable cue-target delay period. Behavioral performance showed that the animals (n=2) had equivalent performance for the two types of cues, indicating similar task difficulty. Preliminary spiking analysis showed a distinct difference of visual responsiveness in both LIP and Pul neurons with respect to receptive field (RF) selectivity after cue onset, which is expected given the location difference of cue location. Further, population spiking activity corresponding to exogenous cues showed clear attention-modulated sustained activity in the delay period prior to target onset, which was unobservable for endogenous cues. However, despite the differences between the two cue types in the cue-target delay period, attention modulation was especially similar after target onset in LIP and was similar to a more modest extent in Pul. These results suggest that attention-modulated neurons may not similarly encode the locus of attention following endogenous and exogenous cueing, that covert attention may utilize non-overlapping populations in exogenous and endogenous attention, or that the type of cue may elicit different forms of neural modulation.

Disclosures: **R. Boshra:** None. **M.K. Eradath:** None. **K. Dougherty:** None. **S. Kastner:** None.

Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Neural codes for endogenous and exogenous attention in medio-dorsal pulvinar, LIP and FEF

Authors: K. DOUGHERTY[1], R. CHEN[1], R. BOSHRA[1], S. KASTNER[2];
[1]Princeton Neurosci. Inst., [2]Princeton Neurosci. Institute, Dept. of Psychology, Princeton Univ., Princeton, NJ

Abstract: Covert visual attention aids the selection of relevant visual information. In some cases, visual attention is voluntary, or endogenous, allowing us to deliberately monitor one location; other times, attention is involuntary, or exogenous, and selects highly salient stimuli. Congruent with this distinction, exogenous and endogenous attention operate on fast and slow time scales, respectively. Exogenous and endogenous attention engage the frontal-parietal attention network, including lateral intraparietal cortex (LIP) and the frontal eye fields (FEF). LIP and FEF share an overlapping projection zone in medio-dorsal pulvinar (mdPul), which is also known to be engaged in exogenous attention. While overlapping regions are involved, differences in the neuronal mechanisms supporting exogenous and endogenous attention are unclear. Here we simultaneously recorded spiking activity with linear electrode arrays from mdPul, FEF, and LIP while two macaques performed a spatial attention task. For each trial, the animal fixated before a cue was presented briefly to indicate the location of an upcoming target. On some trials, the cue was a salient disk that appeared at the target location (exogenous attention). On other trials, the cue was a small arrow that appeared at fixation and indicated the behaviorally relevant location (endogenous attention). After a delay, a visual array consisting of six barrel and bowtie shapes appeared in the eccentricity, centered around fixation. The item over the previously cued location changed color after a second variable delay. Macaques were rewarded with juice when they made a saccade to the target within 600 ms of its onset. We isolated hundreds of neurons across mdPul, FEF and LIP. Attentional modulation of firing rates was significant in all three regions on exogenous trials; this modulation was reduced on endogenous trials. To elucidate whether spiking within each region differentiated exogenous and endogenous trials, we employed supervised learning algorithms and examined the performance of classifiers in decoding the location where attention was directed. Preliminary results from

single-trial based analyses suggest that mechanisms of endogenous and exogenous are distinct within this corticothalamic network.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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KU Leuven C14/21/111

Title: Causal role and electrophysiological correlates of spatial attention shifts in the medial superior parietal lobule of the macaque.

Authors: *M. DE VITIS[1,2], P. F. BALAN[2], R. VOGELS[2,3], C. GALLETTI[1], P. FATTORI[1], W. VANDUFFEL[2,3,4,5];
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Abstract: In everyday life, we are continuously confronted with far more information than our brain can process. Selective attention partially mitigates this problem by facilitating perception of attended stimuli while suppressing that of competing distractor stimuli. Human fMRI and TMS studies revealed the crucial role of the superior parietal lobule (SPL) in covert shifts of spatial attention. However, despite hints from monkey electrophysiology and fMRI, little is known about the underlying neuronal mechanisms. Guided by monkey fMRI, we recorded neural activity from shift-selective regions in medial SPL (areas V6/V6A) using laminar probes. Subsequently, we reversibly inactivated these areas to assess their causal contribution in shifting and sustaining spatial attention. Our experimental paradigm was similar to that of previous human and monkey fMRI experiments, and allowed us to dissociate attentional shift, stay and motor events (used to probe the allocation of attention). Stimuli consisted of 2 pairs of yoked shapes, each containing a relevant and irrelevant stimulus. The display always contained one of the two pairs. A replacement of the first stimulus pair by the second could induce a spatial attention shift when the relevant stimulus position changed to the opposite visual hemifield (shift event). Alternatively, when the relevant stimulus of the new pair appeared at the same position as the relevant stimulus of the preceding pair, this corresponded to a stay event. The allocation of attention was probed behaviorally by dimming events of the relevant/irrelevant stimuli, separated

in time from the shift/stay events. We recorded from 388 multi-units in areas V6/V6A of 2 rhesus monkeys and found that the average population activity was higher for shift than for stay events when the direction of shifts pointed towards the visual hemifield contralateral to the recording hemisphere. Reversible inactivation of V6/V6A using muscimol injections resulted in a significant increase in error rate for shift trials as opposed to stay trials. Overall, our results show a strong correlate of shifting spatial attention within areas V6/V6A in the absence of overt behavior, and also suggest a causal contribution of these areas in shifting spatial attention.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Attention compensates for very large, stimulus-induced firing rate differences in V1/V2 between competing visual stimuli

Authors: *L.-P. RAUSCH[1], M. SCHÜNEMANN[2], E. DREBITZ[1], D. HARNACK[3], U. A. ERNST[2], A. K. KREITER[1];
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Abstract: Selective attention serves to focus on behaviorally relevant stimuli, while ignoring irrelevant ones. In the visual system, shifts in spatial attention are associated with modulations of neuronal firing rate responses and switches in effective connectivity between neuronal populations (routing-by-synchrony). However, it is unclear how the switches in the interareal synchrony that are accompanying the attentional shifts are controlled. Computational studies suggest that a small rate advantage for neural populations passing the attended signal to downstream areas suffices to establish selective processing through interareal synchronization. For stimulus constellations with large imbalances in stimulus-driven activity between competing stimuli, this hypothesis implies unusually strong attentional modulations in order to establish a rate advantage for a weaker target stimulus. We investigated this challenging situation by recording multi-unit activity in areas V1/V2 of two macaque monkeys (*Macaca mulatta*) performing a demanding shape-tracking task. Using luminance contrast to modulate neuronal activity, we quantified the capacity of attentional modulations to compensate for stimulus-driven activity differences between neuronal populations that represent closely spaced competing

stimuli. It turned out, that compensation was indeed possible for very large activity differences of up to 300% (mean 95.8%, SD 84.7). This was mainly achieved by target facilitation (TF, mean 76.8%, SD 69.9) and to a lesser extent by distractor suppression (DS, mean 16.6%, SD 14). TF depended on stimulus context and scaled linearly with the difference in firing rates evoked by the low and high contrast stimulus. Linear regression showed that TF compensated for about 74% of the relative rate difference ($r = 0.89$, $p < 0.01$). In contrast, DS was independent of stimulus context but correlated to the stimulus-induced activity without attention. To explain these findings, we propose a model which combines a simple, contrast-independent attentional control mechanism with surround inhibition and a compressing output non-linearity. Our model reproduces all experimental findings by explaining more than 90% of response variance across all stimulation conditions. Furthermore, it closely captures the observed variability of neural responses and their modulation by attention and stimulus context over different recording sites. Our findings reveal a surprising capacity for attention-dependent rate modulations in V1/V2, and in conjunction with computational models provide a mechanistic explanation for attention-mediated signal selection and processing.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

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Topic: H.01. Attention

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Title: Attention changes the efficacy of information transfer between layers in visual area V1 by adjusting their gamma-phase relations

Authors: *E. DREBITZ, L.-P. RAUSCH, E. DOMINGO GIL, A. K. KREITER;
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Abstract: Attention guides the selection of relevant information. On the neuronal level, attention implements this selection by synchronizing the rhythmic activity of receiver neurons to the rhythmic activity of those subsets of sender neurons that deliver the currently relevant information. This selective synchronization increases the efficacy of information transfer between the receiver and sender neurons: routing by synchrony. Selective synchrony has so far been observed between neuronal populations of distant visual areas. However, we think an attentional enhancement of information transfer would benefit even between neurons of the same visual area encoding only one stimulus. We hypothesize that attention modulates the phase relations between the rhythmic activities of neurons in supragranular and granular layers in such a way that it enhances their effective connectivity and ultimately enhances information transfer.

We tested our hypotheses by performing laminar resolved recordings in area V1 of two macaque monkeys (*Macaca mulatta*) performing a selective attention task. One stimulus was located in the receptive field of the recorded V1 population and was cued to be attended or not on different trials. We measured the gamma-phase difference between supragranular and granular layers. After calculating the phase differences between the layers, we extracted periods of stable phase difference (± 30°). In order to measure information transfer in these periods, we changed the luminance of the stimulus randomly every 10ms. This flicker signal was detectable in the local population activity (current source density, CSD). We calculated the spectral coherence between the flicker signal and the CSD to quantify stimulus-related information. We found (1) a clear peak in the distribution of phase difference angles for periods with stable phase relation, pointing to a preferred phase relation between both layers. (2) When the stimulus was attended, we found even more periods with this stable preferred phase relation. On top, the periods exhibiting this relation became longer. (3) The level of stimulus-related information in the CSD of supragranular layers strongly depended on the phase relations of the CSDs of supragranular and granular layers. These results show that attention modulates the phase relations between neurons of the same cortical column. Furthermore, due to the attention-dependent increases of periods with the preferred difference angle and the attention-dependent increase in the level of the stimulus-related signal, we assume that this particular phase relation provides an optimal mode of information transfer between cortical layers.

Disclosures: **E. Drebitz:** None. **L. Rausch:** None. **E. Domingo Gil:** None. **A.K. Kreiter:** None.

Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Response gains and variability decreases in visual cortex are associated with different computational subcomponents of attention

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Abstract: In this study, we asked whether the extent of attentional modulations on individual neurons is not uniform or random, but rather depends on whether and how a neuron provides task-relevant information.

Studies have found attention gain on visual neurons' responses relates to tuning strength. However, global tuning strength does not totally capture neurons' contributions to task performance, since detecting a visual change requires (1) representing the original stimulus and (2) sensitivity to small input changes. These requirements may rely on different neurons based on their tuning details. Neurons preferring the current stimulus signal it well but are less sensitive to small changes because the gradient of their tuning around the stimulus is zero. Whereas, neurons whose tuning curves are steepest around the stimulus (high Fisher information) are highly sensitive to input changes, but do not signal the current input as well. We reasoned these distributed demands on visual neurons needed for change-detection are associated with different forms of attention modulations: response gain and decreased variability. That is, while decreased variability is always beneficial for neural coding, response gain would not be beneficial for neurons with high Fisher information about the stimulus, thus these neurons would have little gain.
Two macaques performed fine orientation change-detection with a spatial attention manipulation while we recorded V4 activity. The task context was switched: the orientation of the stimuli before the change could be 45 or 135 degrees, randomly selected for each trial. Thus, to solve the task, the animal needed to represent task context, and also detect the small orientation change. From neurons' orientation tuning curves, we extracted two types of task-relevant information: task tuning (the ability to discriminate between two task contexts), and Fisher information around the standard orientations. As predicted, we found neurons with strong task tuning but low Fisher information (highT-lowF) had stronger attentional gain, compared to neurons with weak task tuning but high Fisher information (lowT-highF). Also, their ability to represent task context was enhanced with attention. Meanwhile, variability of response was stabilized by spatial attention for lowT-highF neurons but not for highT-lowF neurons, despite lowT-highF neurons having much lower variability overall. We propose a model to explain such neuronal behavior: a strong attention signal targeting highT-lowF neurons increases their firing rates, resulting in enhanced task representation, and stabilized response of lowT-highF neurons via a normalization mechanism.

Disclosures: **S. Liu:** None. **A. Snyder:** None.

Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Priming alters cortical columnar attentional processing differently for targets vs. distractors in V4

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Abstract: We deploy attention to scrutinize objects in our cluttered environment. This is associated with enhanced processing of the sensory representations of attended objects and suppressed processing of distracting objects. At the neural level in sensory cortex, this is observed as increased activity for neurons (or neural populations) representing the part of visual space containing the attentional target and decreased activity in those representing the distractors. Notably, these attentional modulations are dynamic. Factors such as selection history (e.g., what has been selected for attentional deployment previously) can alter the timing of target enhancement and distractor suppression such that pertinent objects are more rapidly scrutinized. Sensory cortex is involved in priming-induced attentional processing changes, however, what role cortical columnar structure plays in these priming-induced changes has not been investigated. Cortical columns present an attractive opportunity to investigate changes associated with attentional priming as models such as the canonical cortical microcircuit (CCM) afford inferential power in determining the putative origins of these changes. In this study, we sought to investigate whether simultaneous changes in target vs. distractor processing manifest through the same or distinct mechanisms as indicated through laminar processing differences in sensory cortical columns. We exploited the canonical microcircuitry of visual cortex in two macaque monkeys, through neurophysiological recordings with laminar resolution, to determine whether the spatiotemporal profiles of these processes indicate changes to attentional modulations through the same or distinct mechanisms. During recording, monkeys performed pop-out search for an attention-capturing oddball stimulus. Trials were organized to elicit selection history effects (i.e., priming of pop-out). Attentional selection in the population neural responses was present across all layers and varied in time as a function of selection history. However, target enhancement and distractor suppression did not covary in space or time. Target enhancement manifested earlier and nonuniformly across the layers of cortex whereas distractor suppression came about later and relatively more uniformly across layers. These findings suggest that (1) changes in attentional target vs. distractor processing can be observed at the level of cortical columnar activity and (2) these laminar dissociations indicate distinct mechanisms for the adaptations of target vs. distractor processing with selection history.

Disclosures: J.A. Westerberg: None. **A. Maier:** None. **J.D. Schall:** None.

Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Laminar microcircuitry underlying target selection in marmoset posterior parietal cortex

Authors: *J. SELVANAYAGAM, K. D. JOHNSTON, S. EVERLING;
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Abstract: Investigations in primary sensory cortex suggest a basic, laminar structure shared by all areas of neocortex. In the frontoparietal network of the rhesus macaque, investigations have revealed neurons with important roles in oculomotor control and visual attention. However, due to the difficulty in accessing key nodes of this network that are embedded within sulci (e.g., FEF and LIP) in the macaque, knowledge of the laminar microcircuitry of these areas remains limited. To address this gap, we exploited the relatively lissencephalic cortex and homologous frontoparietal networks of the common marmoset (*Callithrix jacchus*). We carried out laminar recordings in the posterior parietal cortex (PPC) of 3 adult marmosets (2 female, 26-32 months), targeting regions identified using fMRI as having strong BOLD responses in a visuomotor task and resting-state functional connectivity with superior colliculus. Recordings were conducted with ultra-high-density laminar probes (neuropixels; 384 electrodes spanning 3.84mm), while the animals completed a visual target selection task in which they were required to make a saccade to a target stimulus with or without a distractor in the opposite hemifield. Marmosets correctly made a saccade to the target in the absence of a distractor with shorter reaction times (median RT: 129.4 ms) and more accurately (mean accuracy: 95.1%) than in the presence of a distractor (146.7 ms; 77.5%).
We recorded the activity of 2261 single units sorted automatically in Kilosort and manually curated in Phy. Of these, 484 exhibited significant visual or saccade related activity. 204 of these discriminated between target and distractor stimuli prior to making a correct response. To investigate whether this varied with cortical depth, we assigned cortical layers using a current source density analysis. For all visual units, we computed the onset latency of the visual response. The earliest visual responses were observed in layer 4, followed by layers 2/3 and finally in layers 5/6. Next, for units that discriminated between target and distractor, we computed the latency at which each unit's discharge activity selected for its preferred stimulus. This discrimination time also varied across depth, occurring first in layers 2/3, then layers 5/6 and finally in layer 4.
In sum, we found that marmoset PPC neurons discriminate between target and distractor stimuli in a visual target selection task. Further, the latency of the visual response and the discrimination time of neurons varied across cortical layers. Taken together, these findings support a model of cortical circuitry where layer 4 serves as an input and layers 2/3 facilitate target discrimination.

Disclosures: J. Selvanayagam: None. **K.D. Johnston:** None. **S. Everling:** None.

Poster

317. Mechanisms of Attention: Non-Human Primates

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Support: NIH Grant EY017921

Title: Frequency-dependent differential effects of feature attention on LFP power across the visual processing stream

Authors: *A. UMOREN, D. MENDOZA-HALLIDAY, R. DESIMONE;
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Abstract: Visual feature attention selectively modulates the firing rates of feature-selective neurons across multiple processing stages of the primate visual stream, with effects becoming stronger as one moves downstream. Feature attention also modulates the power of local field potential (LFP) oscillations. However, no study has systematically compared the effects of feature attention on LFP power across multiple visual processing stages under the same experimental conditions. To investigate this, we trained two macaque monkeys to perform a spatially-global feature attention task in which they selectively attended to one of two full-screen overlapping random dot surfaces moving in opposite directions, cued by the direction of a prior surface. We recorded neuronal activity simultaneously from 5 laminar probes in cortical areas across multiple visual processing stages - MT, MST, LIP, MIP, and LPFC. To measure the effects of feature attention, we compared the LFP power between conditions with opposite attended motion directions. We found that feature attention modulated broadband LFP power in all areas, with modulation strength being lowest upstream (MT) and increasing downstream. Furthermore, in upstream areas, attentional modulation was strongest in the lowest frequencies and progressively weaker in higher frequencies. Traversing downstream, this relationship gradually reversed, with modulation being weakest in the lowest frequencies and progressively stronger in higher frequencies. Among downstream areas (LPFC, LIP, and MIP), the distribution of attentional modulation strength over the frequency domain showed two peaks: one in the low (below 20 Hz) and one in the high (40 to 80 Hz) frequencies. We further found that these peaks represent two partially separate populations of LFP sites with attentional modulation mainly in the low or high frequencies. The proportion of high frequency-modulated sites was minimal in upstream areas and increased progressively downstream. Lastly, we examined whether the feature preference of LFP power during attentional modulation was the same across frequencies. We found that in most areas, motion direction preference was mostly preserved within each of three distinct frequency bands - θ, α/β, and γ, but differed between these bands in a substantial fraction of LFP sites. This indicates that for LFP sites, feature preference during attention is not uniform across the frequency domain. Together, our results show that feature attentional modulation of LFP power differs broadly across visual processing stages and frequencies and that such effects vary systematically in a downstream progression.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Investigating the relationship between attentional modulation and task-relevant feature selectivity among V1 neurons

Authors: *S. SHAH[1], M. MANCARELLA[1], J. HEMBROOK-SHORT[2], V. L. MOCK[2], F. BRIGGS[1];
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Abstract: Despite being constantly flooded with massive amounts of sensory information, our brains can focus on the most critical information using attentional processes. Attention has been shown to modulate multiple aspects of neuronal responses, including gain modulation of neuronal firing rates. Attentional modulation of neuronal activity varies across cell types, laminar compartment locations, and brain areas. One factor contributing to this variability may be neuronal feature-selectivity. Previous research has suggested that attentional modulation of neuronal firing rates depends on the match between neuronal feature-selectivity and the feature attended in the task (Treue & Martinez-Trujillo, 1999). A dependence of attentional modulation on task-relevant feature tuning has been demonstrated across neurons in multiple visual areas, including the macaque primary visual cortex (V1; Hembrook-Short et al 2017). Specifically, these studies have shown that firing rates of neurons selective for task-relevant feature are enhanced while task-irrelevant neuronal activity is often suppressed by attention. However, most studies employ tasks in which only one feature is modulated. Thus, it is possible that attention modulates specific subpopulations of neurons, agnostic to neuronal feature-selectivity. To rigorously test whether gain modulation according to task-relevant feature-selectivity is a generalizable attentional mechanism, we conducted an experiment in which task-relevant features were altered across trials within the same recording session. Recordings were made within and across functional columns in V1, while monkeys performed multiple attention tasks requiring discriminations of stimulus contrast, orientation, and color. We also assessed feature-selectivity for the same V1 neurons independent of the attention tasks. We investigated the linear dependence of attentional modulation in each task on neuronal feature-selectivity using univariate and multivariate regression models. We also investigated these relationships using multidimensional feature-selectivity values per neuron, computed with unsupervised clustering methods. Preliminary data do not support a strictly linear dependence of attention on task-

relevant feature selectivity, but functionally distinct subpopulations are distinctly modulated by visual attention across tasks.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Attentional effects in macaque area V1 mediate selective V1-to-V4 communication: A DCM study with multi-start Variational Bayes

Authors: *C. KATSANEVAKI[1,2], A. M. BASTOS[1,3], H. CAGNAN[4,5], C. A. BOSMAN[6,7], K. J. FRISTON[4], P. FRIES[1,2,6];
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Abstract: Selective attention implements preferential routing of attended stimuli, likely through increasing the influence of the respective synaptic inputs on higher-area neurons. As the inputs of competing stimuli converge onto postsynaptic neurons, presynaptic circuits might offer the best target for attentional top-down influences. If those influences enabled presynaptic circuits to selectively entrain postsynaptic neurons, this might lead to selective routing. Indeed, when two visual stimuli induce two gamma rhythms in macaque area V1, only the gamma induced by the attended stimulus entrains gamma in V4. Here, we modeled this selective entrainment with a Dynamic Causal Model (DCM) for Cross-Spectral Densities. Models were fitted to ECoG Local Field Potential (LFP) data using a newly implemented multi-start scheme with the standard Variational Bayes algorithm that is used to fit DCMs. We found that selective entrainment and communication in the gamma band can be explained by attentional modulation of intrinsic V1 connections. Specifically, local inhibition was decreased in the granular input layer and increased

in the supragranular output layer of the V1 circuit that processed the attended stimulus. Thus, presynaptic attentional influences and ensuing entrainment were sufficient to mediate selective routing.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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Title: Top-down control of exogenous attentional selection is mediated by beta coherence in prefrontal cortex

Authors: *A. DUBEY[1], D. MARKOWITZ[1], B. PESARAN[2];
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Abstract: Salience-driven exogenous and goal-driven endogenous attentional selection are two distinct forms of attention that guide selection of task-irrelevant and task-relevant targets in primates. During conflict i.e., when salience and goal each favor the selection of different targets, endogenous selection of the task-relevant target relies on top-down control. Top-down attentional control mechanisms enable selection of the task-relevant target by limiting the influence of sensory information. Lateral prefrontal cortex (LPFC) is known to mediate top-down control, however the neuronal mechanisms of top-down control of attentional selection are poorly understand.
To investigate the neural mechanisms of top-down control in LPFC, we simultaneously recorded spike and field potentials from a 32-channel drive in two monkeys, during a two-target, free-choice luminance-reward selection (LRS) task. The LRS task dissociated exogenous and endogenous processes by manipulating relative luminance and reward differences. We found that visual-movement (VM) neurons and not visual neurons or movement neurons encode exogenous and endogenous attentional selection. Selection times estimated from VM neurons firing activity were faster for exogenous than endogenous selection (mean ST: 49 vs 116ms). Neural activity during the pre-target baseline period reflected a coherent beta (15-30 Hz) network. We show that coherent-beta activity selectively modulates mechanisms of exogenous selection specifically during conflict and consequently may support top-down control. Exogenous selection but not

endogenous selection showed a decrease in VM firing with an increase in baseline beta power. Our results reveal the VM-neuron-specific network mechanisms of attentional selection and suggest a functional role for beta-frequency coherent neural dynamics in the modulation of sensory communication channels for the top-down control of attentional selection.

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Poster

317. Mechanisms of Attention: Non-Human Primates

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Topic: H.01. Attention

Title: Spatiotemporal coding of attention in the marmoset posterior parietal cortex

Authors: *A. ALIZADEH[1], K. D. JOHNSTON[2], S. EVERLING[2], **L. MA**[1,3];
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Abstract: How do we know if a brain region is encoding a task parameter? If significant information can be decoded from its activity, the answer is an easy yes; but when the decoding fails, it may still be that our algorithm fails to emulate the one employed by the brain. To better understand how neuronal ensembles encode information, it is important to consider both the spatial and the temporal firing patterns; especially now that both aspects are readily accessible through high-density recording techniques. One approach that meets all these needs is space-by-time non-negative tensor factorization (stNTF). Here we applied the stNTF to spiking activities in the posterior parietal cortex (PPC), to decode and predict the attentional engagement of marmosets in a saccadic task. In this task, the marmosets maintained fixation on a central dot until its disappearance. The peripheral target may appear to the left or right, to which the animals made a prompt prosaccade to obtain a reward when they were attentionally engaged or failed to do so when they were not. The latter case was rare early on in each session but occurred more often towards the end of the sessions. Using PPC ensemble activities recorded during fixation, stNTF successfully predicted the correct response 87.18±8.5% of the time, while linear discriminant analysis (LDA) had a success rate of 60.2±7%, given a 50% chance level. During the subsequent response period, LDA's success rate rose to 68.73±16% whereas stNTF succeeded at 89.61 ± 8% of the time. Thus, stNTF appeared to have captured information reflected in firing patterns that were not as well captured by LDA. We went on to demonstrate that it was possible to predict the animal's response on trial *t+1*, using activities recorded during *t-n* to *t*, with an *n* greater than 8. We then examined the mechanism by which the successful decoding was achieved. Intriguingly, the success rate during fixation stayed above 80% with the removal of any neuron, showing a redundancy for task engagement in the PPC. The temporal pattern that contributed to the decoding during fixation reflected persistent activity at the

ensemble level, whereas that for saccadic response reflected a relay of information. The spatial pattern may reflect a topography for task engagement in the PPC.

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Poster

317. Mechanisms of Attention: Non-Human Primates

Location: SDCC Halls B-H

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Program #/Poster #: 317.14

Topic: H.01. Attention

Title: Optogenetic manipulation of covert spatial attention in the nonhuman primate

Authors: *L. N. KATZ[1], C. MEJIAS-APONTE[2], M. A. SOMMER[3], M. O. BOHLEN[4], R. J. KRAUZLIS[5];
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Abstract: Determining the neuronal circuits governing covert spatial attention is a major goal in systems neuroscience. Optogenetic manipulations afford a causal way to interrogate neuronal circuits by providing circuit-specific control at precise times to affect behavior. The technique has been successfully wielded in nonhuman primates, but the behavioral effects observed to date have been largely confined to the realm of saccadic eye movements and the effectiveness of optogenetics in studying higher-order aspects of behavior in primates remains unclear. Here we tested whether optogenetic manipulation in the primate superior colliculus (SC), a midbrain structure crucial for attention, leads to behavioral effects in a covert attention task, separately from any effects on saccades. To this end, we used a motion-change detection task in which one of two motion stimuli was cued, and the monkey was required to respond to a change in motion direction at the cued peripheral location by releasing a joystick, while maintaining central fixation. In one monkey, we injected SC with a viral construct expressing the halorhodopsin *Jaws* and later delivered red light through a tapered optic fiber to suppress neuronal activity. Electrophysiological recordings during light delivery confirmed robust neuronal suppression, and a guided saccade task to an array of targets in visual space was used to confirm the spatial extent of the suppression ("the suppressive field"). To test the effect of optogenetic suppression on covert spatial attention, we first obtained a positive control by documenting the effects of light delivery on saccadic reaction times to targets placed in the center of the suppressive field. Next, we positioned one of the two motion stimuli used in the covert attention task in the center of the suppressive field, and the other in the opposite hemifield. Light delivery coincident with the change in the cued stimulus disrupted the monkey's ability to detect the motion change, leading to reduced hit rates for that location. Hit rates for the opposite location were unaffected, indicating that the effect was spatially specific and not a motor deficit. Hit rates for trials in which only one stimulus was presented were not as affected, indicating that the effect was not

due to a low-level visual impairment but depended on selection amongst distractors. The ability to use optogenetics to cause brief and spatially specific deficits in visual attention - even in a task without saccades - opens up new opportunities for interrogating the neuronal circuits that control visual attention in the primate.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Title: Homologous Cortical Signals of Reward Prediction Error in Human and Mouse

Authors: *P. KEHRER[1], J. F. CAVANAGH[2], C. PIRRUNG[3], G. SINGH[3], J. L. BRIGMAN[4]; [2]Dept. of Psychology, [3]Psychology, [4]Dept. of Neurosciences, [1]Univ. of New Mexico, Albuquerque, NM

Abstract: Title: Homologous Cortical Signals of Reward Prediction Error in Human and Mouse
James F Cavanagh[1], Chris Pirrung,[1] Penelope Kehrer[1,2], Garima Singh[1], & Jonathan L. Brigman[2]
[1]University of New Mexico, Department of Psychology[2]University of New Mexico Health Sciences Center, Department of Neuroscience
The reward positivity is an EEG feature that has recently been shown to common to humans and mice. Using reward prediction errors (+RPE) we can define the common information in spectral signals between species. Previous studies have shown that humans and mice both perform well at probabilistic selection tasks, and time frequency plots of high vs. low probability rewards (pseudo-+RPE differentiation) reveal a common delta band time-frequency Region-of-Interest (tf-ROI). Whereas the prior work used dura screw recordings in mice, the current study aimed to reveal the source of this signal. Mice were implanted with sixteen-lead arrays in the pre/infralimbic (n=11xx) or cingulate (n=12xx) cortex .cortex. After a suitable recovery period the mice then performed a probabilistic learning task where they chose between a fan image or a marble image. The fan image was rewarded 80% of the time ,whereastime, whereas the marble image was rewarded. Mice displayed approximate matching behavior over 600 trials (10 days) of assessment (~80% accuracy).Findings). Findings suggest that the pre/infralimbic area is the major contributor of +RPE in the tf-ROI. The cingulate gyrus also captures some relevant variance, but this is not specific to reward..reward. This suggests that the pre/infralimbic areas may be homologous to the ventromedial areas that generate the reward positivity in humans,

whereas cingulate areas respond to generic surprise. Future work will continue to test homologies between this rodent cortical signal and human studies.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.02

Topic: H.03. Decision Making

Support: Wellcome Trust 095495
Wellcome Trust 204811

Title: Systematic comparison of risky choice preference reversals in human and monkey

Authors: *L. SEAK, S. FERRARI-TONIOLO, W. SCHULTZ;
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Abstract: Monkeys have been used to study the neuronal mechanism of risky decision-making for more than a decade. Although some previous studies tried to compare the decision-making of humans and monkeys, these studies did not investigate the consistency of risk preference across these two primate species. We here designed a task allowing direct comparison of the two species' behavior in a systematic way. We used a well-defined economic test for choice consistency, based on the independence axiom of expected utility theory. We measured the similarity in human's and monkey's stochastic choice behavior in terms of violations of the independence axiom. More specifically, we tested whether adding a common gamble to two gamble options will change the preference of the subject (across 34 sessions and 26 sessions for two monkeys respectively). With this task, we found that monkeys exhibited preference reversal patterns that, to some extent, are similar to humans. Moreover, our machine learning models built with monkey data predicted human behavior and vice versa, supporting the general preference reversal consistency across primates. Our study not only systematically compared risky choice behavior in humans and monkeys, but also provided the basis for using monkeys in single-cell electrophysiology experiments, which would allow us to study the neuronal mechanisms of choices in concept-defined tests of stochastic choice preference consistency.

Disclosures: L. Seak: None. **S. Ferrari-Toniolo:** None. **W. Schultz:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Topic: H.03. Decision Making

Support: Wellcome Trust and the Royal Society, Sir Henry Dale Fellowship 206207/Z/17/Z (F.G.)
Taiwan Ministry of Education Fellowship for Overseas Studies (F.-Y. H.)

Title: Nutrient and sensory coding of anticipated food reward in primate amygdala neurons

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Abstract: Nutrients and sensory food qualities influence the subjective valuation of foods. For example, sugar and fat make foods attractive because of their sweet taste and rich oral texture. Recently, it has been shown that when monkeys chose nutrient-defined liquids, they preferred fat and sugar to low-nutrient alternatives consistent with the assignment of subjective values to choice options (Huang et al., 2021). Importantly, subjective valuations of oral-texture parameters explained the monkeys' fat preferences, suggesting that a texture-sensing mechanism may underlie nutrient values. Here we investigated how single neurons in the amygdala process the sensory and nutrient components of food rewards and related subjective values. We trained two adult male rhesus macaques (*Macaca mulatta*) in a conditioning task in which visual stimuli predicted liquid food rewards with well-defined nutrient and sensory properties. The rewards included flavored dairy-based liquids varying in fat and sugar content, oral texture and control stimuli that mimicked the flavor and texture of the test liquids. Each reward was associated with two distinct conditioned stimuli for visual control. We found that the responses of amygdala neurons to visual conditioned stimuli reflected the sensory and nutrient components of the anticipated food rewards and their subjective values. Specifically, we regressed the responses of 237 recorded neurons on oral-texture parameters, fat and sugar levels, and subjective values inferred from choices. A substantial number of neurons signaled the sugar and/or fat content of anticipated rewards. Almost all fat-sensitive neurons also encoded oral texture, consistent with behavioral evidence that the influence of fat content on choices is mediated by oral-texture parameters (Huang et al., 2021). A significant number of neurons encoded subjective value based on the rewards' nutrient and sensory components. The responses typically emerged within 350 ms after visual-stimulus onset and were not explained by visual cue properties. Thus, primate amygdala neurons encode the sensory and nutrient properties of anticipated food rewards and related values. Our findings identify the amygdala as an integration site that translates information about nutrients and sensory food properties into subjective values important in reward learning and decision-making.
Ref: Huang, F.-Y., Sutcliffe, M. P. F., & Grabenhorst, F. (2021). Preferences for nutrients and sensory food qualities identify biological sources of economic values in monkeys. *Proceedings of the National Academy of Sciences*, 118(26).

Disclosures: F. Huang: None. **F. Grabenhorst:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

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Topic: H.03. Decision Making

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Title: Amygdala inputs to primate frontal cortex contribute to the encoding of reward timing

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Abstract: BACKGROUND: Interaction between the amygdala and frontal cortex (PFC) is essential for adaptively guiding learning and decision-making based on reward (Murray and Fellows 2022). The amygdala plays a key role in representing the reward value of outcomes following reward-guided choices, but less is known about the role of amygdala in tracking the timing of reward, specifically when rewards start and end. Here we investigated how parts of frontal cortex represent the timing of reward delivery both before and after excitotoxic lesions of the amygdala in two distinct tasks.
METHODS: Macaques performed a two-alternative forced-choice task for fluid reward while neural activity was recorded from areas 11 and 13 in orbitofrontal cortex (OFC) and the areas 9 and 24 in anterior cingulate cortex (dACC) both before and after bilateral amygdala lesions. In one task macaques made choices between familiar stimuli (object discrimination) whereas in another they chose between novel stimuli (learning).
RESULTS: Neurons in both OFC and dACC exhibited an outcome-related signal associated with the timing of the end of reward. Before lesions of the amygdala more neurons in OFC, compared to dACC, encoded when rewards ended in the object discrimination task compared to the learning task. This pattern was altered after amygdala lesions; more neurons in ACC signaled the reward end in the object discrimination task. Notably, the higher proportion of reward timing encoding neurons in the object discrimination task was largely preserved in OFC after amygdala removal.
CONCLUSIONS: Amygdala input to PFC is critical for valuation and learning. Here we show that amygdala inputs also play a role in signaling the timing of reward in frontal cortex; critically, this role differs depending on the familiarity of the chosen stimuli and, more generally, depending on the learning environment.

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Poster

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/AEI/10.13039/501100011033 MCIU AE
EU HBP SGA3 Human Brain Project Specific Grant Agreement 3 (945539)

Title: A mechanism for view-based decisions in the primate amygdala

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Abstract: To obtain the best rewards, primates forage visually. By shifting their view from one object to the next, they compare the values of sequentially viewed objects and decide on the best option from a distance, even before acting. Thus, in many natural situations, consideration of choice options is inherently sequential and defined by current view or attentional focus. However, it is unclear whether a dedicated neural mechanism supports such view-based decisions, how it transforms object values to abstract, view-based representations and eventually recovers the chosen object's identity, which is critical for guiding action. Here we recorded the activity of amygdala neurons while two adult male macaques chose between sequentially viewed objects that differed in reward value. Importantly, the animals could form a choice for the currently viewed or last-viewed object covertly, well before they could plan a saccade toward the chosen object. We found that amygdala neurons encoded the monkeys' decisions in a representation defined by current view but not by specific object or reward properties. These view-based choice signals preceded conventional object-choice signals, and were shown in control experiments to be independent of physical features of visual objects and rewards. View-based choice signals emerged gradually across amygdala subdivisions: an object-based code in the lateral nucleus accurately tracked values for specific objects and transitioned to a view-based code in the basolateral nucleus that showed signatures of decision computation and predicted view-based choice. Neural-network modelling identified a sequence of computations by which amygdala neurons implemented view-based decision-making and eventually recovered the chosen object's identity when the monkeys acted on their choice. These findings reveal a neural mechanism that derives object choices from abstract, view-based computations, suggesting an efficient solution for decision problems with many objects.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Topic: H.03. Decision Making

Support: Wellcome Grant WT 095495
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European Research Council Advanced Grant 293549

Title: Diverse coding of subjective economic value in monkey dopamine neurons

Authors: *S. FERRARI-TONIOLO, L. CHI U SEAK, W. SCHULTZ;
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Abstract: Introduction. In value-based decisions, we choose the option with the highest subjective value. Several economic theories define subjective value as resulting from the nonlinear evaluation of objective reward quantities (e.g., reward magnitude m and probability p in risky choice). This could represent a brain mechanism for value computation, one in which the objective reward quantities are nonlinearly transformed and combined into a subjective scalar quantity. We investigated whether and how midbrain dopamine neurons, fundamental units for reward processing, encode economic subjective value during risky choice.
Methods. Compatible with modern economic theories, we defined economic value as the product of nonlinear transformations of reward magnitude (utility, u(m)) and reward probability (probability weighting, w(p)). The resulting economic value function ($V(m,p) = u(m) \cdot w(p)$) represented the subjective value assigned to each choice option. We inferred the economic value function that best described monkey choices through a maximum likelihood estimation procedure. While monkeys made choices, we recorded the activity of single dopamine neurons (N=165, from 2 animals) and analysed the neuronal responses to the gamble cues. We then fitted the economic value function to the neuronal data, obtaining a neuronal economic value function.
Results. We analysed the activity of single dopamine neurons in response to the choice options' cues. The neuronal responses increased monotonically with the value of the chosen option, in terms of both reward magnitude and reward probability. Fitting the economic value function to the neuronal activity highlighted a nonlinear coding of the objective reward quantities. This nonlinearity varied across neurons, indicating the heterogeneous coding of subjective value in a population of dopamine cells.
Conclusions. Our data showed that dopamine neurons' responses to the objective reward quantities were nonlinear and compatible with the economic model. As the coding of value varied across neurons, the neuronal economic value did not systematically reflect the economic value inferred from choice. We are currently applying this framework to understand how a diverse neuronal population is able to encode subjective values compatible with specific choice

biases. This approach provides a basis for understanding the origin of different risk attitudes and choice fallacies.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Program #/Poster #: 318.07

Topic: H.03. Decision Making

Support: WT 095495
WT 204811

Title: Dopamine neurons signal internal subjective value estimates at a population level on a moment-to-moment basis

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Abstract: Dopamine neurons are thought to be a critical neural substrate for subjective valuation. Subjective value is estimated as a function of experienced reward and internal value 'calibration' specific to each individual. This internal calibration is likely a function of unobservable factors (e.g., behavioral history, phenotypic variation), making subjective value a moving target that is difficult to measure experimentally. Typical methods to elicit subjective value suffer three key shortcomings: 1) subjective value is not directly reported but rather inferred from choices; 2) choice sets are often ordinal and complicate the study of value cardinality (i.e., probing 'whether' choice options differ rather than 'by how much'); and 3) hundreds of trials are required to infer choice probabilities, obscuring moment-to-moment changes in value. For these reasons, the fidelity of dopamine neuron encoding of subjective value remains unclear. To more precisely understand how dopamine neurons encode subjective value from moment-to-moment, we trained rhesus monkeys to bid in a Becker-DeGroot-Marschak (BDM) auction task to elicit their willingness-to-pay for juice rewards while recording from midbrain dopamine neurons. The BDM is incentive compatible, meaning the optimal strategy is to bid one's true value; each bid offers an accurate trial-by-trial report of the monkey's internally generated subjective value that can be correlated with dopamine neuron activity. Neuronal responses to reward cues varied with subsequent animal bids even when reward magnitude was held constant. Although the fidelity of bid coding within single dopamine neurons was relatively low, support vector regression revealed that bids were accurately decoded when groups of neurons were analyzed, indicating that value estimates are constructed at a population level. These data show that dopamine responses reflect an intrinsic value estimate of goods prior to

actions taken to acquire them, guiding behavior to obtain the highest subjective value for that individual in a given moment.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Program #/Poster #: 318.08

Title: WITHDRAWN

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

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Program #/Poster #: 318.09

Topic: H.03. Decision Making

Support: NIH Grant MH123713

Title: A grid code for value-based decision-making

Authors: *M. A. ORLOFF[1], S. A. PARK[3], J. BLUMWALD[2], P. DOMENECH[4], E. D. BOORMAN[1];
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Abstract: Grid cells, originally identified for their role in representing physical space (Hafting et al., 2005), have recently been shown to encode an individual's location in abstract space (i.e., a non-physical 2D space), for example in a 'bird' space of neck length and leg length (Constantinescu et al., 2016) or a social space of popularity and competence (Park et al., 2020, 2021). Other neuroeconomic work shows that decisions utilize a common neural currency, where rewarding things such as juice, money, and social stimuli are similarly encoded in the brain (Levy & Glimcher 2012). At the intersection of this work on abstract space and value representation, a grid code for 'value space,' comprised of a magnitude and a probability dimension, has recently been identified in nonhuman primates (Bongioanni et al., 2021). Here, we ask if human primates use a grid code for this value space. To test this, we administer two tasks which both use two sets of shapes that vary along two continuous dimensions (such as

frequency of a grid and orientation) corresponding to magnitude ($) and probability (%), respectively. We first trained 11 adults (8/2/1 F/M/O) to identify the dimensions which correspond to the probability and magnitude for both sets of shapes. We then administered two behavioral tasks where individuals were asked to make a series of binary choices between shapes. First, by calculating the proportion of choice trials participants chose the shape with the higher expected value, we show that individuals make decisions based on the magnitudes and probabilities of each shape in both tasks (task 1: 81.9% ± 5.7%, $p = 4.4e{-}09$; task 2: 76.7% ± 3.9%, $p = 6.1e{-}10$). Second, we implement a mixed effects logistic regression model to further show that, in both tasks, individuals combine probabilities and magnitudes of each shape by multiplying them together to make decisions (task 1: $\beta = 2.6$, $SE = 0.22$, $p < 2e{-}16$; task 2: $\beta = 1.9$, $SE = 0.15$, $p < 2e{-}16$). Lastly, we demonstrate a relationship between response time and decision difficulty according to this multiplicative model (task 1: $\beta = -0.10$, $SE = 0.013$, $p = 1.4{-}e05$; task 2: $\beta = -0.10$, $SE = 0.020$, $p = 3.8e{-}04$). These results mirror the behavioral results of similar work done in nonhuman primates (Bongionanni et al., 2021) and suggests that individuals may utilize a grid code when making decisions in value space. Our future work will utilize intracranial electroencephalography (iEEG) and functional magnetic resonance imaging (fMRI) to examine neuronal firing and BOLD signals, respectively, for evidence of a neural grid code for value space.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Topic: H.03. Decision Making

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Title: Planning during an abstract strategy board game in monkeys

Authors: *M.-Y. PARK[1], B. V. OPHEUSDEN[4], H. LIANG[2], M. OEMISCH[3], K. OSBORNE[3], W. MA[5], D. LEE[1];
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Abstract: Sequential choices during iterative social interactions can be planned according to the current state and desired outcomes. To investigate the dynamics of sequential strategic decision-making and the underlying neural mechanisms, we have trained two monkeys to play a competitive strategy game of four-in-a-row. During this game, the monkeys placed their stones one at a time using a joystick on a 4-by-9 board while taking turns with a computer opponent.

Each player could win by placing 4 pieces in a row horizontally, vertically, or diagonally, and the game outcome could be a win, loss, or tie for the animal, which was rewarded only for wins. The game required the animals to learn the goal of the game, how to plan and execute sequential choices, and how to evaluate and update these plans based on their outcome. Both animals successfully learned how to place their stones sequentially to make a four-in-a-row in multiple ways while avoiding the computer pieces on the board. Moreover, they learned how to block the computer opponent in order to complete their four-in-a-row before the opponent does. This implies that the animals were able to appropriately estimate and compared the values of connecting their own stones and those of blocking the opponent's similar efforts.
To quantitively examine how the animal's choices were affected by the strategy of the computer opponent, we applied multinomial logistic regression model to the animal's behaviors observed against defensive vs. aggressive component opponents. In this model, the value of each potential move was computed by weighted sum of 11 features that was grouped into 3 categories, including the position of the stone on the board, the position to increase the number of stones in a straight line (connecting), and the position to block the opponent from placing their stones in a straight line (blocking). The weights for each feature was chosen to minimize the discrepancies between the actual moves made by the animal and those predicted by the model. We found that the weights for connecting and blocking were low and high, respectively, against aggressive opponents, whereas the opposite was true for defensive opponents. These results showed that monkeys could adjust their strategies during an iterative board game according to the strategy of the opponent and suggest that abstract board games can be a useful platform to investigate the neural mechanisms of strategic decision making.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Topic: H.03. Decision Making

Support: 2019M3E5D2A01060293
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Title: Value-related Dimension of Neural Population Activity in Primate Dorsolateral Prefrontal Cortex Represents Rapid Choices: Application of Non-Motoric Brain-Machine Interfaces

Authors: *M.-K. KIM[1], Y. CHO[2], H. PARK[3], G. KIM[2], J.-W. SOHN[4];
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Gangneung, Korea, Republic of; [4]Med. Sci., Catholic Kwandong Univ., Yeonsu-gu, Korea, Republic of

Abstract: The dorsolateral prefrontal cortex (DLPFC) is functionally involved in multiple cognitive processes, one of which is to estimate values of stimuli or actions for decision making to maximize rewards. However, it remains unclear how a value-related dimension of neural population activity from DLPFC modulates decision-making during rule-changing in probabilistic reward learning. This study aims to find the value-related dimension that projects decision-making based on rewards. To test whether such dimension space is manipulable by subject, we build a non-motoric brain-machine interface for rapid choice based on states confined in that dimension. We also investigate how neural dynamics confined in the value-related dimension are represented during the current decision-making depending on whether rewarded or unrewarded in the previous trial. To this end, we recorded neural activities in the four different cortical regions (DLPFC, primary motor cortex, posterior parietal cortex, and visual cortex) while one rhesus macaque performed a two-alternative forced choice (2AFC) task with the five probabilistic rewards, where only the data from DLPFC was used for the analyses. Inferring the value-related dimension from neural population activity was performed by dimensionality reduction methods, and this was utilized in the 2AFC online control. We found that changes in neural dynamics at 200ms after the target presentation were significantly distinct in terms of target choice for locations (left- and right-sided targets) compared to colors (red or blue). We could successfully translate the neural dynamics associated with changes in the internal state toward decision-making into cursor movements in the 2AFC online control. These results suggest that the value representations in the DLPFC, depending on the previous rewards, are integrated with non-motoric cognitive processes associated with predicting the decision-making.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Title: Decoding working memory of economic values from the prefrontal cortex

Authors: *K. NI[1], X. CAI[1,2];
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Abstract: Neurons in the orbitofrontal cortex (OFC) encode economic value and their activity is causally linked to economic choices. However, in choice tasks in which subjective values must be stored in working memory (WM) for subsequent choices, single-unit analysis of spiking activity failed to identify value-encoding WM signals in the OFC. In this study, we leverage the power of population decoding to examine whether economic value as WM content can be decoded from OFC and/or lateral prefrontal cortex (LPFC). We trained two monkeys to perform an economic choice task with sequentially presented offers (consisting of offer 1, memory, offer 2 periods) in which the animal must memorize the value of offer 1 then compare it with that of offer 2 to make a choice. Using multi-channel recordings (u/v-probes, Plexon Inc), we recorded ~1300 neurons (on average >10 neurons simultaneously recorded per experimental session) from the OFC and LPFC, respectively. Although few neurons showed persistent value-selective activity in either area, pseudo population decoding analysis revealed that the value of offer 1 was maintained in WM and retrieved in offer 2 period in both areas. To probe the temporal dynamics of population coding patterns across the trial, we performed a cross-temporal decoding analysis. Namely, across the course of a trial, we trained a decoder using the data in one time bin and tested the decoding accuracies of the same decoder in all other time bins (bin size = 100 ms, step = 10 ms). The outcome suggests that both OFC and LPFC populations exhibited fairly stable and significant coding for economic values during the WM period, while dynamic coding of value was more prevalent outside the WM period. A demixed principal component analysis (dPCA) confirmed the findings from the decoding analysis in that stable value-related components can be extracted from the population activities of both areas. These results to our knowledge provided the first neuronal evidence of population level parallel value-WM representation in the OFC and LPFC. They also resonate with current views emphasizing the distributed nature of WM, which, in contrast to localized WM, is more robust against perturbation/distraction. Overall, our finding suggests that value-WM may be instantiated in the interactive network involving OFC and LPFC.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

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Program #/Poster #: 318.13

Topic: I.06. Computation, Modeling, and Simulation

Support: NIMH Grant R01MH123687

Title: Gaze-contingent microstimulation of the striatum and anterior cingulate cortex affects flexible learning of feature values in non-human primates

Authors: *R. L. TREUTING[1], K. BANAIE BOROUJENI[2], T. WOMELSDORF[2,1];
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Abstract: Flexible behavior involves enhancing responses to newly valuable objects and inhibiting responses to unrewarding or punishing objects. The fast learning about better and unlearning of bad objects is believed to be supported by the anterior cingulate cortex (ACC) and anterior striatum (aSTR). However, causal manipulations of activity in the ACC and aSTR in previous studies largely induced more static and permanent changes of behavior including the biasing of choices toward specific stimuli or the facilitation of learning more permanent stimulus-response mappings. Here, we set out to investigate whether causal neuromodulation in the ACC or aSTR can also affect how fast reward associations of objects are learned and unlearned. We tested changes of fast and flexible learning by micro-stimulating the ACC or aSTR while non-human primates (NHP) fixated on either gain-associated (Sr+) or loss-associated (Sr-) objects during the learning of feature reward values. Electrical stimulation used a cathodal leading biphasic pulse train triggered when the NHP's fixated on one of three objects to collect reward tokens and avoid losing tokens that were later cashed out for fluid reward. Learning took place at variable cognitive loads and motivational contexts. Cognitive load varied across learning blocks by changing the number of features that could be possible reward targets. The motivational context varied by associating good and bad objects with variable gains and losses of tokens. We found that gaze-contingent ACC stimulation slowed reward learning in Sr+ blocks, particularly at higher cognitive load. In contrast, aSTR stimulation significantly improved reward learning, particularly in Sr- blocks.These findings provide causal evidence that gaze-contingent micro-stimulation enhances or reduces flexible learning depending on the brain area within the fronto-striatal network. With regard to the ACC, the deterioration of learning during Sr+ stimulation is consistent with electrical perturbation enhancing outcome uncertainty and impairing credit assignment for specific object features. For the aSTR, the improved learning in Sr- blocks is consistent with electrical stimulation facilitating inhibitory plasticity of negative-reinforced stimulus-reward associations. These findings provide causal evidence for a functional dissociation of ACC and aSTR to support flexible learning of abstract feature values irrespective of specific sensory-motor mappings.

Disclosures: R.L. Treuting: None. **K. Banaie Boroujeni:** None. **T. Womelsdorf:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.14

Topic: I.06. Computation, Modeling, and Simulation

Support: NIMH Grant R01MH123687

Title: Routing states in spiking networks are gated by oscillatory bursts and attention and amplified during decision making

Authors: *K. BANAIE BOROUJENI[1], T. WOMELSDORF[2];
[1]Vanderbilt Univ., [2]Dept. of Psychology, Vanderbilt Univ., Nashville, TN

Abstract: Information routing in the brain relies on temporally ordered interactions of spiking activity between interconnected neural ensembles. However, so far, it has been unresolved which factors determine the direction and gain of ordered spiking interactions. Here we address these questions by first generating predictions in a realistic model of interconnected spiking networks and testing the model predictions in extracellularly recorded neural activity in lateral prefrontal cortex (LPFC), anterior cingulate cortex (ACC) and the striatum of nonhuman primates performing an attention demanding learning task. We quantified lead/lag relationships between single neurons and neuronal ensembles using spike-weighted spike-triggered multiunit activity that faithfully estimated the temporal ordering of spiking activity between areas.We found that spiking networks intrinsically generate rhythmic spiking activity that systematically lead or lag spiking activity across anatomically connected areas at fast time scales (±20msec). In neuronal recordings we confirmed that on average, spiking activity in LPFC led spiking activity in anterior cingulate cortex (ACC) and striatum by a few milliseconds. This temporal lead was locally modulated by high power and transient local field potential (LFP) bursts. In ACC, neurons that lagged LPFC activity became leaders during LFP theta/alpha bursts (8-14 Hz). In LPFC, the network of spike-leading neurons over striatal activity was expanded during LFP beta bursts (15-25 Hz), which enhanced the temporal lead of LPFC over the striatum. Selective attention and choice behavior modulated the temporal lead-lag relationships in the network. During a choice, neurons in ACC and LPFC increased their spike-lead over neuronal activity in the striatum. During selective attention, prior to making a choice, neuronal ensembles in ACC changed their lead-lag relationships. With attention, neural ensembles in the ACC that were already leading over the striatum amplified their lead, while within the ACC-LPFC network, the ACC neural ensembles that showed spike lead during 8-14 Hz LFP bursts switched to lead neural activity in the LPFC.Taken together, these findings illustrate that the temporal precession of spiking activity can be quantified in the fronto-striatal network to estimate network wide routing states at high temporal resolution. These routing states dynamically were switched or amplified during frequency-specific LFP bursts, selective attention and decision making.

Disclosures: K. Banaie Boroujeni: None. **T. Womelsdorf:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.15

Topic: H.03. Decision Making

Support: NIH Grant R01 DA049147
NIH Grant R01 NS086104

Title: Supplementary Eye Field encodes temptation-modulated monitoring and evaluative signals to guide behavior during intertemporal choice

Authors: *K. LEE[1], J. HWANG[3], V. STUPHORN[2];
[1]Psychological and Brain Sci., [2]Mind/Brain Inst., Johns Hopkins Univ., Baltimore, MD; [3]Section on Neurobio. of Learning and Memory, Lab. of Neuropsychology, NIMH/NIH, Bethesda, MD

Abstract: In real-life situations a choice may not lead to an immediate reward, instead requiring a wait before reward delivery. In these situations with temporally separated choice and reward, information must be retained after choice to successfully evaluate its outcome. Moreover, there must be continuous monitoring for relevant environmental changes that can occur during the delay and change the value of the upcoming reward. How an individual evaluates and monitors in the context of delayed rewards are therefore integral to understanding how we pursue and achieve long-term goals. However, it remains unclear how neuronal activity supports these processes. The Supplementary Eye Field (SEF) is known to carry context-dependent action value signals (i.e., the subjective value of selecting an option) and also monitoring and evaluative signals, making the SEF a potential candidate for this role. Here, we examined how the SEF represents sustained task-related signals throughout the delay, and whether they are used to inform behavior in the context of delayed rewards. We recorded the activity of 201 single neurons from the SEF of two monkeys performing a self-control task, a modified intertemporal choice task in which for a small proportion of trials, the monkey is presented with the temptation to switch from his initial choice to the other option. First, we found that SEF neurons continue to represent task-related value information throughout the delay and reward delivery periods. In particular, SEF activity after reward delivery predicted choice in the subsequent trial, suggesting that the SEF retains value information for evaluation and for modulating future choice. Second, we found that the sustained value representation in the SEF reflects the unexpected appearance of temptation. Finally, we found that SEF activity predicts for how long the monkey remains at his initial choice of the larger delayed reward before ultimately giving into the temptation to switch to the more immediate smaller reward. Together, this work demonstrates that the SEF continues to encode task-related variables after choice to support monitoring and evaluative roles in the context of delayed rewards.

Disclosures: K. Lee: None. **J. Hwang:** None. **V. Stuphorn:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.16

Topic: H.03. Decision Making

Title: Electroencephalographic differences in reward and punishment during the stages of decision-making

Authors: *F. A. IRIBE BURGOS, J. P. GARCÍA HERNÁNDEZ, P. M. CORTES ESPARZA, M. HERNÁNDEZ GONZÁLEZ, M. GUEVARA PÉREZ;
Inst. De Neurociencias, Inst. De Neurociencias, Guadalajara, Mexico

Abstract: Decision-making (DM) has been defined as the choice of one option among multiple alternatives. Regarding the cognitive processes involved, DM can be divided into 3 stages: forming preferences, execution of action (s), and experiencing the outcome. Since outcomes may be pleasant (reward) or unpleasant (punishment) they may modify behavior and the strategies used in decision-making to allow individuals to adjust their behavior and preferences towards pleasant options. The prefrontal, temporal, and parietal cortices participate in these processes to enable the transition from stimuli evaluation to action. The goal of this study was to determine electroencephalographic coherence (hEEG) during the three stages of a DM task with reward (shorter execution time) or punishment (longer execution time). Twenty healthy, right-handed men aged 20-35 participated voluntarily. EEG activity was recorded in the dorsolateral prefrontal (F3-F4), frontopolar (Fp1-Fp2), temporal (T3-T4), and parietal (P3-P4) cortices. EEG interhemispheric (hINTER) and intrahemispheric (hINTRA) coherence were analyzed for each EEG band: theta (4-7 Hz), alpha1 (8-10 Hz), alpha2 (11-13 Hz), beta1 (14-19 Hz), beta2 (20-30 Hz), and gamma (31-50Hz). The ANOVA analysis showed interaction between the DM stages and the experimental conditions, specifically with increases of coherence in the last stage in the reward condition, for hINTER in the alpha2 and beta1 bands between the left and right temporal cortices (T3-T4) [alpha2, FInteraction = 3.52; p (F) = 0.032; beta1, FInteraction = 3.30; p (F) = 0.039], for hINTRA in the alpha2 band between the left frontopolar and parietal cortices (F1-P3) [FInteraction= 4.61; p (F) = 0.012], and between the right frontopolar and temporal cortices (F2-T4) [FInteraction= 4.13; p (F) = 0.018]. Data show that the type of feedback modified cortical EEG coupling between the DM stages. The hINTER between temporal cortices could be associated with the affective processing of pleasant stimuli as the end of the DM task approached in the reward condition, while the hINTRA between frontopolar and posterior areas could participate in the processing of the semantic meaning of the reward. In conclusion, the type of feedback received during DM processes can modify relations among brain structures during the experiencing the outcome stage of DM.

Disclosures: F.A. Iribe Burgos: None. **J.P. García Hernández:** None. **P.M. Cortes Esparza:** None. **M. Hernández González:** None. **M. Guevara Pérez:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.17

Topic: H.03. Decision Making

Title: Eeg activity related to contingency changes during decision-making

Authors: *J. P. GARCÍA HERNÁNDEZ, P. CORTES, F. A. IRIBE BURGOS, M. HERNÁNDEZ GONZÁLEZ, M. A. GUEVARA;
Univ. De Guadalajara, Univ. de Guadalajara, Guadalajara, Jalisco, Mexico

Abstract: Decision-making (DM), understood as the ability to process multiple alternatives, has been divided into three stages: 1) formation of preference; 2) execution of an action; and 3) outcome evaluation. DM is a flexible process that allows individuals to adapt their behavior in response to contingency changes in order to reach a goal. Formation of preference and outcome evaluation are essential for these changes in behavior. One technique for evaluating how such changes modify behavior utilizes reversal learning paradigms in which the stimulus-reward association is modified. Performance on these tasks has been related to changes in the activity of the ventromedial and orbitofrontal prefrontal cortices; specifically, during inhibition of a previously established response. The prefrontal cortex thus works in conjunction with posterior areas, such as the parietal and temporal cortices, due to their role in evaluating stimuli during information updating. A description of how electrical activity in these cortices changes between stimuli presentation and outcome evaluation in a contingency change paradigm could offer insights into the neural bases of behavioral adaptation in DM. The objective of this study was to evaluate frontopolar, dorsolateral, parietal, and temporal electroencephalographic (EEG) activity during the formation of preference and outcome evaluation stages of DM in a reversal learning condition. The EEG activity of 22 young men was recorded while they executed a DM task that was divided into 3 blocks (each of 40 trials): 1) initial learning condition: subjects made a stimuli-reward association; 2) retention condition: the previous association was reinforced; 3) reversal learning condition: the stimuli-reward association was changed. During the formation of preference stage in the reversal learning condition of the DM task, there was a higher rEEG between P3 and P4 in the alpha2 band, while in the outcome evaluation stage there was a higher AP at F3 in the theta band and a higher rEEG between F2 and F4 and F2 and T4 in the alpha1 band. The increased rEEG in parietal areas during the formation of preference stage in the reversal learning condition could be related to the semantic processing involved in the re-association of value; whereas the changes in prefrontal-temporal activity during outcome evaluation in this condition may be associated with the detection of contingency changes and the storage of new information in working memory.

Disclosures: J.P. García Hernández: None. **P. Cortes:** None. **F.A. Iribe Burgos:** None. **M. Hernández González:** None. **M.A. Guevara:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.18

Topic: H.03. Decision Making

Support: ERC/DYNERFUSION/865003

Title: EEG-informed high resolution imaging of human midbrain activity during reward and punishment learning

Authors: *J. CARVALHEIRO, M. G. PHILIASTIDES;
Sch. of Psychology & Neurosci., Univ. of Glasgow, Glasgow, United Kingdom

Abstract: Learning to make choices that lead to rewards while minimise punishments is critical for adaptative behaviour. During reward learning, midbrain dopaminergic neurons have been shown to encode outcome value and salience signals, which unfold in rapid succession and drive learning. In contrast, the involvement of the dopaminergic pathway in punishment learning remains an active debate topic in the literature. In humans, fMRI has been the primary tool for probing the neural representations of value and salience signals. However, the low temporal resolution of fMRI precludes a rigorous identification of the temporal dynamics of these signals which could be multiplexed at the level of macroscopic BOLD activity. Here, we exploit high temporal precision EEG collected simultaneously with 3T fMRI (with tailored sequences to image the SN/VTA complex), to identify rapidly unfolding signals of value and salience and use the endogenous variability in these signals to tease apart their spatial representations in the midbrain via EEG-fMRI fusion. We collected preliminary data (N=12) during a probabilistic reversal-learning task, in which participants had to choose between two visual stimuli carrying different outcome probabilities, in separate reward and punishment contexts. The two contexts led to win/no-win (reward context) and loss/no-loss (punishment context) outcomes. We used multivariate discriminant analysis of the EEG to find spatial projections in the data that discriminated between win vs. loss (to test for value) and win/loss vs. no-win/no-loss (to test for salience). We found reliable discrimination performance across wins vs. losses as well as in win/loss vs. no-win/no-loss in the outcome-locked EEG data, in the range of 260-600ms post-outcome, albeit with distinct spatiotemporal patterns observed across the different dimensions of value and salience. These results are suggestive of parametric value signals as well as separate salience signals unfolding near simultaneously across reward/punishment contexts. Given the temporal proximity of value and salience signals, in a next step we will use the endogenous, trial-by-trial variability in these electrophysiological signatures, to build EEG-informed fMRI predictors and decouple these cascading signals in the BOLD activity. In doing so, we aim to understand the extent to which these cortical representations could explain BOLD variability in the SN/VTA complex, thereby offering a more comprehensive picture of the spatiotemporal dynamics of value and salience in midbrain dopaminergic structures.

Disclosures: J. Carvalheiro: None. **M.G. Philiastides:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.19

Topic: H.03. Decision Making

Title: Evidence for value normalization circuits in human BOLD signals

Authors: *B. SHEN[1], K. LOUIE[2], P. GLIMCHER[1];
[1]Neurosci. Inst., NYU Grossman Sch. of Med., New York, NY; [2]Ctr. for Neurosci., New York Univ., New York, NY

Abstract: Contextual value coding has been shown in both neural activity and context-dependent behavior. However, the precise form of contextual coding is controversial, with proposed models including divisive normalization, range adaptation, and subtractive competition. Here we developed a novel mathematical tool to differentiate predictions of different theories and examined fMRI activity in a pilot sample of human subjects performing a contextual economic choice task. Human subjects were scanned in a two-stage evaluation and choice task (258 trials). In the first step of choice trials, the participant viewed one, two, or three consumer product(s). In the second step, the items were withdrawn. In the third step, either two items were presented - one item from the previous set and a new item or two items from the previous sets were redisplayed. The participant chose one within 2.5s. The steps were designed to elicit value representation, working memory, and action selection signals in a manner that dissociates value signals from choice probability signals. From the behavior, we found that the choice accuracy (assessed by a prior bid-based valuation) between two items changes with contextual value (the value of the extinguished items) as a 'U' shape - initially decreasing but rebounding at high levels of contextual value, consistent with previous findings and the prediction of divisive normalization. Mathematically, we show that the cross 2nd derivative (X-D^2) of BOLD over direct input value and contextual value differentiates existing theories: zero X-D^2 in range adaptation and subtractive inhibition, and negative X-D^2 in divisive normalization. We approximate the effect of X-D^2 by contrasting the parametric modulations of the item value between high and low values of the other two. In preliminary data, we identified negative X-D^2 in insula and VS during representation, and STG and M1 during memory, suggesting a contextual suppression consistent with divisive normalization. Surprisingly, we also observed positive X-D^2 in distinct areas including pMTG and PM during representation, and insula and VS during memory, suggesting non-linear contextual enhancement in these areas, which may reflect attention, salience, or contrast effects. In conclusion, we identify distinct value-related circuits for both contextual suppression and contextual enhancement in human value coding. Together, these results reinforce the canonical nature of normalization in neural computation and emphasize the importance of a strict mathematical analysis in brain-wide disambiguation of alternative decision-making models.

Disclosures: B. Shen: None. **K. Louie:** None. **P. Glimcher:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

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Program #/Poster #: 318.20

Topic: H.03. Decision Making

Support: Research Talent Development Fund of the University of Zurich
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Nomis Foundation

Title: Neural representations of combined consumption in the human brain

Authors: *H.-K. CHUNG[1], P. N. TOBLER[2];
[1]Univ. of Zurich, [2]Univ. of Zurich, Zurich, Switzerland

Abstract: Consumption is a characteristic feature of modern societies and often concerns not only single goods but combinations of goods. Some goods are more desirable when consumed together with other goods than separately. For example, people may prefer consuming cake in combination with coffee more than consuming each food separately. These types of goods are called complementary goods. In contrast, substitute goods serve a similar purpose, they are interchangeable to some degree such as coffee and black tea. While the brain circuits that are critical for valuation and decision-making have begun to be identified in the last decades, there is still a lack of understanding of the conditional valuation processes for different relations between goods. The present study aims to investigate such processes at both behavioral and neural levels. In our experiment, participants provided willingness to pay (WTP) bids for the same food items sometimes in isolation (single condition) and sometimes in combination with other foods (paired condition). In the paired condition, participants were given a free food on top of the food item they were bidding for. This novel design allowed us to investigate how WTP differed between single and paired conditions ($\Delta WTP = WT_{paired} - WTP_{single}$) while controlling for the intrinsic value of the food item. To independently determine the interaction between goods, participants also provided ratings of perceived complementarity and substitutability for each food pair after the experiment. We predicted that the valuation of a particular food item would change as a function of which other items it was paired with.
As predicted, ΔWTP increased when the combined foods were rated as higher complementary but decreased when combined foods were rated as higher substituting. These findings indicate that complementary and substituting relations between goods differentially affect valuation. Moreover, regression analyses revealed a positive relationship between increased valuation of an item and the self-reported frequency with which participants consumed the two items in combination. The preliminary fMRI results replicate previous findings that medial orbitofrontal cortex and striatal regions encode subjective value as measured by WTP. More importantly, such value regions, but also the substantia nigra/ventral tegmental area and parahippocampal regions process the value change (ΔWTP). Together, our behavioral and neural findings are compatible with the notion that memory contributes to the enhancement of value when goods are higher complementary.

Disclosures: H. Chung: None. **P.N. Tobler:** None.

Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

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Program #/Poster #: 318.21

Topic: H.03. Decision Making

Support: NIDA Grant 261086

Title: Individual differences in substance use associated with elevated temporoparietal junction responses to rewards shared with friends

Authors: *O. ZAFF[1], D. SAZHIN[1], J. B. WYNGAARDEN[1], J. DENNISON[1], A. VAFIADIS[1], A. NGUYEN[1], J. M. JARCHO[1], L. ALLOY[1], J. M. CHEIN[1], D. S. FARERI[2], D. V. SMITH[1]; [1]Dept. of Psychology & Neurosci., Temple Univ., Philadelphia, PA; [2]Psychology, Adelphi Univ., Garden City, NY

Abstract: Study Objective: A growing number of studies have found a link between self-reported substance use and aberrant reward responses in the striatum. Less is known, however, about whether this effect is independent of an individual's trait reward sensitivity when experiencing rewards within social contexts. We set out to probe whether reward-related striatal responses in substance users will differ independently of self-reported reward sensitivity across social and non-social contexts. We hypothesized that the effect of substance use would be such that its association with striatal activation varies independently of trait reward sensitivity during receipt of social reward. Methods: Participants (N=52) underwent fMRI while they played a card guessing game. Monetary outcomes were shared with three partners--(1) a close friend of the participant, (2) a stranger, or (3) the computer, thus manipulating the social context in which rewards were experienced (Fareri et al., 2012). Trait Reward Sensitivity was assessed via a composite of the Behavioral Activation Scale (Carver & White, 1994) and Sensitivity to Punishment/Reward Questionnaire (Torrubia & Tobeña, 1984). Substance use was assessed via a composite of the Alcohol Use Disorders Identification Tests and Drug Use Disorders Identification Tests (Babor et al., 1992). We excluded 7 participants based on pre-registered criteria (aspredicted.org/SFX_MXL) for head motion and missed trials leaving a final sample of N = 45 for all analyses. Results: We found that social and nonsocial rewards evoke activation in the ventral striatum (VS). Consistent with prior work with this task (Fareri et al., 2012) we observed increased striatal activation to rewards shared with a friend than with a stranger. A preliminary whole-brain analysis did not support the prediction that VS response to social reward is associated with substance use. However, we found that increased activation of the temporoparietal junction (TPJ) for rewards experienced with friends, relative to strangers and computers, was associated with increased substance use (FWE cluster correction = 0.05). Conclusions: These results replicate previous research on striatal activation for rewards experienced in social as opposed to non-social contexts, and provide evidence that TPJ activation in these conditions may be moderated by substance use. Future analyses will explore the

interaction between substance use and reward sensitivity. Understanding the links between neural response to social reward, trait reward sensitivity, and substance use may provide new insights into the mechanisms underlying addiction.

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Poster

318. Neural Mechanisms of Value Based Decision Making in Non-Human Primates and Humans

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 318.22

Topic: H.03. Decision Making

Support: NIH Grant R01HD097619

Title: Neural mechanisms underlying the expectation of rewards resulting from effortful exertion

Authors: *A. KIM[1,2], V. S. CHIB[1,2,3];
[1]Biomed. Engin., Johns Hopkins Sch. of Med., Baltimore, MD; [2]Kennedy Krieger Inst., Baltimore, MD; [3]Kavli Neurosci. Discovery Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Expectations shape our decisions to engage in effortful activities. If we exert a large amount of effort while expecting payment in return, but receive less than expected, we might feel dissatisfied with the outcome. However, if we receive more rewards than expected, we might feel more satisfied. In this way, reward expectations can serve as a reference point that motivates effortful activity. Despite the fundamental role of reference points in human performance, little is known about how the brain processes expectations to guide motivated exertion. In this experiment, 34 participants (24 ± 7 years, 17 F) completed a reward-based effort task and we used functional magnetic resonance imaging (fMRI) to investigate the neurobiology of reference-dependent effort exertion. During each trial, participants were presented with a risky option that would either result in a fixed monetary payment, regardless of their effort exertion, or a piece-rate payment where the payment was in proportion to the amount of effort exerted. Each of these options had an equal probability of occurring, and we varied the fixed payment so that participants had different expectations. The actual payment was realized after individuals had an opportunity to exert effort, allowing us to infer how the brain encoded expectation as a reference point in relation to outcomes. We found that on average, participants exerted more effort as the fixed payment increased (linear mixed-effects model: $t=7.28$, $p<0.001$, cohen's $d=0.45$), suggesting that the fixed payment influenced the reference point for effort exertion. To examine how the brain processes expectation to guide effort exertion, we explored brain activity when individuals received the outcome of the risky option (i.e., fixed payment or piece-rate payment). We found that activity in the ventral striatum was significantly correlated with the deviation from

the payoff to reward expectations (p=0.005, k=20), consistent with previous studies which showed that ventral striatum is responsible for encoding reward expectations and predictor error. Overall, these results suggest that value-related brain areas, particularly the ventral striatum, encode expectations of reward as a reference point to motivate effort exertion. Understanding why individuals think such effort is worth exerting is the key question in the neuroeconomics of effort-based decision-making. Our study sheds light on how reward expectation, encoded in well-known value-related brain areas, can contribute to evaluating this decision-making process for effortful activities.

Disclosures: A. Kim: None. **V.S. Chib:** None.

Poster

319. Executive Function and Inhibitory Control

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 319.01

Topic: H.04. Executive Functions

Support: Nippon Telegraph and Telephone East Corporation

Title: Prolonged eSports playing induces cognitive fatigue in association with pupil diameter reduction rather than the perceived sense of fatigue

Authors: *T. MATSUI, S. TAKAHASHI, Y. MINE, A. KAWAMOTO, S. YASUDA, S. YOSHITAKE, H. MATSUOKA;
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Abstract: Esports, a form of competition using video games, has emerged worldwide as a new sport, and many young people often play esports for a long duration, seeking entertainment and self-actualization. Acute and chronic video games played for moderate durations enhance cognitive functions, particularly executive functions in relation to prefrontal activities. Still, prolonged mental activities on visual display terminals can cause a decline in executive functions, called cognitive fatigue, together with a reduction in pupil diameter as an indirect indicator of prefrontal activity. We here tested the hypothesis that prolonged esports play causes cognitive fatigue in association with pupil diameter reduction. Thirteen young men who like video games wore heart rate monitors and blood glucose sensors, and they played 180 min of eFootball (Konami Digital Entertainment Co., Ltd.) against a gaming artificial intelligence. Mood (perceived sense of fatigue and enjoyment etc.) and executive functions (flanker, Stroop, and Simon tasks) were assessed at five time points: pre-play, 60, 120, and 180 min after the start of play, and 30 min after the end of the play. Physiological parameters (heart rate and pupil diameter) were also measured constantly during play. Only at 180 min was the perceived sense of fatigue assessed by a visual analogue scale significantly higher than the pre-play levels. Enjoyment peaked at 120 min of play and then returned to pre-play levels. Arousal levels measured by the two-dimensional mood scale were higher at 60 and 120 min of play but lower

after 180 min, compared to pre-play levels. Flanker interference was significantly shortened at 60 min of play compared to pre-play levels, but it was delayed at 120 and 180 min. The percentage of correct responses to the flanker task and the surface temperature of the fingertips were lower only at 180 min. Pupil diameter decreased with playing duration. Delayed flanker interference did not positively correlate with a perceived sense of fatigue, but it did negatively correlate with changes in pupil diameter (Δ pupil diameter). Stroop and Simon task performance levels, heart rate, and blood glucose levels remained unchanged throughout the experiment. These results reveal that esports play lasting over 120 min induces cognitive fatigue in association with a reduction in pupil diameter as a possible biomarker of declined prefrontal activity. Cognitive fatigue during prolonged esports playing could diverge from the perceived sense of fatigue, which is different from exercise and physical sports, proposing pupil diameter as a non-invasive neuro-biomarker to aid the perception of cognitive fatigue during esports play.

Disclosures: T. Matsui: None. **S. Takahashi:** None. **Y. Mine:** None. **A. Kawamoto:** None. **S. Yasuda:** None. **S. Yoshitake:** None. **H. Matsuoka:** None.

Poster

319. Executive Function and Inhibitory Control

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 319.02

Topic: H.04. Executive Functions

Support: Asahi Soft Drinks Co., Ltd.

Title: Carbonated water mitigates cognitive fatigue with prolonged esports play

Authors: *S. TAKAHASHI[1], S. YOSHITAKE[1], H. MATSUOKA[1], Y. MINE[1], A. KAWAMOTO[1], Y. TAKAGI[1], S. YASUDA[1], W. KOSUGI[2], S. MIZUNO[2], T. MATSUI[1]; [1]R&D Ctr. for Sport Innovation, Univ. of Tsukuba, Tsukuba, Japan; [2]Asahi Soft Drinks Co., Ltd., Tokyo, Japan

Abstract: Although moderate esports play enhances executive function (EF), prolonged play over 120 minutes declines EF. This EF decline is called “cognitive fatigue”, which occurs along with a reduction in pupil diameter as an indirect indicator of prefrontal activities. Carbonated water stimulates the brainstem via capsaicin-sensitive neurons in the pharynx, and it ameliorates sense of fatigue and task performance during cognitive tasks. We thus hypothesized that drinking carbonated water improves cognitive fatigue with prolonged play of esports. To test this hypothesis, we here conducted a crossover design experiment with 15 healthy adults (14 males and 1 female, 22.9 ± 2.5 years) in fresh water (FW) and carbonated water conditions (CW). Participants played efootball 2021 (Konami Digital Entertainment Co., Ltd., Japan) against standard-level gaming artificial intelligence for 180 minutes while drinking fresh or carbonated water. Their heart rate, blood glucose, pupil diameter, and game stats were measured throughout play. Psychological parameters (sense of fatigue, hunger, enjoyment, and EF by flanker task)

were measured before (Pre), during (60, 120, and 180 minutes), and 30 minutes after playing (Post 30 minutes). Statistical analysis performed by Two-way ANOVA. Maximum heart rate in CW was higher than in FW. Blood glucose levels decreased depending on playing durations, independent of FW/CW conditions. Sense of fatigue and hunger increased depending on play duration in FW, but it did not increase in CW. Enjoyment levels with play in CW were higher than those in FW. Interference and correct response rate for the flanker task decreased after 120 and 180 min of play in FW, but there was no significant decrease in CW. Mean pupil diameters in FW were decreased depending on playing duration in association with declined flanker interference, but it was not observed in CW. Among the game stats, the number of fouls in CW was lower than FW. Our findings provide evidence that drinking carbonated water mitigates cognitive fatigue in associations with pupil diameter as an indicator for prefrontal activity, and improves fairness during prolonged esports play. Carbonated water is likely to serve as an ergogenic aid in prolonged modern cognitive activities such as e-sports, providing an alternative to caffeine and carbohydrates.

Disclosures: **S. Takahashi:** None. **S. Yoshitake:** None. **H. Matsuoka:** None. **Y. Mine:** None. **A. Kawamoto:** None. **Y. Takagi:** None. **S. Yasuda:** None. **W. Kosugi:** A. Employment/Salary (full or part-time):; Asahi Soft Drinks Co., Ltd. **S. Mizuno:** A. Employment/Salary (full or part-time):; Asahi Soft Drinks Co., Ltd.. **T. Matsui:** None.

Poster

319. Executive Function and Inhibitory Control

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 319.03

Topic: H.04. Executive Functions

Support: The Toyota Foundation
HAYAO NAKAYAMA Foundation for Science & Technology and Culture

Title: Esports increases in-play emotional expressions and post-play happy faces in a real offline tournament for non-professional players

Authors: *H. MATSUOKA, S. TAKAHASHI, S. YOSHITAKE, Y. MINE, S. YASUDA, A. KAWAMOTO, T. MATSUI;
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Abstract: Sports serve as an emotional communication tool through the enjoyment of human competition. However, the measurement of facial expressions, which act a critical role in communication, is difficult due to the intense physical movements during sports play; thus, sports-induced face expressions are largely unknown. We here aimed to examine the effect of electric sport (esport) by competing video games, a sport with minimal physical movements, on emotions by analyzing facial expressions. Sixteen university students participated in an offline esport tournament at the University of Tsukuba, Japan. eSport title was eFootball 2021 (Konami

Digital Entertainment Co., Ltd., Japan), and the total of 20 knockout matches were conducted (N= 40, 2 players in each match). The match last 10 min and was played in the physically same space as the opposing player. We recorded the players' face images throughout the pre-play (Pre), in-play, half-time (HT), and post-play (Post). The in-play was divided into the first half (FH) and the second half (SH). Time expressed and maximum intensity of facial emotions, neutral, happy, sad, angry, surprised, scared, and disgusted were analyzed by FaceReader 9 (Noldus Information Technology bv.). The time expressed in emotion was converted to a percentage of the time expressed in the emotion divided by the total time of each match segment. Two-way ANOVA was used, and statistical significance was set at .05. The percentage of time expressed with neutral faces significantly decreased in the Post (42.2±25.2%) compared to the Pre (61.7±24.3%), the FH (75.2±18.8%), and the SH (76.7±18.3%), while the percentage of time with happy facial expression significantly increased in the Post (31.3±24.8%) compared to the Pre (20.3±23.1%), the FH (12.3±12.0%), and the SH (12.7±12.1%). However, the maximum intensity of happy, sad, angry, surprised, and disgusted facial expressions was higher in the FH and the SH compared to the Pre, the HT, and the Post. There was no significant difference in their expressed emotion between the winning and losing players. The percentage of time and maximum intensity of post-play happiness were positively correlated with game stats related to opponent interactions. Correlation coefficients between the percentage of time in the happy face and the number of fouls, interception, opposing FK, and opposing shot probability were .51, .46, .51, and .54, respectively. Our findings provide evidence that eFootball match, a sport with minimal physical movements, induces in-play rich emotional expressions and post-play happy face in a real offline competition setting. eSport likely serves as a sport promoting human emotional communication.

Disclosures: H. Matsuoka: None. **S. Takahashi:** None. **S. Yoshitake:** None. **Y. Mine:** None. **S. Yasuda:** None. **A. Kawamoto:** None. **T. Matsui:** None.

Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.04

Topic: H.04. Executive Functions

Support: NIH/NIDA R01DA048094
NIH/NIDA R01DA038700
NIH/NIDA UG3DA048510

Title: Associations between right inferior frontal gyrus morphometry, cognitive control, and smoking dependence motives in individuals with nicotine dependence

Authors: *A. BROWN, S. UPTON, S. CRAIG, B. FROELIGER;
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Abstract: The hyperdirect pathway - a circuit involved in executing inhibitory control (IC) - is dysregulated among individuals with nicotine dependence (ND). The right inferior frontal gyrus (rIFG), a cortical input to the hyperdirect circuit, has been shown to be functionally and structurally altered among nicotine-dependent smokers. The rIFG is divided into 3 anatomical subregions: opercularis, triangularis, and orbitalis. This study investigated the associations between rIFG morphometry, IC, smoking dependence motives, and smoking relapse susceptibility. Baseline behavioral and magnetic resonance brain imaging (MRI) data from 127 adult smokers smoking at least 5 cigarettes per day (CPD) (M_{age}=42.9, ±11.1, M_{CPD}=18.3, ±7.0) were assessed. A Go-Go/NoGo task, the Wisconsin Index of Smoking Dependence Motives (WISDM) questionnaire, and a smoking relapse analog task (SRT), were used to assess IC, smoking dependence motives, and smoking relapse susceptibility, respectively. MRI data was processed via Freesurfer's cortical reconstruction pipeline. Two complementary approaches were used to assess rIFG morphometry: ROI parcellation via the Desikan-Killiany atlas, and rIFG cluster analysis. Average cortical thickness values within the rIFG ROIs and the rIFG cluster were extracted and analyses were performed in SPSS. Age and education were used as nuisance variables. ROI parcellations revealed that cortical thickness of the right orbitalis was associated with IC task performance (*pr* = .192, *p* = .032), inhibiting ad lib smoking during the SRT (*pr* = .225, *p* = 0.012), and composite WISDM score (*pr* = -.201, *p* = .025). Right triangularis cortical thickness was also associated with composite WISDM score (*pr* = -.265, *p* = .003). rIFG cluster analysis of cortical thickness vs. IC task performance revealed a significant cluster spanning the right triangularis and ventral opercularis (cluster-wise *p*-value = .017, size = 527.73 mm^2). Average cluster cortical thickness was associated with IC task performance (*pr* = .364, *p* < .001), and composite WISDM score (*pr* = -.236, *p* = .008). Post-hoc analyses revealed that IC task performance was associated with inhibiting ad lib smoking during the SRT (*pr* =.189, *p* = .034). These findings support prior research associating rIFG morphometry with cognitive control and smoking relapse vulnerability. Our results suggest that each rIFG subregion's morphometry is associated with different aspects of cognitive control and smoking behavior. Future research assessing rIFG subregion morphometry in smokers may help explain the relationship between rIFG structure and function, allowing for more precise treatment for individuals with ND.

Disclosures: **A. Brown:** None. **S. Upton:** None. **S. Craig:** None. **B. Froeliger:** None.

Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.05

Topic: H.04. Executive Functions

Support: Department of Veterans Affairs Office of Research and Development (I01 CX001816)

Title: Does behavioral impulsivity distinguish the severity of suicidal behavior? Examining response inhibition and severity of recent suicide related events in a high risk sample

Authors: *E. R. BENNETT[1], A. INTERIAN[2], K. RODRIGUEZ[3], A. R. KING[3], M. SEDITA[3], J. DEL POZZO[4], L. ST. HILL[3], R. MILLER[3], M. LATORRE[3], C. DAVE[5], C. E. MYERS[6]; [1]Rutgers, The State Univ. of New Jersey, New Brunswick, NJ; [2]Rutgers University-Robert Wood Johnson Med. Sch., Piscataway, NJ; [3]VA New Jersey Hlth. Care Syst., East Orange, NJ; [4]Montclair State Univ., Montclair, NJ; [5]Rutgers University-Institute for Health, Hlth. Care Policy and Aging Res., New Brunswick, NJ; [6]Dept. of Veterans Affairs, New Jersey Healthcare Syst., East Orange, NJ

Abstract: Suicide is the 10th leading cause of death in the United States. Other suicide-related events (interrupted/aborted attempts, preparatory suicidal behavior, or suicidal ideation-related hospitalizations) are also frequent occurrences and often precede actual suicide attempts, yet these important risk factors are understudied. This crisis demands improved understanding of the full range of suicide behaviors. Deficits in neurocognition are a known vulnerability associated with suicide, and behavioral impulsivity has been implicated in the transition from suicidal ideation to behavior. We studied a cohort of US Veterans (n = 50, 6 female) who presented with a history of suicide-related events. We used a Go/No-Go task (GNG) to evaluate behavior dependent on prefrontal substrates of inhibitory control, in which subjects respond to rapidly presented target stimuli ("Go" response) or withhold responding ("No-Go") to infrequent foil stimuli. We considered two GNG task versions, with varying complexity of foil stimuli, to examine between group and within subject replicability. Subjects were categorized as past-month actual suicide attempt (ASA, n = 10); past month other suicide-related event excluding ASA (OtherSE, n = 26); or neither (noSE, n = 14). Results on the simpler GNG task revealed a significantly higher rate of false alarms (FA) to foils in the OtherSE group as compared with the ASA or noSE groups (robust ANOVA followed by post-hoc Bonferroni-corrected Welch's t-tests). The more challenging GNG task showed a similar pattern, approaching significance. Within-subject reliability of FA rate was r = 0.71 across the two GNG versions. The finding of increased impulsivity in those with prior month suicide-related events is consistent with prior findings of decreased response inhibition related to suicidality; however, the absence of increased FA in those with a prior month ASA is somewhat surprising. Although the current sample size is small, these results indicate important cognitive considerations in the subgroup of high-risk individuals who transition to outright ASA. Results also appear consistent with previous research associating more lethal ASAs with less cognitive impulsivity, perhaps due to the planning and forethought used during a suicide attempt. Future examinations will explore relationships between ASA lethality and GNG performance, as well as history of traumatic brain injury to investigate TBI-induced impulsivity and resultant suicide risk. By extending previous neurocognitive findings, these results show the promise of using impulse control measures for improving predictive tools to identify individuals at high-risk for suicide.

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Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.06

Topic: H.04. Executive Functions

Support: NIH T32-DA037183
NIH K23-MH112867

Title: Hierarchical Drift Diffusion Modeling of Reaction Times of Individuals with Anorexia Nervosa on a Video Foraging Task (WebSurf)

Authors: O. L. CALVIN[1], *A. D. REDISH[2], A. F. HAYNOS[3];
[1]Neuroscience; Psychiatry and Behavioral Sci., [2]Neurosci., [3]Psychiatry and Behavioral Sci., Univ. of Minnesota, Minneapolis, MN

Abstract: Recent theories of anorexia nervosa suggest that the pursuit of weight control reflects a general rigid pursuit of tasks and goals, implying that this excessive rule-based goal pursuit may be evident on other tasks that encourage optimization of some outcome. In this study we explored how individuals (n=30 with anorexia nervosa, n=30 demographically-matched controls with no history of an eating disorder) behaved on the WebSurf task, a cross-species translational foraging task. Participants surfed for enjoyable videos of different categories, choosing to wait out a delay (1s-30s) or skip a video (random time delay at each trial). As in prior studies, subjects (both those with anorexia nervosa and controls) revealed thresholds for each video category, typically staying for delays below threshold and skipping for delays above threshold. The value of an offer was calculated as the difference between that offer's delay and the delay threshold between the participant choosing to stay or skip offers of that type. To better understand the computational process that gives rise to participants' choices, we fit a set of hierarchical drift-diffusion models (HDDM) to subject responses and reaction times on the task. We observed that value-congruent responses (staying for an offer below threshold, skipping above threshold) were generally faster than value-incongruent responses, and became faster as offer values became more extreme. The model that best described the reaction-time pattern changed the bias parameter as a function of the value such that it became more biased towards the value-congruent response as the offer value became more different from the threshold. Other drift-diffusion models that we explored (including standard HDDM models and models in which the rate of integration changed as a function of value) were unable to account for the slower reaction times of value-incongruent responses. The change in bias as a function of value suggests a pre-planning / precommitment rule-like response to the offer. An excess of pre-planning or precommitment could explain often-observed clinical characteristics of anorexia nervosa.

Disclosures: O.L. Calvin: None. **A.D. Redish:** None. **A.F. Haynos:** None.

Poster

319. Executive Function and Inhibitory Control

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Topic: H.04. Executive Functions

Support: NIH/NIDA R01DA048094
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NIH/NIDA UG3DA048510

Title: Effects of Continuous Theta-Burst Stimulation of the Hyperdirect Pathway on Inhibition, Craving, and Tobacco Smoking

Authors: *S. UPTON[1], A. BROWN[1], M. ITHMAN[1], R. NEWMAN-NORLUND[2], G. SAHLEM[3], E. L. GARLAND[4], J. PRISCIANDARO[5], E. MCCLURE[5], B. FROELIGER[1]; [1]Univ. of Missouri, Columbia, MO; [2]Univ. of South Carolina, Columbia, SC; [3]Stanford Univ. Med. Ctr., Palo Alto, CA; [4]Univ. of Utah, Salt Lake City, UT; [5]Med. Univ. of South Carolina, Charleston, SC

Abstract: Nicotine dependence (ND) is associated with dysregulated hyperdirect pathway (HDP: right inferior frontal gyrus [rIFG]; right supplementary motor area [rSMA]; right subthalamic nucleus [rSTN]) function. We previously reported that HDP task state functional connectivity (tsFC) mediates the association between inhibitory control (IC) and smoking relapse vulnerability. We present new results from two studies: **Study 1 (Baseline Study)** examined associations between HDP tsFC and smoking behaviors; **Study 2 (TBS Study)** examined the effects of rIFG neuronavigated continuous TBS (cTBS) and intermittent TBS (iTBS) on IC performance, HDP tsFC, craving and cigarettes per day [CPD] over 48hrs. Safety and feasibility were also assessed. **Study 1:** 127 adult smokers (M_{age}=42.9, ±11.1; M_{CPD}=18.3, ±7.0) completed MRI, questionnaires, and a behavioral smoking relapse analog task (SRT). The SRT presents neutral, emotional, and smoking images across 10, 6-min. blocks. After each block, participants choose to either earn $1 for completing an additional block or quit the task and smoke. **Study 2:** 37 adult smokers (M_{age}=47.6, ±9.5; M_{CPD}=18.8, ±5.7) completed a baseline session and then two counterbalanced rIFG TBS sessions – one while receiving cTBS, the other while receiving iTBS. Task-based fMRI data were collected immediately prior to and following each TBS session, and survey data were collected over 48hrs following each session. MRI data were analyzed in SPM12 and CONN toolbox. **Study 1 Results:** rIFG-rSTN tsFC was positively associated with inhibiting ad lib smoking during the SRT (r=.189, p=.034) and negatively associated with craving (r=-.213, p=.016). **Study 2 Results:** cTBS improved IC performance (M=+3.78, SE=2.0), while iTBS impaired it (M=-3.08, SE=1.6) (p=.007). cTBS marginally strengthened rIFG-rSTN tsFC (M=+0.32, SE=2.1), while iTBS weakened it (M=-1.41, SE=3.0) (p=.114). Both TBS conditions reduced CPD post-24hrs (p's<.04). Only cTBS reduced craving post-24hrs (p=.026). cTBS related changes (post-pre) in: 1) IC performance was associated with less craving at session's end (r=-.355, p=.033) and post-24hrs (r=-.333, p=.047); 2) rIFG-rSTN tsFC was associated with less craving post-24hrs (r=-.334, p=.046) and 48hrs (r=-.366, p=.033); 3) rIFG-rSMA tsFC was marginally associated with less CPD post-24hrs (r=-.321, p=.060). No adverse events were reported. These novel findings provide support for a model of the HDP contributing to inhibiting smoking. Also, rIFG cTBS may strengthen HDP tsFC and have value for treating addiction pathophysiology. Future research examining an extended course of TBS as a therapeutic intervention for ND is warranted.

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Poster

319. Executive Function and Inhibitory Control

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 319.08

Topic: H.04. Executive Functions

Title: Vim stimulation effects on strategic adjustment during action control in ET patients

Authors: *J. KANE[1], J. MCDONNELL[1], T. STEWART[1], S. WYLIE[1], J. S. NEIMAT[2], N. C. VAN WOUWE[2];
[2]Neurosurg., [1]Univ. of Louisville, Louisville, KY

Abstract: Introduction: Although Essential Tremor (ET) is a movement disorder primarily marked by action tremor, previous studies have also found impairments in the cognitive aspects of action control, i.e., the ability to control conflicting or impulsive action tendencies (conflict control). Deep Brain Stimulation (DBS) of the ViM (ventral intermediate nucleus) offers effective improvement of the clinical motor symptoms in ET, however, the effects on cognitive action control are unclear. **Objective:** We investigated whether stimulation of the ViM impacts strategic adjustment during conflict control performance in ET. **Methods:** Three ET patients (data collection ongoing) with bilateral ViM implants performed a Simon conflict control task both OFF and ON stimulation. The Simon task requires a left or right response to a feature of a spatially lateralized stimulus (colored circle). When the color of the circle and the action signaled by its spatial location do not correspond (conflict), the inappropriate action impulse must be suppressed. This leads to slower reaction times and more errors on conflict versus non-conflict trials (Simon effect). We used a modified version of the Simon task with three levels of difficulty, based on the relative amount of conflict and non-conflict trials (30, 50, or 70% conflict trials). Fewer conflict trials make the task more difficult which also increases the likelihood of errors. The parametric manipulation of task difficulty allows us to investigate whether participants can make strategic adjustments in action control either across conditions (macro-adjustments) or post error-trials (micro-adjustments). **Results:** Distributional analyses of the error rates tentatively suggested more fast impulsive actions with DBS ON versus OFF across conflict conditions and this seemed most pronounced in the difficult conflict condition. Distribution analyses also revealed that with slower RTs, impulse suppression improved ON DBS, but only in the relatively easy condition. Further analyses will associate these cognitive measures with clinical motor outcomes. **Conclusions:** Our preliminary results tentatively suggest that bilateral DBS ViM might modulate conflict control, particularly in response to the relative amount of conflict presented. Clinically, this could translate to a recommendation for patients to slow down in highly demanding situations that require quick decision making. Future studies should investigate whether stimulation induced changes in cortico-striatal or cerebello-

thalamocortical circuits are responsible for modulations of action impulsivity and how this correlates with clinical outcome measures.

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Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.09

Topic: H.04. Executive Functions

Support: Gruber Science Fellowship

Title: A transdiagnostic analysis reveals differential brain edge motifs in manic versus non manic patients

Authors: *M. FOSTER[1], S. NOBLE[2], D. SCHEINOST[1];
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Abstract: A transdiagnostic analysis reveals differential brain edge motifs in manic versus non-manic patients Maya Foster[1], Stephanie Noble[2], Dustin Scheinost[1,1]Department of Biomedical Engineering, [2]Department of Radiology and Biomedical Imaging, Yale University, New Haven CT
Mania is defined as a condition in which an individual experiences a period of abnormally elevated activity or extreme changes in mood or energy levels. Its neurobiological basis remains a mystery and it is not well studied in neuroimaging contexts. The benefit and merit of studying mania is that it is a symptom of different disorder types and so it can be thought of as on a transdiagnostic continuum that spans in severity and presentation. Moreover, disorders of which mania is a feature are often conflated with each other - leading to misdiagnosis which makes treatment difficult. By taking a transdiagnostic approach to studying mania, we can better construct a fundamental picture of what it is and modify our understanding such that timely and targeted treatment can be enforced. This project aimed to determine what brain areas, if any, are differentially connected in patients with and without manic symptoms using resting state and task functional magnetic resonance imaging (fMRI). Further, this project aimed to assess if consistent brain areas were differentially connected across task and resting state fMRI. To conduct this analysis, mean time series data was extracted for all patients (N = 142; 116 females; 49 Bipolar I, 50 Schizophrenia, 43 ADHD) from the UCLA Consortium for Neuropsychiatric Phenomics dataset using the Shen-268 atlas. The mean time series were correlated with each other to create a 268 by 268 symmetric matrix connectome. Mania was defined using a non-clinical scale called the Young Mania Rating Scale (YMRS). Scores within the dataset population ranged from 0 to 39, a value greater than 12 indicating the emergence of manic symptoms (35 manic total). An unequal variance two-tailed two-sample t test thresholded at a p

value of 0.05 was conducted to determine significantly different edges in manic vs non-manic populations across the seven tasks and resting state fMRI conditions. Correction for multiple comparisons was then conducted using false discovery rate (FDR). Motor areas were found to have the most consistent significant differences between manic and non-manic connectomes across tasks. Resting state fMRI revealed heavy involvement of the frontoparietal areas. Crucially, although nascent, these results suggest that brain areas involved in mania may be transdiagnostic, spanning several disorders.

Disclosures: M. Foster: None. **S. Noble:** None. **D. Scheinost:** None.

Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.10

Topic: H.04. Executive Functions

Title: Association between biochemical parameters and cognitive performance in first year undergraduate students of Health Sciences at the Autonomus University of thr State of Mexico

Authors: *G. ORTIZ-CABRERA[1], J. ESTRADA[1], M. LÓPEZ-MEZA[3], V. PAREDES-CERVANTES[4], I. CONTRERAS[2];
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Abstract: Association between biochemical parameters and cognitive performance in first year undergraduate students of Health Sciences at the Autonomous University of the State of Mexico
*Ortiz-Cabrera Guadalupe[1], Estrada José Antonio[1], López-Meza M. Sagrario[2], Vladimir Paredes-Cervantes[3], Contreras Irazu[11] Faculty of Medicine. Autonomous University of the State of Mexico. State of Mexico [2] Faculty of Behavioral Sciences. Autonomous University of the State of Mexico. State of Mexico[3] La Raza National Medical Center. Mexican Social Security Institute. Mexico City.
Correspondence: Ortiz-Cabrera Guadalupe, Faculty of Medicine. Autonomous University of the State of Mexico. State of Mexico. email: ortizlupita012@gmail.com.
Adolescence is a stage characterized by emotional, psychological, physical and social changes. The lifestyles of college students usually have unhealthy characteristics, due to long study hours, lack of personal organization skills, and in some cases, separation from the family. It is well known that unhealthy diets are involved in development of chronic degenerative diseases. The objective of this study was to explore a possible association between biochemical parameters and cognitive performance in first year undergraduate students of health sciences at the Autonomous University of the State of Mexico. To this end, 50 first-year, 18 to 21 year-old students attending the Faculty of Medicine were selected to participate in the study. Biochemical blood tests and the

Wechsler Intelligence scale for adults-IV (WAIS-IV), were used to assess parameters including, glycaemia, cholesterol, triglycerides, proteins, billirubins, hepatic enzymes and ionic blood concentrations, along with four cognitive parameters: verbal comprehension index, perceptual reasoning, working memory and processing speed index. Multivariate, Rho and Sperman´s correlations were used to analyze the results. The results show cholesterol and triglyceride concentrations were elevated in both male and female subjects. A possible association (p=0.036, Rho -0.426) was found between working memory
index and blood phosphorus concentrations, as well as between intelligence quotient, glucose and cholesterol (p=0.000, Rho 1.000) for men. In women, a possible association was found between the processing speed index and blood concentrations of cholesterol, triglycerides, and glucose (p=0 .049, Rho 0.350). In conclusion, our data show significant correlation between specific blood biochemical parameters and cognitive performance in first-year students at the Faculty of Medicine, UAEM.

Disclosures: G. Ortiz-Cabrera: None. **J. Estrada:** None. **M. López-Meza:** None. **V. Paredes-Cervantes:** None. **I. Contreras:** None.

Poster

319. Executive Function and Inhibitory Control

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 319.11

Topic: H.04. Executive Functions

Title: Brain PET 18-FDG metabolism and neurocognitive déficit in chronic inhalants users

Authors: *M. A. MENDOZA-MELÉNDEZ[1,2], N. KERIK-ROTENBERG[2], K. RAZÓN HERNÁNDEZ[3], C. AGUILAR-PALOMEQUE[2], C. DıAZ-MENESES[2], T. CORONA-VAZQUEZ[2], V. CALDERÓN-SALINAS[1];
[1]CINVESTAV IPN: Ctr. de Investigacion y de Estudios Avanzados del Inst. Politecnico Nacional, Ciudad de México, Mexico; [2]Inst. Nacional De Neurologia Y Neurocirugia, Mexico City, Mexico; [3]UNAM, Mexico City, Mexico

Abstract: The intoxication by volatile psychoactive substances makes up a public health problem in the world, whose prevalence is approximately 7% among high school students in Mexico and is most prevalent in adolescents. Volatile solvents are contained in many common household products such as spray, paints, thinners, lacquers, and glues and people inhale toluene containing products for its instantaneous intoxicating effects. The aim of this study is to evaluate the chronic consumption effects of inhalant abuse in the brain and status neurocognitive in young adults in abstinence. The present study uses a cross-sectional design to evaluate thirty-five males, from 18 to 40 years old with chronic consumption of inhalants were recruited in Mexico City from 2013 to 2018 and submitted to drug rehabilitation and detoxification to “Centros de Atención y Adicciones” (non-governmental centers for addiction). 18F-FDG PET/CT brain scans studies were acquired and analyzed with SPM12, and evaluate executive functions with

neuropsychological BANFE tests. For the neuroimaging analysis 6 consumer participants in abstinence and 10 nonuser participants were selected. We found hypometabolism bilaterally in the prefrontral cortex, thalamus, putamen, anterior and posterior cingulate, and cerebellar cortex. The results obtained in the BANFE test to evaluate the cognitive deficit show that the group of consumers of cerebral hypometabolism took longer to execute and finish the Stroop Effect form A (101.3 ± 44.5 and 83.2 ± 25.4 seconds, $p < 0.031$) and Stroop effect form B (82.1 ± 35.4 and 69.1 ± 16.7 seconds, $p < 0.041$) in comparison with the group of non-users, obtaining statistical differences, respectively. Likewise, inhalant users presented a greater number of errors in the execution of the Tower of Hanoi test (1.2 ± 1.5 errors;) compared to non-users (0.5 ± 0.9), these differences being significant ($p < 0.008$),between both groups. Glucose metabolic imaging, brain uptake of 18F-FDGit is a useful tool for examining the metabolic impact of toluene abuse. We found hypometabolism bilaterally in the prefrontral cortex, thalamus, putamen, anterior and posterior cingulate, and cerebellar cortex with neurocognitive deficit in executive functions, when evaluating executive functions in people who consume inhalants with severe bilateral hypometabolism through complex neurocognitive tasks, we identified that it took them longer to analyze and abstract information, which most likely indicates that their mental planning processes (dorsolateral prefrontal cortex) and their ability to inhibit a highly automated response (orbitomedial prefrontal cortex) are diminished compared to nonusers.

Disclosures: **M.A. Mendoza-Meléndez:** None. **N. Kerik-Rotenberg:** None. **K. Razón Hernández:** None. **C. Aguilar-Palomeque:** None. **C. Dıaz-Meneses:** None. **T. Corona-Vazquez:** None. **V. Calderón-Salinas:** None.

Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.12

Topic: H.04. Executive Functions

Support: Nationwide Children's Hospital Clinical and Translational Intramural Award
American Heart Association

Title: Network-derived cortical volume and its links with neurocognition in children with critical congenital heart disease and healthy peers

Authors: ***K. R. HOSKINSON**[1], A. MCALLISTER[2], B. LANEY[1], P. THOMAS[1], M. L. MAH[2], K. VANNATTA[1];
[1]Abigail Wexner Res. Institue At Nationwide Children's Hosp., Columbus, OH; [2]Nationwide Children's Hosp., Columbus, OH

Abstract: Objective: As surgical approaches and complex care have improved, many of the roughly 40,000 children born annually with critical congenital heart disease (CHD) in the United States reach adulthood. However, there is growing awareness of neurocognitive morbidities and

disrupted self-regulation that are highly persistent and threaten quality of life. Identifying the neural systems that underlie these deficits is key to characterizing their etiology and potential for remediation. In this pilot, we examined how network-based cortical volume is associated with neurocognitive outcome and self-regulation in children with CHD and healthy peers (HP). Methods: Children with CHD (N=15, M_{age}=12.03y) and HP (N=13, M_{age}=11.55y) completed neurocognitive assessment (WASI-II Full Scale IQ, WISC-V Processing Speed Composite, NIH Toolbox) and parents rated children's self-regulation on the BRIEF-2. Children also underwent MRI on a 3T Siemens Prisma, including a high-resolution volumetric MPRAGE sequence. Network-based composites of the salience (SN), central executive (CEN), default mode (DMN), and mentalizing networks (MN) were computed from Freesurfer 7.0 output.
Results: Children with CHD performed more poorly than HP on the WISC-V PSI (p=.038, d=-0.70), and NIH Toolbox Flanker (p=.004, d=-1.10) and List Sorting Tasks (p=.017, d=-0.85), and were rated by parents as having worse emotional regulation (p=.014, d=-0.90). While not significant in group comparisons, children with CHD performed nearly a full standard deviation below expectation on other NIH Toolbox tasks and were rated by parents as over a standard deviation from the norm on cognitive, behavioral, and global self-regulation. Children with CHD also had reduced volume bilaterally in all composite networks (all ps less than .05, ds: -0.90 to -1.51). Greater network volume was generally correlated with better neurocognitive performance and parent-rated self-regulation, most notably in the left-hemisphere SN, CEN, and MN.
Conclusions: Children with CHD displayed expected difficulties with neurocognitive performance and self-regulation, at times obscured by poorer-than-expected performance by HP. These deficits were associated with underlying fronto-temporal neural networks linked with higher order executive function and cognitive control in other populations. This is a first step to fill in meaningful context for long-term morbidities from CHD. Next steps include identifying shared and distinct network contribution to other morbidities (e.g., emotional distress, social function) and longitudinal evolution of these links across vulnerable periods of neurodevelopment.

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Poster

319. Executive Function and Inhibitory Control

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 319.13

Topic: H.04. Executive Functions

Support: Canadian Institutes of Health Research (FRN114887)
Dr. Julien/Marcelle and Jean Coutu Foundation Research Chair in Community Social Pediatrics

Title: The association of screen time, impulsivity, response inhibition and working memory and their relationship to growth in ADHD symptoms: a longitudinal cohort study among adolescents

Authors: *J. WALLACE[1], E. BOERS[2], J. OUELLET[3], M. AFZALI[2], A. LIVET[2], P. CONROD[2];
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Abstract: With the recent widespread diffusion of screen-based devices in adolescent population, several studies investigated the association between screen time and the growth of attention-deficit/hyperactivity disorder (ADHD) symptoms. Findings suggested an important relationship between digital media use and ADHD symptom severity. However, further studies investigating the causal pathway through the effect of screen time on neuropsychological development in adolescents are needed. With this aim, we carried out a longitudinal cohort study and designed a multilevel model to investigate the association of screen time and ADHD symptoms mediated by impulsivity, response inhibition and working memory. Our sample included 3779 adolescents (1858 girls; mean [SD] age: 12.8 [0.5] years) who were recruited from 31 high schools in the greater Montreal area, and participated in annual surveys for five consecutive years, from 7th to 11th grade. We assessed screen time by asking participants how much time per day they spend on social media, playing video games, watching shows or movies on television and practicing other activities on computer. We assessed impulsivity with five items of the Substance Use Risk Profile Scale. We measured inhibitory control using an adaptation of the Go/No-Go Passive Avoidance Learning Paradigm. We studied working memory applying the spatial working memory task ''Find the phone'' which is based on the Self-Order Pointing Task and the spatial working memory task of the Cambridge Neuropsychological Test Automated Battery. Our multilevel statistical model included three time-variations of both direct and indirect effects of screen time: the concurrent between-person effect, the concurrent within-person effect, and the lagged-within-person effect. Results showed that further increases in screen time in a given year were associated with an exacerbation of ADHD symptoms within that same year (within-person association), over and above potential common vulnerability (between-person association). Impulsivity resulted to be the most robust mediator associating screen time and ADHD symptoms at both between and within-person levels, while response inhibition and working memory only at between-person level. Social media and computer use presented a significant lagged-within-person effect mediated by impulsivity, showing a lasting effect from one year to the next. These findings have important clinical implications not only to help reduce ADHD symptoms among adolescents but also to prevent them to occur.

Disclosures: J. Wallace: None. **E. Boers:** None. **J. Ouellet:** None. **M. Afzali:** None. **A. Livet:** None. **P. Conrod:** None.

Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.14

Topic: H.04. Executive Functions

Support: NIH R01-HD092847

Title: Alterations in brain function in adolescents with Klinefelter syndrome: an fMRI study of executive function

Authors: *E. GHASEMI[1], L. C. FOLAND-ROSS[1], V. R. ALSCHULER[2], J. SUNDSTROM[1], G. WITKIN[3], A. TAHSIN[3], K. KOWAL[3], M. MULLIS[1], M. MARZELLI[1], J. ROSS[3], A. L. REISS[1];
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Abstract: Klinefelter syndrome (KS) is a genetic condition affecting 1 in 500 males that is defined by the presence of an extra X chromosome and altered pubertal development. While boys with KS are at increased risk for ADHD and often exhibit reductions in executive functioning, little is known about the neural bases of such alterations. In the current study, we investigated patterns of brain activation using functional magnetic resonance imaging (fMRI) in 38 adolescents with KS (mean age 12.3 ± 2.2 years) and 43 typically developing control subjects (mean age 11.6 ± 1.7 years) as they performed a go/no-go task, an executive function paradigm. Children underwent scanning and cognitive assessment at 1 of 2 different sites. Group differences in activation occurring during the contrast of "no-go" > "go" were examined while controlling for age and scan site. Results indicated that, despite equivalent task performance between the two groups (as indexed by d-prime; $p = 0.76$), KS-associated decreases in activation were observed in regions subserving response inhibition, including the right inferior frontal gyrus, bilateral caudate nuclei, anterior cingulate gyrus, and anterior insula (ps < 0.05). Ongoing analyses testing potential associations between neural function and pubertal hormones and performance on laboratory- and parental report-based assessments of executive function are in progress.

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Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.15

Topic: H.04. Executive Functions

Support: Midwest Roybal Center for health promotion and translation

Title: Dual-tasking in older adults with mild cognitive impairment: Effect of cognitive-vestibular tasks on reactive balance control

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Abstract: Vestibular function declines with age and is further impaired in older adults with mild cognitive impairment (OAwMCI) affecting balance control and gait function, especially under dual-task (DT) conditions. Recent findings show that compared to cognitively intact older adults (CIOA), OAwMCI exhibits postural instability demonstrating poor ability to recover balance control from unexpected external perturbations. However, the effect of DT with cognitive tasks challenging the vestibular system on reactive balance tasks remains unknown in OAwMCI. This study examined the effect of explicit cognitive tasks involving vestibular and visuospatial function on balance recovery against unexpected external perturbation in OAwMCI and CIOA. OAwMCI (n=15, 18-25/30 on MoCA) and CIOA (n=15, > 26/30 on MoCA) performed vestibular and visuospatial tasks (VVST) in standing and while responding to unexpected stance perturbations on the Active Step treadmill. VVST involved moving a paddle horizontally- 1) to catch a vertically dropped soccer ball while avoiding a distracter quantified by performance error- Target game; 2) to follow a moving target horizontally quantified by sum of errors- Tracking game. Slip-like (prevalent type of accidental falls) perturbations -SPT was quantified by postural stability (shortest distance of the COM motion state, i.e., its position and velocity, to the theoretical boundary). Performance error of the target game was significantly high in OAwMCI compared to CIOA ($p=0.006$) during the SPT. In CIOA, the performance error was significantly high during the SPT compared to standing ($p=0.01$), however, no significant change was observed in OAwMCI. The Sum of errors of the tracking game was significantly high in OAwMCI compared to CIOA ($p=0.008$) while responding to the SPT. Additionally, the sum of errors was significantly high during the SPT compared to standing in both OAwMCI ($p<0.001$) and CIOA ($p<0.001$). Postural stability during SPT was significantly low in OAwMCI compared to CIOA while performing the target ($p=0.04$) and tracking game ($p=0.006$) with no difference in postural stability between OAwMCI and CIOA when SPT was performed alone. Results indicate mainly a motor prioritization in both groups with a deterioration in cognitive but not balance, with DT interference being greater in OAwMCI. Although post hoc revealed inter-individual differences, some showed mutual interference (both cognitive and motor deterioration) as well. Results indicate a potential sharing of higher cortical resources between reactive balance control and VVST which could be impacted due to healthy aging as well as pathology-triggered cognitive impairment.

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Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.16

Topic: H.04. Executive Functions

Support: Eunice Kennedy Shriver National Institute of Child Health & Human Development (NICHD) 1P50HD103536

Title: An acute exercise intervention to ameliorate behavioral and neurophysiological indices of inhibitory control deficits in schizophrenia: A Mobile Brain/Body Imaging (MoBI) study

Authors: *V. A. POPOV[1], E. G. FREEDMAN[3], J. J. FOXE[2];
[1]Univ. of Rochester, [2]Neurosci., Univ. of Rochester, Rochester, NY; [3]Del Monte Inst. for Neurosci., Univ. of Rochester - Neurosci., Rochester, NY

Abstract: Introduction: Schizophrenia spectrum disorders (SSDs) are chronic, debilitating psychiatric disorders characterized by impulsivity and deficiencies in planned behavior. Disruptions in sensory and cognitive processing pathways in people with diagnoses of SSDs are well-established. However, to our knowledge, no studies to date have examined changes in neural activity in individuals with SSDs when walking and cognitive tasks co-occur. Physical activity and the resultant increase in functional connectivity between the default mode network and frontal executive control networks may ameliorate symptoms and improve cognitive performance in individuals with SSDs. **Methods:** Male and female individuals with SSDs and neurotypical, age-matched healthy controls (HCs) completed the Go/NoGo response inhibition (RI) task while sitting and walking. Mobile Brain/Body Imaging (MoBI) was utilized and encompasses high density electroencephalography (EEG) and motion capture during treadmill walking or sitting. **Results:** Preliminary data in participants with SSDs showed clear behavioral improvements on the RI task when walking. In fact, when walking, event related-potential (ERP) RI indices in participants with SSDs were similar to those of HCs. Comparisons were made with both the sitting condition and neurotypical, age-matched HCs.
Conclusion: When walking participants with SSDs showed dramatic improvements in inhibitory control and electrophysiological processes. This is intriguing because walking may improve cognitive functioning, impulsivity, and deficiently planned behavior through targeting identified SSD endophenotypes.

Disclosures: V.A. Popov: None. **E.G. Freedman:** None. **J.J. Foxe:** None.

Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.17

Topic: H.04. Executive Functions

Support: National Dairy Council

Title: Neuroelectric indices of attention are related to academic skills in preschool aged children

Authors: *S. A. KEYE[1], A. M. WALK[2], C. N. CANNAVALE[3], N. A. KHAN[4];
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of Illinois at Urbana-Champaign, Urbana, IL; [4]Univ. of Illinois At Urbana-Champaign, Urbana, IL

Abstract: Cognitive abilities measured behaviorally and using event related potentials (ERPs) have previously been associated with academic success in school-aged children. Although, no study has examined the relationship between ERPs and early academic abilities in preschool aged children. This study assessed the relationship between the P3 component, an index of attentional processes, and academic skills in children 4-6 years old. We expected target P3 amplitudes, recorded during a 2-stimulus auditory oddball task, to be positively related to academic skills whereas latency was expected to be negatively related to academic skills. Participants (n=28, 16 female) completed The Woodcock Johnson Early Cognitive and Academic Development Test to assess general intellectual abilities (GIA), early academic skills, and expressive language. Electroencephalography was recorded during an auditory oddball task to elicit the P3 component at the Cz electrode. Linear regressions were conducted to determine relationships between P3 and early academic skills and expressive language in which step one included age, sex, and GIA and step two included the target P3. Target P3 peak (R^2= 0.608, β=0.356, p=0.029) and mean (R^2= 0.527, β=0.333, p=0.041) amplitude were positively associated with early academic skills. Target P3 peak latency was negatively associated with early academic skills (R^2=0.604, β=-0.038, p=0.033). Contrary to the hypothesis target P3 peak amplitude was negatively associated with expressive language (R^2=0.842, β=-0.222, p=0.023). These data indicate that the P3 component is associated with early academic skills and expressive language in preschool aged children. Therefore, neuroelectric assessments of attention during early childhood may be used as a biomarker of academic achievement. Future studies should consider using ERPs to potentially predict academic skills in children.

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Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.18

Topic: H.04. Executive Functions

Support: CONACYT Student grant 661556

Title: Making-decisions and emotional control in adolescents

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Abstract: During adolescence, cognitive, psychological, and emotional changes succeed influencing the decision-making process. Additionally, emotional control, considered within the behavioral regulation index of executive functions according to Gioia et al. (2000), is developed

in that stage. According to the somatic marker hypothesis, emotional signaling is relevant to the decision-making process affecting daily life during human development. The purpose of this study was to find if exists a relation between the decision-making process and emotional control in student adolescents. Participants were 119 Mexican adolescents (47 men, 72 women), between 15-17 years old (M= 16.4, ±.81), students of hight school, and without TDAH. The study was carried on from January to May 2022, the last part of the COVID-19 pandemic, thus instruments were virtually applied. We used the self-report version of the Behavioral Rating Inventory of Executive Function (BRIEF-SR) and the Columbia Card Task (CCT). The BRIEF-SR includes eight subscales and three indexes, here we are reporting just the emotional control subscale. The CCT contains an emotional condition (with feedback) and another cognitive (without feedback). Results showed a mean score of 15.8 (DE=4.4) in the emotional control subscale. In the CCT, the average of cards turned over in the emotional condition (M=11.1) was lower than the cognitive condition (M=15.2), with significant differences between conditions (t [118] =-6.3, p<.001, d=-.58). The correlation test showed a significant correlation between the emotional condition of CCT and the emotional control subscale of BRIEF-SR (r= .20, p=.027); whereas didn´t present a correlation between that subscale and cognitive condition (r=.113, p=.222). The average number of cards turned over by adolescents was higher in the cognitive condition, which indicates that adolescents make more risky decisions when emotional feedback is absent. Relation between the self-perception of emotional control (more perceived troubles) and emotional condition (more cards turned over) in CCT was expected. Whereas the absence of this relation in the cognitive condition, despite the highest number of cards turned over, suggests that other factors influence greater risky decision-making in adolescents.

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Poster

319. Executive Function and Inhibitory Control

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Topic: H.04. Executive Functions

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Title: Sustained suppression of brain activity induced by transcranial ultrasound stimulation in humans

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Abstract: Low-intensity transcranial ultrasound stimulation (TUS) can stimulate the deep brain structures with high spatial accuracy of a few millimeters. Previous studies have reported that TUS can induce short- or long-term suppression/enhancement of the activity depending on stimulation parameters. However, it is largely unknown whether behavioral impact can be induced by TUS to the human subcortical areas. In this study, we first confirmed the suppression of cortical activity after TUS by measuring motor-evoked potential (MEP) with transcranial magnetic stimulation (TMS) to the human primary motor cortex (M1). MEP from the first dorsal interosseous (FDI) muscle was measured before and after TUS intervention. To identify the FDI-M1 spot in each subject, functional MRI scan was administered (Siemens Prisma, 3T, TR 1 s, TE 30 ms, voxel 2 mm) while the subjects performed a motor task designed to activate the FDI-M1 (Tamura et al., PLOS One, 2019). The FDI-M1 spot in each subject was targeted by TMS and TUS with the aid of an online navigator (Localite). MEPs were recorded by delivering a single-pulse TMS (7-cm figure-of-eight coil and Magstim 200^2, Magstim). A 40-s train of pulsed ultrasound (500 kHz), consisting of 30-ms bursts every 100 ms, was delivered to the target FDI-M1 using a four-element transducer (Neuro FUS, Brainbox). MEPs were suppressed significantly for at least 60 min after TUS ($t(19) = 8.2$, $p = 1.1 \times 10^{-7}$, paired t-test, pre vs. post). Next, we examined the essentiality of the basal ganglia region for response inhibition by TUS intervention. TUS was applied to the right subthalamic nucleus (STN) identified by fMRI during the stop-signal task (Osada et al., J Neurosci, 2019; Osada et al., Cell Rep, 2021). The performance of the stop-signal task was monitored before and after TUS. Sustained prolonged stop-signal reaction time (SSRT), a behavioral index for response inhibition, was observed after TUS to the STN (mean difference: 8.5 msec, $t(19) = 3.4$, $p = 3.4 \times 10^{-3}$, paired t-test, pre vs. post). These results demonstrated that TUS induced the sustained suppression of brain activity and suggest the feasibility of TUS for revealing the functions of both cortical and subcortical areas.

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Poster

319. Executive Function and Inhibitory Control

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Topic: H.04. Executive Functions

Support: USDA/Agricultural Research Service Project 6026-51000-012-000D
USDA/Agricultural Research Service Project 6026-51000-012-06S

Title: Effect of protein vs. carbohydrate intake on inhibitory control in overweight preteens

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Abstract: Preteens with overweight or obesity show problems in inhibitory control observed in behavioral and electrophysiological response as well as brain volumetric. Diet also seems to affect brain activation, and this depends on the type of macronutrients consumed. We hypothesized that the consumption of certain macronutrients may improve inhibitory control process in overweight preteens. To test this hypothesis, fifty-one preteens were provided with one of two equal-calorie shakes (high-carbohydrates (hC) or high-protein (hP)) ninety minutes before collecting event-related potentials (ERPs) during a stop-signal task. Twenty-four of our sample drank hC shake (mean body mass index, BMI =23.2 ±4.9; 10.2 years old), and 27 drank hP shake (mean BMI = 23.5 ±4.7; 10.1 years old). Although no significant differences between groups were observed in behavioral responses, the hC and hP groups differed in the amplitude of N200 and P300a components regardless of experimental condition. The hP group displayed greater negativity than the hC in the left frontal, frontal medial, and right frontal regions of interest (ROIs), reflecting differences between groups in conflict-monitoring. The hC group displayed a greater positivity of P300a than the other group, suggesting differences in attention-inhibition process between groups. The amplitude of N200 and P300a positively correlated with better behavioral performance in the inhibition condition. We conclude that carbohydrates and protein macronutrients have a specific effect on inhibitory control in preteens with overweight. Although both improve participants' behavioral performance, the high protein shake seems to increase their ability to detect the inhibition condition, while the high carbohydrate shake seems to support participants' inhibition processing.

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Poster

319. Executive Function and Inhibitory Control

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Program #/Poster #: 319.21

Topic: H.04. Executive Functions

Support: NIA: R44 AG071063

Title: Executive function assessed remotely and unattended: Preliminary validation of a novel tablet-based digital e-Stroop task developed for the in-home iPACES neuro-exergaming clinical trial for MCI

Authors: *C. ANDERSON-HANLEY[1,2], P. ARCIERO[2], M. DUNNAM[2], I. FERGUSON[2], R. GLATT[3], D. MERRILL[3], S. PANOS[3], J. ROGERS[2], N. ROQUE[4], E. SAULNIER[5], G. WARNER[2], E. ZIMMERMAN[6];

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Abstract: BACKGROUND:Given the nearly ubiquitous availability of the internet and electronic devices, empirically-sound and well-validated digital cognitive assessments are sought after, and the COVID-19 pandemic necessitated innovations of these given the nuanced and proliferating needs (e.g., unattended remote tests). Traditional paper and pencil cognitive assessment via a trained neuropsychological evaluator (NPE) remains the gold standard by which to compare digital tests. Starting a clinical trial at the outset of the pandemic we examined a slate of digital assessments, many of which were not readily adapted to or validated for a fully-remote, unattended, in-home scenario, especially with a cognitively-impaired sample. The current study provides preliminary validation of a novel self-administered tablet-based e-Stroop task developed for the interactive physical and cognitive exercise system (iPACES) clinical trial to evaluate the impact on executive function of neuro-exergaming for mild cognitive impairment (MCI). METHODS:Sixteen older adults [50% MCI/caregiver; age = 70(6) , sex = 43%, gender = 43%, white = 93%] were enrolled in a fully-remote, in-home clinical trial. They completed both traditional neuropsychological assessments administered via videoconference [e.g., the Montreal Cognitive Assessment (MoCA) and screen-shared “paper-Stroop” (p-Stroop; 40-item Kaplan version by van der Elst)], as well as a newly developed electronic Stroop (e-Stroop, designed to mimic the p-Stroop, with the same order and number of stimuli, digitally presented: colored blocks, black words, and incongruent colored words). The e-Stroop took 5-10 minutes to complete, unattended on a study-provided low-cost android tablet.RESULTS:Pearson correlations revealed a significant and positive association between the NPE-administered p-Stroop and the unattended e-Stroop [$r(13) = .53$, $p = .04$]. The e-Stroop also performed similarly to the p-Stroop, in its relationship to overall cognitive functioning on the MoCA [e-Stroop: $r = .49$ vs. p-Stroop: $r = .42$).DISCUSSION:Promising preliminary validation of a novel digital assessment of executive function, the e-Stroop, as self-administered on a tablet by MCI patients and caregivers participating in a full-remote, at-home, clinical trial of neuro-exergaming. The e-Stroop performed well, with a strong and significant correlation when compared with the gold standard evaluator-administered paper test, suggesting that the e-Stroop may provide a way to obtain a quick and valid assessment of executive function, even when self-administered as in this remotely administered, unattended, at-home clinical trial.

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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.; iPACES LLC. **R. Glatt:** 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.; iPACES LLC. **D. Merrill:** 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.; iPACES LLC. **S. Panos:** 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.; iPACES LLC. **J. Rogers:** 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.; iPACES LLC. **N. Roque:** None. **E. Saulnier:** 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.; iPACES LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); iPACES LLC. **G. Warner:** 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.; iPACES LLC. **E. Zimmerman:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.01

Topic: H.06. Social Cognition

Support: NIMH R01MH110750 R00MH099093
NSF Graduate Research Fellowship Program

Title: Oxytocin promotes prosocial behavior via amygdala-mediated alterations in ACC neural activity

Authors: *O. MEISNER[1,2], O. DAL MONTE[4,2], N. FAGAN[2], P. PUTNAM[2], S. W. CHANG[2,3];
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Abstract: Prosocial decisions are integral to human's social lives, yet the specific neural mechanisms underlying these behaviors remain under-explored. The primate basolateral

amygdala (BLA) and anterior cingulate cortex (ACC) are two reciprocally connected brain regions[1] implicated in prosocial behaviors. Notably, when monkeys make decisions about reward allocation for themselves and others, neurons in the BLA and the ACCg selectively enhance spike-field coherence for expressing prosocial preferences in this social decision-making context[2]. Recent work in humans has found that oxytocin, a neuropeptide strongly implicated in a wide range of social behaviors across species[3], helps sustain prosocial learning over time by increasing fMRI functional connectivity involving the ACC[4]. Here, we hypothesize that oxytocin helps mediate BLA-ACC communication to help sustain prosocial preference during social decision-making. To test this, we examined if and how oxytocin alters the communication between BLA and ACCg by focally infusing either oxytocin or saline into the BLA while recording local field potentials (LFP) from the ACC during a social reward allocation task. In this task, actor monkeys made decisions to deliver juice rewards between a conspecific monkey and an empty juice bottle (Other/Bottle context) and between oneself and both monkeys (Self/Both context). Behaviorally, oxytocin infusion in the BLA, compared to saline, resulted in a more sustained preference over time for delivering juice rewards to the conspecific over the bottle in the Other/Bottle context. By contrast, it had no behavioral effect in the Self/Both context, when the monkeys' decisions involved reward delivery to themselves. Neurally, oxytocin infusion in the BLA increased low-frequency (< 30 Hz) LFP power in the ACC associated with the reward outcome for both the actor and conspecific monkeys (Self, Other, Both) but not for the bottle (Bottle). Further, these effects on the ACC LFP signals from infusing oxytocin in the BLA were only present when the monkeys actively made the choices, but not when the same choices were forced on the monkeys. These results support the notion that oxytocin processing in the BLA promotes BLA-ACC communication through low-frequency bands, and that these interregional effects are associated with processing reward outcomes arising from social decisions. Funding: NSF Graduate Research Fellowship and the NIMH. Carmichael, S. T. & Price, J. L. *J. Comp. Neurol.* **363**, 615-641 (1995).2. Monte, O. D., et al. *Nature Neuroscience* vol. 23 565-574 (2020).3. Donaldson, Z. R. & Young, L. J. *Science* **322**, 900-904 (2008).4. Martins, D., et al. *Prog. Neurobiol.* **213**, 102253 (2022).

Disclosures: O. Meisner: None. **O. Dal Monte:** None. **N. Fagan:** None. **P. Putnam:** None. **S.W. Chang:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.02

Topic: H.06. Social Cognition

Support: Swedish Research Council 2018-01578

Title: The drug methylenedioxyamphetamine (MDMA) enhances plasma oxytocin and modulates cortical responses during affective touch

Authors: H. MOLLA[1], G. NOVEMBRE[3], H. DEWIT[2], ***I. MORRISON**[4];
[1]Dept. of Psychiatry and Behavioral Neurosci., [2]Dept. of Biomed. and Clin. Sci., Univerisity of Chicago, Chicago, IL; [3]Linköping Univ., Linköping, Sweden; [4]Linköping Univ., Linköping Univ., Linkoeping, Sweden

Abstract: Oxytocin (OT) is a key mediator of social attachment across many mammalian species, yet surprisingly little is known about endogenous OT neuromodulation of key neural pathways of affective touch in humans. We investigated this question by manipulating perception of touch after administration of 1.5 mg/kg of the drug methylenedioxyamphetamine (MDMA) or placebo during functional magnetic resonance imaging (fMRI) in n = 22 healthy adult volunteers (age 21-40, 13 male; 4-40 times lifetime MDMA use). MDMA, also known as ecstasy, is an amphetamine derivative associated with prosocial effects, as such as increasing self-reported feelings of empathy and social connection (Bedi et al., 2010). MDMA has also been shown to enhance the pleasantness of affective touch (Bershad et al., 2019) and to facilitate the release of OT in the periphery (Kirkpatrick et al., 2014). In this study, each participant completed two counterbalanced fMRI sessions (drug and placebo, separated by at least 72 hours), in which they experienced gentle stroking by a soft brush at two velocities (3 cm/s, experienced as more pleasant, and 30 cm/s, experienced as less). OT samples were collected pre- and post-session to measure any change in endogenous OT (extracted and analyzed using enzyme-linked immunoassay). MDMA increased subjective effects of drug, measures of friendliness, blood pressure, heart rate, and a range of affective and social self-report ratings, as well as pleasantness of touch at both stroking speeds. Plasma OT (n = 18) showed main effects of drug and OT change, with greater OT change during the MDMA session. Hemodynamic responses in the brain showed a main effect of MDMA vs placebo in bilateral precentral gyri, alongside an interaction between OT, drug, pleasantness, and stroking velocity in medial prefrontal (mPFC) and inferior temporal gyrus (ITG) clusters. These findings indicate that MDMA influences endogenous OT and cortical responses to touch, and that mPFC and ITG responses are modulated in a manner that depends on touch pleasantness, together with the influence of MDMA and the influence of endogenous OT release. Such neuromodulation of these regions points to their critical role in augmenting the perception of social touch.

Disclosures: H. Molla: None. **G. Novembre:** None. **H. DeWit:** None. **I. Morrison:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 320.03

Topic: H.06. Social Cognition

Support: NIH NIMH R01 MH107513
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NIH NIMH R37 HD 090153

Title: Simultaneously acquired functional Near Infrared Spectroscopy (fNIRS) and electroencephalography (EEG) signals during flickered gaze at live human faces show complementary changes in face processing mechanisms

Authors: *M. KELLEY[1], X. ZHANG[2], J. A. NOAH[2], T. PARKER[1], J. HIRSCH[3];
[1]Interdepartmental Neurosci. Program, [3]Dept. of Psychiatry, [2]Yale Sch. of Med., New Haven, CT

Abstract: Introduction: Past research found that the right temporoparietal junction (rTPJ)[1,2] as well as the N170[3] differentiates face viewing from non-face viewing. How these differences emerge is unclear. One theory is that visual acquisition patterns influence neural processing. If true, then disrupting eye movements during live face viewing will correspond with decrease in activity in the rTPJ and the N170. To assess this, we acquired hemodynamic, electrocortical, and behavioral data via simultaneous fNIRS, EEG and eye tracking during a live face viewing task with and without a visual flicker to disrupt eye-movements. We hypothesized that disrupting the view of the face (flicker) compromises visual acquisition, preventing social information carried by live faces from being acquired or encoded. **Methods:** Subjects (n=6) completed 2 tasks that alternated live human face viewing events with rests. Face viewing was allowed or prevented via a divider with electronically controlled transparency/opacity placed between the subject and a human partner. Both tasks had a total of 3.2S of face exposure per event. The control condition consisted of one undisrupted face view per event. The experimental condition subdivided the face view into 400ms periods separated by a 200ms flicker of the divider. Hemodynamic data were collected via a 128 channel Shimadzu LabNIRS system; scalp EEG via a 32-channel g.USBamp system; and eye behavior via a desk mounted Tobii Pro system. **Analysis:** fNIRS was analyzed with a general linear model of task and rest convolved with HRF. Eye tracking was analyzed via fixations per second (nFix) and mean fixation length (mFix) within a box around the partner's face. EEG Events were averaged. **Results:** Eye behavior significantly differed for the tasks (Ctrl: nFix=2.0/S of face viewing, mFix=519.6ms; Flicker: nFix=3.6/S, mFix=270.4; $p<0.01$). Significant activity was found in the rTPJ during the control (Peak T=2.97, P=0.02) but not the flicker. N170 differed in amplitude for control (peak amplitude=-3.8uV) and flicker conditions (peak amplitude=-1.2uV). **Conclusions:** These preliminary data support the theory that visual sensing influences neural processing of live faces. As a consequence of the flicker, the social information associated with live faces may not be acquired or encoded, diminishing rTPJ and N170 activity. Sensory motor systems associated with faces are activated suggesting a ventral "back-up" system for face processing. Thus the face may still be perceived without the finer social-related details being acquired.[1]Kelley et al, *Fronts in Robotics* (2021); [2]Noah et al, *Fronts in Human Neuro* (2020); [3]Dravida et al, *Neurophotonics (2019)*

Disclosures: M. Kelley: None. **X. Zhang:** None. **J.A. Noah:** None. **T. Parker:** None. **J. Hirsch:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.04

Topic: H.06. Social Cognition

Support: F32MH20872
Max Planck Florida Institute for Neuroscience

Title: Region-specific encoding responsibilities in social recognition learning

Authors: *M. PHILLIPS, P. PARRA-BUENO, R. YASUDA;
Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: Determination of the appropriate social behavior is an essential and dynamic neural calculation in communal animals dependent on proper function of numerous brain regions connected by long-range circuitry. Regions involved in social recognition memory include dorsal CA2 , ventral CA1, and the medial prefrontal cortex, which form a continuous, unidirectional circuit. Previous work using optogenetic and chemogenetic approaches has found that perturbation of these regions is sufficient to disrupt social memory. However, the mechanism of these approaches causes off-target effects and does not promote delineation of the unique aspects of social memories each region is responsible for encoding via synaptic plasticity. These technical limitations have made it difficult to parse the unique roll of each region in social recognition memory. To address this, we utilize a photo-activatable AIP2 to inhibit CaMKII in order to impair synaptic plasticity without affecting neuronal firing patterns. We systematically expressed paAIP2 in each region and wirelessly activated paAIP2 during a freely interacting social learning paradigm. By using computer vision to score a plethora of naturalistic behaviors, we have determined the necessity of region-specific CaMKII-dependent synaptic plasticity to shape social behavioral choices. Our data indicates that CaMKII-dependent synaptic plasticity in dCA2 is involved in, but not required for, social habituation and is required for social discrimination. Alternatively, plasticity in vCA1 is not involved in social habituation but is required for robust social discrimination. Finally, synaptic plasticity in the mPFC is required for social habituation, but not for social discrimination. These data provide much needed detail as to the mechanism of social memories.

Disclosures: M. Phillips: None. **P. Parra-Bueno:** None. **R. Yasuda:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.05

Topic: H.06. Social Cognition

Support: NIMH R01 MH110750

Title: Subtle differences in social gaze target discriminability by broad-spiking and narrow-spiking neurons in the primate prefrontal-amygdala circuits

Authors: *P. GANGOPADHYAY[1], S. FAN[1], O. DAL MONTE[2], N. A. FAGAN[1], S. W. CHANG[1];
[1]Dept. of Psychology, Yale Univ., New Haven, CT; [2]Dept. of Psychology, Univ. of Turin, Turin, Italy

Abstract: Gaze interactions among conspecifics make up an important part of social communication. Neurons in the primate prefrontal-amygdala circuits have been shown to encode multiple interactive social gaze-related variables (Dal Monte, Fan, et al., 2022, Neuron). However, the specificity of social gaze processing by different cell types remains unexplored. Here we examined if putative excitatory and inhibitory neurons in the primate prefrontal cortex and the amygdala signal social gaze variables differently during real-life social gaze interaction between pairs of macaques. We recorded single-neuron activity from the basolateral amygdala (BLA) and three prefrontal areas - the rostral gyrus of the anterior cingulate cortex (ACCg), orbitofrontal cortex (OFC), and dorsomedial prefrontal cortex (dmPFC). Based on the peak-to-valley distance of mean waveforms, we classified individual neurons into three clusters - broad-spiking (putative excitatory), narrow-spiking (putative inhibitory), and others that did not belong to either distribution. We then contrasted the spiking activity of neurons pertaining to gaze fixations to the face of the partner monkey with gaze fixations to a non-social object. Although the activity from many neurons distinguished social from non-social stimuli in the BLA (n = 201/454), OFC (n = 27/96), ACCg (n = 56/191), and dmPFC (n = 45/151), only the BLA and ACCg showed subtle differences in the proportions of broad-spiking and narrow-spiking neurons for differentiating social from non-social fixations. In the BLA, compared to narrow-spiking neurons, a larger fraction of broad-spiking neurons discriminated social from non-social fixations (140/293 [47.8%] broad-spiking vs. 61/161 [37.9%] narrow spiking, p = 0.042, chi-sq prop. test). Among the significantly discriminating BLA cells, most broad-spiking neurons had higher activity when the monkeys looked at the face compared to the object, but this proportion was equivalent for narrow-spiking neurons. By contrast, a larger fraction of narrow-spiking neurons in the ACCg differentiated social from non-social fixations compared to broad-spiking neurons (13/63 [20.6%] broad-spiking vs. 43/128 [33.6%] narrow-spiking, p = 0.064, chi-sq prop. test). A fraction of narrow-spiking (n = 12/43), but not broad-spiking (n = 0/13), neurons also discriminated looking at the eyes versus the non-eye region of the face. Taken together, these results suggest that there are only subtle differences in social gaze target discriminability between putative excitatory and inhibitory neurons in the investigated regions in the primate brain.

Disclosures: P. Gangopadhyay: None. **S. Fan:** None. **O. Dal Monte:** None. **N.A. Fagan:** None. **S.W. Chang:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.06

Topic: H.06. Social Cognition

Support: CONACYT, grant number 709993

Title: Neural dynamics associated with learned sociability in adult Drosophila melanogaster

Authors: *V. LOBATO RIOS, P. RAMDYA;
Neuroengineering Laboratory, Brain Mind Inst. & Interfaculty Inst. of Bioengineering, EPFL, Lausanne, Switzerland

Abstract: Sociability, the ease with which individuals interact with conspecifics during E.g., collective foraging, coordinated prey avoidance, and courtship, is critical for an animal's survival. Studies in several model organisms including mice, flies (*Drosophila melanogaster*), and worms (*C. elegans*) have identified environmental factors, genes, and neural circuits whose disruption affects sociability [1,2,3]. However, the neural dynamics associated with sociability remain largely unexplored. We raised female wild-type flies in one of two social contexts: isolated or grouped. Grouped flies show sociable behaviors, including active leg-body interactions. By contrast, isolated animals showed a constellation of behaviors including social fear and avoidance. We found that these animals adapt to their neighbor and became sociable over several hours. Thus, sociability in *Drosophila melanogaster* is a learned state. Isolation dramatically alters an animal's reactions to same-sex, conspecific neighbors. We next asked which sensory cues mediate this learning. We found that learned sociability is largely multimodal: it is mediated by visual experience and exposure to conspecific odors. Finally, to uncover neural dynamics associated with this social adaptation, we recorded descending neuron populations (~100 neurons) in tethered isolated animals (female flies expressing GCaMP6f and tdTomato) exposed to a freely behaving conspecific neighbor during two-photon microscopy over the course of one hour. Our results reveal how neural population dynamics adapt through social experience and open the door to studying how sensory cues impact the formation of social memories. This can have an impact on our understanding of social disorders including autism spectrum disorder. [1] Yamada K, Hirotsu T, Matsuki M, Butcher RA, Tomioka M, Ishihara T, Clardy J, Kunitomo H, and Iino Y. Olfactory plasticity is regulated by pheromonal signaling in Caenorhabditis elegans. *Science* 329, 1647-1650 (2010). [2] Yost RT, Robinson JW, Baxter CM, Scott AM, Brown LP, Aletta MS, Hakimjavadi R, Lone A, Cumming RC, Dukas R, Mozer B. Abnormal social interactions in a Drosophila mutant of an autism candidate gene: neuroligin 3. *International journal of molecular sciences* 21, 4601 (2020). [3] Lee DK, Li SW, Bounni F, Friedman G, Jamali M, Strahs L, Zeliger O, Gabrieli P, Stankovich MA, Demaree J, Williams ZM. Reduced sociability and social agency encoding in adult Shank3-mutant mice are restored through gene re-expression in real time. *Nature neuroscience* 24, 1243-1255 (2021).

Disclosures: V. Lobato Rios: None. **P. Ramdya:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

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Program #/Poster #: 320.07

Topic: H.06. Social Cognition

Support: MOST Grant 107-2320-B-006-072-MY3
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Higher Education Sprout Project, Ministry of Education to the Headquarters of University Advancement at NCKU

Title: Microbial metabolites regulate social novelty preference via CaMKII neurons in the BNST

Authors: *C.-W. LIOU[1,2], S.-J. CHENG[3], T.-H. YAO[2], W.-L. WU[1,2,4];
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Abstract: Social novelty preference is a cognitive process that is essential for animals to interact strategically with conspecifics based on their prior experiences. The commensal microbiome in the gut modulates social behavior through various routes, including microbe-derived metabolite signaling. Short-chain fatty acids (SCFAs), metabolites derived from bacterial fermentation in the gastrointestinal tract, have been previously shown to impact host behavior via unknown mechanisms. Herein, we demonstrate that the delivery of SCFAs directly into the brain disrupts social novelty preference through distinct neuronal populations. We are the first to observe that infusion of SCFAs into the lateral ventricle disrupted social novelty preference in microbiome-depleted mice. The deficit in social novelty preference can be recapitulated by activating calcium/calmodulin-dependent protein kinase II (CaMKII)-labeled neurons in the anterodorsal bed nucleus of the stria terminalis (adBNST). Conversely, chemogenetic silencing of the CaMKII-labeled neurons and pharmacological inhibition of fatty acid oxidation in the adBNST reversed the SCFAs-induced deficit in social novelty preference. Our findings suggest that microbial metabolites impact social novelty preference through a distinct neuron population in the adBNST.

Disclosures: C. Liou: None. **S. Cheng:** None. **T. Yao:** None. **W. Wu:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.08

Topic: H.06. Social Cognition

Support: NSF NeuroNex 1707408

Title: Linking the cerebello-cortical circuit during social interaction by synchronized electrophysiology and miniature microscopy (E-Scope)

Authors: *S. HUR[1], K. SAFARYAN[1], H. T. BLAIR[2], S. C. MASMANIDIS[3], D. AHARONI[1], P. GOLSHANI[1];
[1]Dept. of Neurol., [2]Dept. of Psychology, [3]Neurobio., UCLA, Los Angeles, CA

Abstract: The cerebellum, alongside other cortical areas, such as the anterior cingulate cortex (ACC), has been well demonstrated as being strongly involved in social behavior and implicated in social deficits, However, what the physiological output of the cerebellum, specifically the right Crus I (RCrus I), during social interaction is and how the activity patterns of these socially related structures co-modulate during social behavior is not well understood. Here, we developed an electrophysiology integrated miniature microscope (E-Scope); a custom-made miniaturized microscope equipped with electronics to acquire and multiplex imaging and 32-channel electrophysiological recordings through a single coaxial cable. Utilizing the advantages of this device, we performed multiple single unit electrophysiological recordings using silicon probes from the RCrus I Purkinje Cells (PCs) or dentate nucleus (DNs) neurons synchronously with calcium imaging from hundreds of left ACC neurons during performance of social interaction or object interaction. We find a sizable subpopulation of PCs inhibited by social interactions but not object interactions. In addition, a subpopulation of DNs is selectively activated by social interaction. In the ACC, both socially activated or inhibited populations were observed, however the amount of over lapping cells for each task were minimal. Ongoing work is focused on characterizing fine-scale coordination of these different populations of neurons during social and object interactions. Therefore, a subpopulation of cerebellar neurons output a distinct activity which may affect the subpopulation of cells in the ACC upon social interaction.

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Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.09

Topic: H.06. Social Cognition

Support: 22-CoE-BT-03

Title: Frontal cortex regulates strategic cooperation

Authors: *B. KIM, H. CHOE;
DGIST, Daegu, Korea, Republic of

Abstract: Cooperation is the process in which different individuals are working or acting together to achieve a common benefit. To obtain a benefit, we perform a task alone or together. The reason why we cooperate is that cooperating together is more efficient than performing a task alone. Cooperation leads to increase evolutionary fitness. When there is only one trial, there may not be much difference in efficiency between performing a task alone and performing it cooperatively. However, if this trial is repeated several times, it will be more advantageous to cooperate in the end. However, neurological mechanisms that regulate cooperation remain unclear. We design a strategic cooperation paradigm in that mice can choose single or cooperation and show that mice can cooperate with conspecific. In this behavioral paradigm, two mice can move freely and they should press two levers within a time interval to drink water. At this time, to press the two levers, the mice can choose whether to press them alone (single) or together (cooperation). Single requires more effort than cooperation, and for mice to drink water easily, they must cooperate. Using chemogenetic manipulation, we find that inhibition of the orbitofrontal cortex (OFC) decreases the number of cooperation, but not single, and inhibition of the prelimbic cortex (PL) decreases the number of single, but not cooperation, suggesting that the frontal cortex is involved in strategic cooperation. This study shows that mice can cooperate strategically and the frontal cortex regulates cooperation.

Disclosures: B. Kim: None. **H. Choe:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.10

Topic: H.06. Social Cognition

Title: Rapid, feedforward processing of social touch observation in humans

Authors: *H. LEE MASSON, L. ISIK;
Cognitive Sci., Johns Hopkins Univ., Baltimore, MD

Abstract: Visually observed touch evokes a strong social and emotional response in humans. Our prior work found that enhanced communication between brain networks, including somatosensory cortex and social brain regions, underlies our ability to extract the social-affective meaning of others' touch interactions. Yet, the direction of information flow across these networks and the overall neural dynamics of these processes remain unknown. Such results have important implications for theories of social perception and embodied simulation. In particular, understanding whether social-affective meaning is extracted quickly via perceptual signals or based on simulation from the somatosensory cortex. To answer this question, we conducted an electroencephalography (EEG) experiment during which 20 participants watched 500ms video clips showing social and non-social touch interactions. Using representational similarity analysis, first, we tracked the temporal dynamics of visual and social-affective processes during touch observation. We find that EEG neural patterns are explained by low-level visual features

beginning 100 ms post video onset. Social features, including sociality, arousal, and valence of observed touch, are processed shortly after, explaining neural patterns beginning at 160ms. Next, we tracked the spatial-temporal neural dynamics by combining the EEG data with fMRI data from our prior study using EEG-fMRI fusion methods. We examined information flow across three key brain regions: early visual cortex, the temporoparietal junction (TPJ, a key region in the theory of mind network), and somatosensory cortex. We find that neural information first arises in early visual cortex 80 ms post video onset, and then is processed by TPJ at 130 ms, and finally somatosensory cortex at 190ms. These results suggest that social-affective meaning of observed touch is first extracted by social brain regions followed by the later involvement of the somatosensory cortex. Our findings also show that social touch is processed quickly by the brain, well within the timeframe of feedforward visual processes. This fast processing may underlie our ability to quickly and effectively use social touch for interpersonal communication.

Disclosures: H. Lee Masson: None. **L. Isik:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.11

Topic: H.06. Social Cognition

Support: NIH T32 NS115753-01
ARCS Foundation
NIH R01HD054453
NIH R01NS117597

Title: Neural circuits underlying social touch behavioral deficits in mouse models of autism

Authors: *T. CHARI[1], A. HERNANDEZ[1], C. PORTERA-CAILLIAU[2];
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Abstract: Individuals with autism spectrum disorders (ASD) are apprehensive to social touch, but the circuit mechanisms are not known. Differences in social touch in the context of ASD have been relatively understudied in rodent models, though some assays exist. We designed a novel assay to investigate maladaptive behavioral responses to social touch, while simultaneously recording neural activity, in two ASD mouse models (the genetic Fragile X Syndrome model and the environmental Maternal Immune Activation model). In this assay, a head-fixed *test* mouse (free to run on a floating polystyrene ball) is allowed to voluntarily interact (whisker-whisker) or forced to interact (snout-snout) with a head-fixed *stranger* mouse on a motorized stage. Controls include similar interactions with an inanimate novel object. Each interaction type occurs in several bouts to mimic typical mouse to mouse/object interactions. High speed videos (>50 FPS) are recorded during the assay to analyze avoidance behaviors, aversive facial expressions, pupil diameter, and locomotion using custom MATLAB routines and

DeepLabCut. We find mice of ASD models avoid stranger mice and display aversive facial expressions to a greater degree than healthy controls during social touch compared to 'no-contact' or novel object interactions. We also observed reduced behavioral adaptation of pupil size (arousal) to social touch in ASD mice. We then used Neuropixels probes to chronically record single-unit activity of behaviorally relevant neuronal populations in barrel cortex (S1BF), dorsal striatum and basolateral amygdala (BLA), all of which had high cFos expression during social touch. We identified individual neurons in all three regions whose activity is modulated by social touch. Furthermore, we found significantly reduced adaptation to repetitive bouts of social touch in the activity of touch-responsive cells of ASD mice in S1BF and BLA. We will also present data that correlates neuronal activity in these brain regions to maladaptive behaviors during social touch. Thus, our novel assay for mice shows a strong translational potential for investigating behavioral responses to social touch in ASD.

Disclosures: T. Chari: None. **A. Hernandez:** None. **C. Portera-Cailliau:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.12

Topic: H.06. Social Cognition

Support: UConn SLAC
UConn IBACS
Peter and Carmen Lucia Buck Foundation
UConn IDEA Grant

Title: The effect of demonstrator-observer relationship in rat observational learning

Authors: *R. TROHA[1], M. GOWDA[2], S. TAVAKOLI[2], E. J. MARKUS[3];
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Abstract: Observational learning can be defined as a change in behavior that follows the observation of another animal performing a task rather than personally performing the task. From an evolutionary aspect, it is beneficial to learn from a conspecific rather than expending energy or exposing oneself to danger. Such social learning is seen both in primates and certain non-human primates. In humans, social learning impairments are seen in certain disorders such as Autism Spectrum Disorder and Schizophrenia. Our lab has developed an observational learning paradigm, where two conjoined operant chambers are used. One chamber contains the observer and one contains the demonstrator. Each chamber has a nose poke on each side, one on the left side and one on the right side. Observers must learn to watch a demonstrator respond at one nose poke and then choose the nose poke on the same side in their own chamber. The automated nature of this task results in rapid social learning. In addition, precise behavioral assessment of

both the observer and demonstrator rats is possible through a machine learning program. In the past we showed that observers were more oriented to a live demonstrator than a non-social cue (plastic object). Successful observation is of course based on the behavior of the observing rat, however the behavior of the demonstrator rat is also important. To better understand the role of a demonstrator, rats were trained with a familiar demonstrator and then placed with a novel demonstrator to determine how this would affect their observational learning. Once learning criteria was reached, observers were then tasked with observing a demonstrator of either a novel strain or opposite gender. Performance of the observer rats was measured, as well as heading orientation to the demonstrator throughout the task. This experiment helps to better understand the dynamics of social learning in rats. In the future, this task will also help understand how different conspecifics may be represented in the brain during learning. In addition, we looked at how the inactivation of the ventral hippocampus through DREADDs would affect success through the observational trial.

Disclosures: **R. Troha:** None. **M. Gowda:** None. **S. Tavakoli:** None. **E.J. Markus:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.13

Topic: H.06. Social Cognition

Support: NIH Grant R01MH116176

Title: Anatomical and functional characterization of the dorsal endopiriform cortex (EPd): a novel area for shifting gears from surveillance to attention?

Authors: *S. B MANJILA, S. SON, Y.-T. WU, R. BETTY, Y. KIM;
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Abstract: Endopiriform nucleus (EPd) is a small group of neurons medial to the piriform cortex and ventral to the claustrum, mainly suggested to have role in temporal lobe epileptogenesis. However, cellular resolution anatomical characterization and functional implications of the area remains unclear mainly due to lack of cell type specific marker for the region. Here, we have investigated the whole brain anatomical connectivity and functional role of Epd neurons using oxytocin receptor (OxtR) Cre mice that densely label the EPd. Our whole brain anterograde mapping revealed major connectivity of EPd to medial prefrontal cortex, olfactory cortices, and higher olfactory centers. Retrograde monosynaptic mapping revealed strong inputs from olfactory areas as well as the basal forebrain area. Patch clamp recordings in brain slices confirmed that these neurons fire continuously upon exposure to Oxt that is abolished by OxtR antagonists. Next, we performed an in-vivo miniscope recording in the EPd and found tonic firing during resting state which is attenuated during isoflurane induced anesthesia. The firing rate was also significantly reduced when new objects was introduced in a home cage. When

another mouse was introduced, we saw robust reduction in the neuronal activity much more pronounced than the introduction of novel objects. This result suggests that OxtR positive neurons in the EPd are active during general surveillance and their activity are attenuated upon novel cues. Our anatomical characterization of Epd reveal complex inputs that might help attenuate the activity when surveillance mode needs to be shifted to attention. Further studies are ongoing to identify the specific inputs that drive this activity pattern.

Disclosures: S. B manjila: None. **S. Son:** None. **Y. Wu:** None. **R. Betty:** None. **Y. Kim:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 320.14

Topic: H.06. Social Cognition

Support: NIH Grant R01MH111729 (AS)
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MGH ECOR Scholars Program Grant (AS)
Tosteson/MGH

Title: A circuit-based enhancer of Dyrk1a function reverses Dyrk1a-associated social cognition impairment

Authors: *Y.-T. SHIH[1,2,3,4], J. ALIPIO[1,5,3,4], A. SAHAY[1,2,3,4];
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Abstract: Autism spectrum disorder (ASD) is a class of heterogeneous neurodevelopmental disorders that affects 1 in 100 children around the world and is characterized by impaired social cognition, repetitive behaviors and seizure risk. Genetic studies and large-scale exome-sequencing efforts have identified haplo-insufficient loss of function (LOF) mutations in the Dual specificity tyrosine-phosphorylation-regulated kinase *Dyrk1a* gene in syndromic ASD. However, the functional impact of hemizygous *Dyrk1a* LOF on synaptic and circuit mechanisms underlying social cognition remain poorly understood. This challenge is magnified because Dyrk1a phosphorylates more than 70 substrates and Dyrk1a LOF is likely to disrupt many biological processes during development. Currently, there are no drugs that enhance or restore Dyrk1a function. Together, these observations underscore the need to identify cell-type and circuit-specific mechanisms that may be targeted to reverse Dyrk1a associated social cognition impairments. Here we show that Dyrk1a in dentate granule cells dictates mossy fiber recruitment

of GABAergic inhibition in CA3/CA2 through phosphorylation of Ablim3, a cytoskeletal regulator, to facilitate social recognition. Targeting Ablim3 in dentate gyrus (DG) or activating Parvalbumin inhibitory neurons in CA3/CA2 of adult Dyrk1a hemizygous mice is sufficient to reverse developmental deficits in DG-CA3/CA2 circuitry and social cognition. Our studies illuminate a distinct approach to identify and harness Dyrk1a synaptic substrates as "enhancers" to restore hippocampal GABAergic inhibition and social cognition in ASD.

Disclosures: Y. Shih: None. **J. Alipio:** None. **A. Sahay:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.15

Topic: H.06. Social Cognition

Support: NIH Grant P30GM122734

Title: Complex changes in brain dynamics observed following transcranial direct current stimulation (tDCS) to facilitate social learning in ASD: a MEG study

Authors: *C. TESCHE[1], J. E. WILSON[2], A. A. VAKHTIN[3];
[1]Univ. of New Mexico, Albuquerque, NM; [2]Dept. of Communication Disorders, New Mexico State Univ., Las Cruces, NM; [3]The Mind Res. Network/Lovelace Biomed. and Envrn. Res. Inst., Albuquerque, NM

Abstract: Recent studies indicate a potential for transcranial direct current stimulation (tDCS) to enhance social cognition in autism spectrum disorder (ASD). A major challenge lies in the multiplicity of choices for the stimulation parameters. Based on our previous studies, we hypothesized that tDCS in combination with social skills training would induce changes in network-level brain dynamics in individuals with ASD or with high autistic traits (AT). Characterization of participant-specific brain dynamics may contribute to the development of more effective therapeutic interventions. Participants (4M, 5F; age17-26) viewed social skills training videos and human/dog facial images during two (verum/sham: double-blinded) sessions of tDCS (anodal electrode over right temporoparietal junction, cathodal on ipsilateral deltoid: 2 mA, 30 minutes). Spectro-temporal plots were extracted from an atlas-based analysis of MEG data recorded following each stimulation session. Statistical significance of changes in spectral peak frequencies and amplitudes were evaluated on ~40 individual trials/participant/condition using a permutation t-test followed by a false discovery rate (FDR) correction for multiple comparisons (threshold $p = 0.05$). Regions experiencing significant current flow (right temporal cortex, including fusiform gyrus, and right inferior cerebellum) showed changes in theta-, alpha-, beta- and low and high gamma-band oscillatory activity. tDCS also induced network-level changes of brain dynamics in the left hemisphere. Results depended on autism quotient score and included frequency-dependent mixed effects of both up- and down-regulation within the same

brain region. Anodal stimulation is often considered to be excitatory, leading to up-regulation of activity beneath the stimulation electrode. Frequency-specific effects are typically neither expected nor reported. Importantly the effects of the second (cathodal) electrode are often ignored. We found a complex pattern of frequency-dependent effects across multiple brain regions. The changes in oscillatory features observed in bilateral inferior cerebellar cortex as well as left cortical regions suggest that the stimulation may induce modulation of cerebellar control of frontal and posterior dynamics in a patient population known to have atypical cerebellar function. Importantly, the significant variation of results across participants suggests that design of frequency-specific stimulation protocols should be informed by knowledge of intrinsic brain dynamics in this heterogeneous population.

Disclosures: C. Tesche: None. **J.E. Wilson:** None. **A.A. Vakhtin:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.16

Topic: H.06. Social Cognition

Support: NIH Grant R01MH120292

Title: The encoding of social information in the hippocampal CA2 region may mediate the effect of social familiarization to decrease social aggression

Authors: *A. VILLEGAS, S. SIEGELBAUM;
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Abstract: Memories of individuals are imbued with rich shared experiences. These familiar experiences guide and prepare future interaction, whereas novel social encounters require flexible cognitive effort. In the hippocampus, the dorsal portion of the CA2 sub-region plays a critical role in both social recognition memory and in the promotion of social aggression. Whether and how social aggression and social memory are linked remains uncertain. Here, we designed a multi-day modified resident-intruder assay of social aggression to test how social experience and familiarization regulate aggressive behavior towards a familiar compared to novel conspecific. We find that repeated presentation of a novel conspecific intruder over a period of several days increases aggressive behavior (defined as one or more biting attaches). In contrast, repeated presentation of the same intruder leads to a fixed level of aggression over several days, with a level of biting attacks significantly less than that seen in novel conspecifics (**p<.01 by mixed model; n= 8/group). Next, we performed miniscope calcium recording of CA2 neural activity at the single cell level as mice engage with familiar or novel conspecifics. Consistent with our behavioral findings, imaged resident mice displayed relatively constant levels of aggression to a familiarized conspecific (ns by mixed model; n = 11/group), whereas presentation of a novel intruder significantly increased aggression above levels seen with the

familiar intruder (***p<.001 by mixed model; n= 11/group). We used both manual labeling and supervised segmentation of exploration, dominance, and attack behaviors to characterize CA2 neural population representations using a linear support vector machine decoding approach to explore the relationship between days of experience and behavior. We find that CA2 population activity could accurately decode exploration versus dominance versus attack behaviors in binary comparisons, both during encounters with familiar and novel conspecifics (***p<.001 by SVM classifier, n=~500 neurons, 5 mice). Together, these findings suggest that social experience is integrated by CA2 to guide future behavior and provides insight into how aggressive behavior may be influenced by social memory, with novel animals triggering increased aggression.

Disclosures: A. Villegas: None. **S. Siegelbaum:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.17

Topic: H.06. Social Cognition

Support: JSPS KAKENHI Grant JP19K24681
JSPS KAKENHI Grant JP20K16232

Title: The role of the lateral habenula in coordinative social signaling behavior in a mouse model of autism

Authors: *H. ARAKAWA, Y. HIGUCHI;
Grad. Sch. of Med., Univ. of the Ryukyus, Okinawa, Japan

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disease characterized by social disconnection, including atypical social behavior and uncooperative communication. Neural mechanisms underlying these behavioral characteristics are poorly understood. BTBR T^{+} $Itpr3^{tf}$/J (BTBR) mice as an idiopathic animal model of autism display typical behavioral phenotypes resembled with human ASD symptoms such as a lack of social motive and interaction and excessive stereotypy behavior. Then we wonder if BTBR mice exhibit a deficit in social communication abilities? Male mice particularly use urinary scent odor to signal surroundings their social status and conditions, called as scent marking behavior. The scent marks are usually deposited as a delayed signaling response in a confronted situation dependent manner. By comparison with C57BL/6J (B6) mice as a highly social strain, male BTBR mice are less responsive to the presence of social stimuli by depositing scent marks. At the same time, the expressions of c-Fos as a marker of neuronal activity in brain regions were evaluated in these mice during scent marking sessions. A blunted c-Fos expression along with stationary scent marking was found in selected brain areas including the medial amygdala and lateral habenula (LHb) in BTBR mice. Immunohistochemical analysis on the neuronal projections of social peptides indicated scarce axonal projections of vasopressin neurons and fewer matured neurons

in the LHb of BTBR mice compared to B6 mice. Finally, chemogenetic manipulation of LHb region demonstrated that inhibition of LHb neural activity induced decreased scent marking in both B6 and BTBR mice. Our data indicate that male BTBR mice are less responsive to the presence of social stimuli in response as social signaling, which is associated with morphological features in LHb of BTBR mice, including faded vasopressinergic signal that could be responsible for the dysfunction of social communication in BTBR mice as a mouse model of ASD.

Disclosures: H. Arakawa: None. **Y. Higuchi:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.18

Topic: H.06. Social Cognition

Support: Diversity Supplement R01 MH120069-01

Title: Investigating a novel role for the cerebellar domain, paraflocculus, in ASD-relevant behaviors

Authors: *B. RAMIREZ[1], P. TSAI[2];
[1]Med. Scientist Training Program, [2]Neurol., UTSW, Dallas, TX

Abstract: Social interactions entail complex and nuanced behaviors requiring the processing of different sensory inputs to adapt and elicit an appropriate response. The goal of these experiments is to better understand the neuronal activity and connectivity important for social interactions and the behavioral consequences when these processes go awry in disorders such as Autism Spectrum Disorders (ASD). Two of the most consistently implicated regions are within the cerebellum; right crus I (RCrusI) and lobule IX, also known as the cerebellar tonsil and analogous to the paraflocculus (Pfl) in mice. While RCrusI has been more extensively studied, little is known about how lobule IX is implicated in ASD and social behaviors. Human imaging studies have linked this region to cortical areas involved in social cognition and language processing. Additionally, structural studies in ASD revealed that, decreased grey matter in lobule IX correlated with increased symptoms and severity of social deficits. However, despite these studies, the contribution of lobule IX/Pfl to social and other ASD-relevant behaviors remains unknown. We hypothesize that this clinically implicated region and its interaction with other socially relevant lobules of the cerebellum such as RCrusI, has critical roles in the regulation of social behavior. In this study we use chemogenetic manipulations to modulate neuronal activity of the Pfl and assess its necessity for ASD-relevant behaviors. We observe behavioral changes upon inhibition of this region in wildtype mice, demonstrating that neuronal activity of the Pfl is necessary for typical social behavior in mice. The results of this study reveal a novel potential therapeutic target for social deficits and lead to a better understanding of the cerebellar role in social behavior.

Disclosures: **B. Ramirez:** None. **P. Tsai:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 320.19

Topic: H.06. Social Cognition

Support: NIH Grant F31MH129025

Title: Social stimuli elicit glutamatergic neuronal activity in the posterior intralaminar complex of the thalamus in male and female mice

Authors: *A. LEITHEAD[1], A. GODINO[2], H. HARONY-NICOLAS[1];
[1]Icahn Sch. of Med. At Mount Sinai, Icahn Sch. of Med. At Mount Sinai, New York, NY; [2]Nash Family Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: The posterior intralaminar (PIL) complex of the thalamus is considered a relay center in the brain by which sensory stimuli are perceived and communicated to downstream regions for the generation of social behaviors. The PIL has previously been implicated in maternal social behaviors in female rats and mice; however, it remains unknown whether the PIL is involved more broadly in other forms of social interactions in both male and female rodents. To address this question, we first used viral retrograde tracing and RNAscope *in situ* hybridization in male and female mice to demonstrate that the PIL sends monosynaptic glutamatergic inputs to the paraventricular nucleus of the hypothalamus which contains many cell types, including oxytocin neurons, that are highly implicated in social behaviors. Next, we performed a behavioral experiment in which adult male and female mice (n = 3/sex/condition) were exposed to either a novel same-sex juvenile social stimulus or a novel object stimulus for 1 hour of free interaction. Afterward, brains were collected and analyzed for immunohistochemical staining of the immediate early gene *c-fos*. We observed significantly more *c-fos+* cells in the PIL of mice exposed to social stimuli compared to those exposed to object stimuli ($p = 0.02$), indicating that the PIL is selectively activated during social interaction. To confirm these results in real-time, we used fiber photometry to record neural activity of glutamatergic neurons in the PIL during interactions with social and non-social stimuli in male and female mice (n = 9/sex). The neural activity of PIL glutamatergic neurons of males and females was increased when mice were engaged in social interaction with a same-sex juvenile ($p = 0.005$) or opposite-sex adult ($p < 0.001$), but not an object toy rat. Interestingly, glutamatergic neural activity was also increased in females ($p < 0.001$) when interacting with an opposite-sex adult odor (urine sample); however, this pattern of increased activity was not observed in males. Together, these findings suggest that glutamatergic PIL neurons respond to social stimuli in both male and female mice, but activity may vary depending on sex and sensory modality. Future research will parse out the contributions of different sensory modalities on glutamatergic neuronal activity in the PIL of males and females. Additionally, chemogenetic tools will be employed to inhibit the activity of

glutamatergic neurons in the PIL to measure the impact of this inhibition on the perception of social stimuli and behavior. We expect our findings to identify novel roles for the PIL which may further contribute to our understanding of neural mechanisms involved in social behaviors.

Disclosures: **A. Leithead:** None. **A. Godino:** None. **H. Harony-Nicolas:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 320.20

Topic: H.06. Social Cognition

Support: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 814302

Title: The role of early dyadic episodes during parent child interaction in shaping infant neural processing of sensory information within 1st year of life

Authors: *T. BATABYAL[1], A. RADKOWSKA[1], A. MALINOWSKA KORCZAK[2], A. ANZULEWICZ[1], P. TOMALSKI[2];
[1]Psychology, Univ. of Warsaw, Warsaw, Poland; [2]Inst. of Psychology, Polish Acad. of Sci., Warsaw, Poland

Abstract: The role of social behavior on shaping development in functional and structural activity of brain circuits and pathways, particular to the postnatal development of the cerebral neocortex in infants is broadly an idea presented in the theory of interactive specialisation framework by Mark Johnson (2000). Majority of the time spent in social interaction between infants and caregivers is associated with episodes of dyadic interaction. It involves episodes of infants gaze direction to faces (mutual gaze) and toys or objects (parallel attention) and serves as a cue to learn from the environment.In the current study, the measures of percentage and number of dyadic episodes of mutual gaze and parallel attention of infants at the age of 5 months have been longitudinally associated with resting state EEG power spectral measures to dynamic faces of rhyming woman (social-S) and moving toys (nonsocial-NS) acquired at 11 months. 122 infants participated in the study across two time points of 5 months (164.86 days ±15.29) and 11 months (350.18 days ± 10.27) of age. Data of 44 (M=25, F=19) infants have been analyzed longitudinally. EEG power was estimated for channel groups of Frontal(F), Central(C) ,Occipital (O), Left Temporoparietal (LTP) and Right Temporoparietal (RTP) for Theta (3-5Hz), Alpha(6-9 Hz), Beta(13-20 Hz), Lower Gamma (21-30 Hz) and Upper Gamma (31-45 Hz). The results show that Beta power during NS condition in central electrodes positively correlates (spearman rho r=0.320,p=0.034) with percentage of mutual gaze (PMG). Upper Gamma power during NS condition in central electrodes positively correlates with both number of mutual gaze (NMG) (spearman rho r=0.335,p=0.026) and PMG (spearman rho r=0.338,p=0.025). Alpha , beta and

lower gamma power during NS condition in frontal electrodes are negatively correlated to percentage of parallel attention (PPA) (Pearson r = -.326,p=.031,r=-.323,p=.032,r=-.307,p=.043).Beta, lower gamma and upper gamma power during S conditions in central electrodes are negatively correlated to number of parallel attention (NPA) (spearman's rho r=-.327,p=.030,r=-.321,p=.033,r=-.333,p=.027).Beta, lower gamma and upper gamma power during NS condition in central electrodes are negatively correlated to NPA (spearman's rho r=-.378,p=.011,r=-.350,p=.020,r=-.334,p=.027).Upper gamma power during S condition in central electrodes is negatively correlated to PPA (spearman rho r=-.308,p=0.042). Thus, the results indicate the possible relationships of dyadic episodes of mutual gaze and parallel attention to sustained attention, object representation and attention shifting neural correlates of infants within the first year.

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Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.21

Topic: H.06. Social Cognition

Support: Human Frontier Science Program Postdoctoral Fellowship

Title: Ensemble representations of social and non-social behavioral goals in the medial prefrontal cortex

Authors: E. A. AMADEI, R. BÖHRINGER, B. EHRET, B. F. GREWE;
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Abstract: Natural environments intrinsically offer a large variety of behavioral options, but how animals represent and select among these possibilities remains poorly understood. Previous work has implicated the medial prefrontal cortex (mPFC) in encoding the relative value of competing behaviors but has focused on non-social behaviors and rewards (e.g. water seeking). However, animals must often choose between non-social and social behaviors, which have distinct benefits (e.g. self-preservation, social information). To study how the mPFC represents competing non-social and social behaviors, we developed a decision-making task in which water-deprived C57BL/6 mice choose between sweet milk or social interaction with a novel conspecific. To measure mPFC population activity in behaving mice during this task, we employed *in vivo* calcium imaging. We therefore expressed the genetically encoded calcium indicator GCaMP6m under the CaMKIIa promoter in mPFC combined with a head-mounted miniaturized microscope system. Out of 707 imaged mPFC neurons (n = 5 mice), we identified two distinct subpopulations that significantly discriminated between the two behaviors (social > milk activity or milk > social activity; 48.8% of total population). These social- and milk-selective neurons

were sufficient to decode subjects' behavioral choice once animals started to run towards their behavioral target. Our preliminary results suggest that mPFC population activity tracks the pursuit and attainment of social and non-social behavioral goals, via specific subpopulations dedicated to the individual behavioral options. Our future work will include further characterizing these subpopulations based on molecular profiles or projection targets and directly manipulating their activity to test causality.

Disclosures: E.A. Amadei: None. **R. Böhringer:** None. **B. Ehret:** None. **B.F. Grewe:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

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Program #/Poster #: 320.22

Topic: H.06. Social Cognition

Support: NIH
Seaver

Title: Studying the impact of Shank3-deficiency on the mesoaccumbens pathway of reward

Authors: *M. BARBIER[1], K. THIRTAMARA RAJAMANI[1], S. NETSER[2], S. WAGNER[2], H. HARONY-NICOLAS[3];
[1]Mount Sinai Sch. of Med., Mount Sinai Sch. of Med., New York, NY; [2]Univ. of Haifa, Haifa, Israel; [3]Psychiatry/Neuroscience, Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Social deficits are a core symptom of autism spectrum disorder (ASD). Clinical studies have implicated the mesoaccumbens reward circuit in autism spectrum disorder. However, the causality between alterations in this system and social deficits has not been established. The ventral tegmental area (VTA), a core node of the mesolimbic pathway, is interconnected with the nucleus accumbens (NAc) via the VTA dopaminergic projections. Despite the role of the reward system in social interaction, little is known about the impact of ASD associated mutations on processing social reward and on the functional integrity of this pathway. In this work, we study the effect of a mutation in an ASD high-risk gene, *Shank3,* on the mesoaccumbens pathway in rats. We hypothesize that *Shank3* mutation impacts neural activity in the mesoaccumbens pathway, causing abnormalities in accumbal dopamine transmission and leading to impairments in processing social reward. To identify abnormalities in dopamine transmission in *Shank3*-deficient rats that correlate with deficits in processing social reward, we used fiber photometry to record in the VTA in combination with a dopamine sensor in the NAc during a social reward paradigm (n = 15 per genotype, WT, HET and *Shank3*-KO; and for the behavioral experiment, n = 40 per genotype). In this paradigm we introduced two rewarding stimuli, social and food, during satiety and food deprivation and examined investigation time for each reward during the two conditions ($p < 0.001$ between WT and *Shank3*-KO at food deprivation). To control for attentional deficits, we used the same paradigm,

but replaced the social stimuli with a moving toy rat (behavioral experiment, n = 30 per genotype). To rule out reduced motivation to food or impairment in food consumption, we assessed food consumption (n = 20 per genotype). We found that *Shank3*-deficient rats have deficits in processing reward that are associated with perturbation in VTA neural activity and an intact attention and food consumption. Our study demonstrates that *Shank3*-deficient rats have deficit in processing reward and provides a first step toward understanding the role of *Shank3* in the reward system, and how *Shank3*-deficiency may leads to social deficits.

Disclosures: M. Barbier: None. **K. Thirtamara Rajamani:** None. **S. Netser:** None. **S. Wagner:** None. **H. Harony-Nicolas:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.23

Topic: H.06. Social Cognition

Support: ZIAAA000440

Title: Fever-like temperatures without immune activation alleviate cognitive deficits in the Scn2a autism mouse model

Authors: *Y. SHEN, J. MEZA, J. ABRAMOVITZ, M. W. ANTOINE;
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Abstract: According to the Centers for Disease Control, one in fifty-four children has Autism Spectrum Disorder (ASD) and treatment options for its associated cognitive deficits are limited. For unknown reasons, up to 83% of ASD patients experience improved cognition during fever, a phenomenon called the “fever effect”. Fever occurs when body temperature (T_b) exceeds 38°C and is usually induced by infection. It remains unclear whether improvements are facilitated by T_b increase and/or the immune component of fever. To test this, we developed a “thermal fever” (TF) protocol to elevate mouse T_b into fever range using infrared light. To assess the effect of immune challenge during fever, the TF protocol is combined with an immune challenge. The immune challenge alone did not elicit fever. Using the Sodium Voltage-Gated Channel Alpha Subunit 2 (Scn2a) haploinsufficient mouse model of ASD and intellectual disability, mice were evaluated on their ability to locate the position of the sole escape hole on a 20-hole maze over a two-day period, during exposure to the TF protocol, with and without immune system stimulation. At normal T_b, *Scn2a*$^{+/+}$ mice displayed significantly reduced escape times on day 2 versus day 1 trials, suggesting normal learning and cognitive function. However, such improved performance was not observed in *Scn2a*$^{+/-}$ littermates. Both *Scn2a*$^{+/+}$ and *Scn2a*$^{+/-}$ mice under the TF protocol alone and those with TF and immune challenge, displayed significantly reduced escape times over the two-day period. However, due to the possible effects of lethargy, *Scn2a*$^{+/-}$ mice with immune challenge had significantly longer escape times than mice in the other

conditions. TF protocol *Scn2a*$^{+/-}$ mice had the fastest escape times of all mice. To examine the mechanism mediating improved behavior in the TF condition, we simulated the elevated thermal component in acute brain slices by increasing temperature from 30° to 36°C and 39°C. Measurements of evoked activity in cortical Scn2a$^{+/-}$ L2/3 pyramidal (PYR) neurons showed that spike probability was significantly enhanced at 30°C and 36°C, but normalized at 39°C. To confirm that improved behavior was mediated by a fever-temperature induced decrease in PYR neuron spiking, at normal T_b, we chemogenetically decreased PYR neuron spiking in the cortex during the learning task on days 1 and 2, and this manipulation promoted normal learning. In summary, our results demonstrate that T_b increase into the fever range improved cognitive function in Scn2a$^{+/-}$ mutants by reducing abnormal spiking activity in brain networks. These findings may prove relevant in developing therapeutic strategies that reproduce the beneficial effects of fever in ASD.

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

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.24

Topic: H.06. Social Cognition

Title: Oxytocin receptor signaling in hilar mossy cells modulates discrimination of social stimuli

Authors: *Y.-C. HUNG[1], Y.-J. WU[2], M.-E. CHIEN[2], Y.-T. LIN[3], K.-S. HSU[4];
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Abstract: Hippocampal oxytocin receptor (OXTR) signaling is essential for discrimination of social stimuli to guide social recognition, but circuit mechanisms and cell types involved remain incompletely understood. Here, we report a crucial role for OXTR-expressing hilar mossy cells (MCs) of the dentate gyrus in mediating social stimulus discrimination by regulating granule cell (GC) activity. Using a Cre-loxP recombination approach, we found that selective genetic ablation of *Oxtr* from MCs impairs discrimination of social, but not non-social, stimuli in mice. Cell-type-specific deletion of MC *Oxtr* increases spontaneous firing frequency in GCs, synaptic excitation to inhibition (E/I) ratio of MC-to-GC circuit, and GC firing when temporally associated with lateral perforant path inputs. Optogenetic activation of MC-to-GC circuit can rescue social discrimination deficit in MC OXTR deficient mice. Together, our results uncover a previously unknown role of MC OXTR signaling for discrimination of social stimuli and delineate a MC-to-GC circuit for social information processing. These findings may also provide a novel therapeutic avenue to target specific neuronal cell types in treating social cognitive disabilities commonly observed in autism spectrum disorders and other psychiatric disorders.

Disclosures: **Y. Hung:** None. **Y. Wu:** None. **M. Chien:** None. **Y. Lin:** None. **K. Hsu:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.25

Topic: H.06. Social Cognition

Support: T32 AG49688

Title: Anterior cingulate cortex neurons encode social but not nonsocial image identity during a decision-making task

Authors: *J. SIMON, IV, E. L. RICH;
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Abstract: Humans and nonhuman primates live in complex social environments that require them to process unique social information. Processing of this information has been linked to many brain regions including the anterior cingulate cortex (ACC), specifically the ACC gyrus. Here, we asked if this region more strongly encodes information from a social context relative to a nonsocial context when the information and desired outcome are the same. To test this, we have designed a reward localization task where monkeys had to use social, or nonsocial, visual guides to locate a reward target. Social stimuli were pictures of monkeys' faces gazing to the left or right, or arrows pointing to the left or right directions. Monkeys had to choose between two identical squares, requiring monkeys to use visual cues to help make correct choices. We also used nondirectional stimuli in the form of forward gaze (i.e., monkeys looking straight ahead or double-headed arrows). This manipulation dissociates stimulus identity from its informative qualities. We targeted the ACC gyrus for single unit recordings in two rhesus monkeys performing the task. We also recorded from the frontal eye field (FEF), as this region has not been reported to specialize in social information. We analyzed single neurons in both regions (N = 101, ACC; N = 102, FEF). We performed a regression to determine the effects of image type on neuron activity. A binomial test found that there was a significant difference between social and nonsocial in the ACC, but not the FEF. Specifically, the proportion of recorded ACC neurons encoding a social image is significantly greater than nonsocial images ($p < 0.02$). This difference was not observed in the FEF. Thus, these data suggest that neurons in the ACC, but not FEF, are selective for stimulus context. Our next steps, are to determine to what degree information context (i.e., directional vs nondirectional/forward) is encoded in our recorded neurons.

Disclosures: **J. Simon:** None. **E.L. Rich:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 320.26

Topic: H.06. Social Cognition

Support: NSF Award #1707408

Title: Long-term instability of nucleus accumbens neural population codes in a model of autism

Authors: *P. ZHAO, X. CHEN, A. BELLAFARD, D. AHARONI, P. GOLSHANI;
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Abstract: Impaired social interaction is one of the core deficits of autism spectrum disorder (ASD). The social motivation theory of autism posits that these impairments arise because social interaction may be less rewarding in autism. Critical questions that remain are whether and how nucleus accumbens (NAc), a key hub of reward circuitry, encodes and drives social interaction and whether NAc neural population codes for social interaction are disrupted in ASD. To answer these questions, we performed calcium imaging using miniaturized microscopy (UCLA Miniscope), and identified NAc ensembles which specifically encode social interactions. At the population level, NAc activity patterns and specifically D1-recepter expressing medium spiny neurons (D1-MSNs) population activity predicted social interaction epochs. NAc-based decoders showed higher decoder performance than decoders trained with medial prefrontal cortex (PFC) and hippocampus CA1 activity. Although there was high turnover of NAc neurons modulated by social interaction across days, cross-day decoding revealed a stable social representation in NAc. Intriguingly, this stability was impaired in the *Cntnap2*$^{-/-}$ mouse model of ASD. Therefore, social interactions are preferentially, specifically, and dynamically encoded by NAc neurons and social representation is degraded in autism model. Further work will aim at identifying specific NAc cell type in mouse model of ASD which fails to steadily carry population codes for social interaction. Since social behavioral deficits is one of the major symptoms of ASD as well as many other psychiatric disorders, including depression and schizophrenia, understanding how NAc neurons dysfunction can impair social interaction and mediate social rewards will be critical for finding better cell-type specific therapies for social interaction deficits across a range of conditions.

Disclosures: P. Zhao: None. **X. Chen:** None. **A. Bellafard:** None. **D. Aharoni:** None. **P. Golshani:** None.

Poster

320. Social Cognition: Circuits and Neural Mechanisms I

Location: SDCC Halls B-H

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Program #/Poster #: 320.27

Topic: H.08. Learning and Memory

Support: DBT/Wellcome Trust India Alliance intermediate grant (IA/I/14/1/501306)
DST-Cognitive Science Research Initiative (DST/CSRI/2017/271)
Council of Scientific and Industrial Research, Govt. of India

Title: Role of synaptic inhibition in multimodal learning of pheromone locations

Authors: *S. D. MARATHE, M. PARDASANI, N. M. ABRAHAM;
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Abstract: Pheromone signalling plays a vital role in driving the social and reproductive behaviours of rodents. Learning and memorizing pheromone locations involve both somatosensory and olfactory systems as rodents can encounter pheromones streaked on objects of varying shapes and sizes in their natural habitat. To probe the neural basis of this behaviour, we developed a pheromone place preference paradigm wherein mice have to use both whisker and olfactory systems to gather information about pheromones[1]. Briefly, we trained female mice to sense the urinary volatiles emanating from a chamber closed with a door having orifices of a specific size or shape and bedding with non-volatiles kept in front of the pheromonal chamber. Animals were then trained to learn this specific pheromone location and differentiate it from the location of a neutral stimulus, water. On completing the training of 15 days, mice were challenged to memorize these locations after 1-2 weeks in absence of volatile and non-volatile cues. Here, we investigated the role of inhibitory synaptic signalling in modulating pheromone location learning and memory. As most of the inhibitory interneurons in main and accessory olfactory systems express the 65-kDa *isoform* of glutamic acid decarboxylase (GAD65)[2], we aimed to delete the subunits of ionotropic glutamate receptors, GluA2 subunit of AMPA receptors and NR1 subunit of NMDA receptors, from GAD65 interneuron population in different cohorts of mice. The calcium influx and thereby, the synaptic functioning can be bi-directionally modulated by these modifications[3]. We generated heterozygous knockouts (KOs) of GluA2 and NR1, targeting GAD65 interneuron population. The pheromone detection abilities were similar among GluA2 KOs, NR1 KOs, and wild-type female mice. However, their memory of pheromone locations was significantly reduced. Further, correlated changes were observed in the expression levels of activity-regulated cytoskeletal *(Arc)* protein, which is critical for memory consolidation, in the associated brain areas. These results prove the role of inhibitory synaptic signalling in modulating multimodal learning of pheromone locations.
1. Pardasani M, Marathe SD, Purnapatre MM, Dalvi U, Abraham NM. Multimodal learning of pheromone locations. *Faseb J* **35**, (2021).2. Parrish-Aungst, S et al. “Quantitative analysis of neuronal diversity in the mouse olfactory bulb.” *The Journal of comparative neurology* vol. 501,6 (2007)3. Abraham NM, *et al.* Synaptic Inhibition in the Olfactory Bulb Accelerates Odor Discrimination in Mice. *Neuron* **65**, 399-411 (2010).

Disclosures: S.D. Marathe: None. **M. Pardasani:** None. **N.M. Abraham:** None.

Poster

321. Hippocampal-Subcortical Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 321.01

Topic: H.08. Learning and Memory

Support: NIH Grant R01MH112143

Title: Correlated activity of cerebellar Purkinje cells and hippocampal sharp-wave ripples in mice

Authors: *Y. LIU, B. L. CORREIA, M. B. FOX, D. H. HECK;
Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Several recent studies show that spatial and memory functions of the hippocampus also involve the cerebellum. We recently showed that spatial working memory (SWM), a cognitive function involving both the hippocampus and prefrontal cortex, is modulated by Purkinje cell activity in the cerebellar lobulus simplex (LS). In an earlier study we showed that Purkinje cells in LS and Crus I represent information about the phase and phase differences of neuronal oscillations in the medial prefrontal cortex (mPFC) and dorsal hippocampus (dCA1). Here we investigated the interactions between the hippocampus and cerebellum during sharp-wave ripples (SWR) in the hippocampus. SWRs are considered crucial for memory consolidation. We examined the relationship between the occurrence of SWRs measured in the dCA1 of the hippocampus in awake mice and changes in Purkinje cell simple spike firing in LS. Experiments were performed on five adult male C57BL/6J(B6) mice. The Purkinje cell activity in cerebellar lobulus simplex and dCA1 local field potential (LFP) were simultaneously recorded in awake head-fixed mice at rest, i.e. during periods without body movements. Purkinje cells spike activity was identified by the appearance of complex spikes. Simple spike activity of 35 Purkinje cells (units) were analyzed. Spike-times were converted into waveform (SPW) using normal distribution density function (Matlab code: normpdf; sigma=0.05). The upper-envelop of band-pass filtered signal (130-200 Hz) from the dCA1 LFP were used to represent SWR activity for a cross-correlation analysis between the SPW and SWR envelope functions. Our results suggest that there are 3 types of Purkinje cells defined by the modulation of their spike firing during SWRs: for 20 units (57.1%) SPW-SWR correlation was positive, for 5 units (14.3%) the correlation was negative and 10 units (28.6%) showed no change in firing during SWRs. Further analysis of the SWR envelope function suggests a possible systematic relationship between SWR amplitude and the correlation with SPW activity. SWRs with the highest average peak-amplitude paired with SPWs that showed no correlation with SWRs, SWRs with the smallest amplitude paired with negatively-correlated units, with the positive-correlated units pairing with SWRs of intermediate average amplitude. These findings suggest that the occurrence of SWRs in the hippocampus differentially modulates cerebellar cortical output in a subset of Purkinje cells. It remains to be shown whether this interaction is relevant for memory consolidation, the presumed main function of hippocampal SWRs.

Disclosures: Y. Liu: None. **B.L. Correia:** None. **M.B. Fox:** None. **D.H. Heck:** None.

Poster

321. Hippocampal-Subcortical Circuits

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Program #/Poster #: 321.02

Topic: H.08. Learning and Memory

Support: NIH Grant R01-NS104071
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University of Minnesota's MnDRIVE (Minnesota's Discovery, Research and Innovation Economy) Initiative
University of Minnesota McKnight Land-Grant Professorship

Title: A novel GABAergic projection from the hippocampus to the supramammillary area

Authors: *L. R. GLASSBURN[1], E. I. KROOK-MAGNUSON[2];
[1]Univ. of Minnesota Grad. Program In Neurosci., Minneapolis, MN; [2]Neurosci., Univ. of Minnesota, Minneapolis, MN

Abstract: The supramammillary area (SuM) is a region of the hypothalamus which projects directly to the hippocampal dentate gyrus and CA2, and has been shown to impact hippocampal theta oscillations, spatial and social processing, and memory. In contrast, hippocampal influence over the SuM is largely uncharacterized. We have identified hippocampal inhibitory cells projecting to the SuM, cells which we refer to here as inhibitory hippocampal-hypothalamic cells (IHH cells). With injections of AAVs labeling GABAergic neurons into the dorsal hippocampus of mice (n=10), we find that the majority of IHH fibers are in the lateral SuM and near neurons which project back to the hippocampal dentate gyrus (SuM->DG cells). We additionally injected Cre-dependent AAVs into the dorsal hippocampus of mice expressing Cre either in somatostatin (n=5) or neuronal nitric oxide synthase (NOS)-expressing cells (n=5). These injections show that IHH fibers are largely from somatostatin+ neurons, with a smaller contribution from NOS+ neurons (i.e. LINCs). Using retrograde tracing with fluorogold injected into the SuM of mice (n=2), we similarly find that IHH cells are primarily somatostatin-expressing cells. We also find that IHH cells originate from the dentate gyrus, CA3, and CA1 across the septo-temporal hippocampal axis. We have additionally found that IHH cells form functional synapses with SuM neurons (n=4 cells, 3 mice) using optogenetic stimulation of IHH fibers during ex vivo whole-cell patch clamp recordings of SuM neurons. Given that IHH fibers are near SuM->DG neurons, IHH cells are in a unique position to modulate SuM inputs to the hippocampal dentate gyrus and possibly suppress SuM->DG spatial novelty signals. We hypothesize that IHH cells encode a spatial recognition signal that can inhibit SuM spatial novelty signals and influence spatial memory encoding. Future experiments will further analyze the connectivity and functions of IHH cells.

Disclosures: L.R. Glassburn: None. **E.I. Krook-Magnuson:** None.

Poster

321. Hippocampal-Subcortical Circuits

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Topic: H.08. Learning and Memory

Support: Marie Skłodowska-Curie Individual Fellowship (EU proposal 841301 – DREAM; European Commission; Horizon 2020 – Research and Innovation Framework Programme)

Title: Pontogeniculooccipital waves in the naturally sleeping rat brain

Authors: *J. RAMIREZ-VILLEGAS, D. K. RANGEL-GUERRERO, P. BARACSKAY, J. CSICSVARI;
Inst. of Sci. and Technol. (IST) Austria, Klosterneuburg, Austria

Abstract: Sleep has various physiological functions, including the consolidation of memories. Current theories establish that memories are transiently stored in the hippocampus and transferred to the neocortex for long-term storage during sleep. These processes involve brain-wide plastic changes, heralded by different types of macroscopic electrical activities that occur upon changes in the neuromodulatory activity of the brainstem. The brainstem prompts periods of both enduring and transient changes of neuronal excitability that affect the activity of other sub-structures in a precise manner. Thus, brainstem activity is likely critical for long-range coordination of several brain structures underlying memory formation. However, the specific neural mechanisms that allow these subcortical regions to participate in memory formation remain poorly understood. Here, 4 wild-type Long Evans rats were chronically implanted with recording micro-drives incorporating three bundles of movable recording electrodes targeting the dorsal hippocampus, the dorsal lateral geniculate nucleus (dLG) of the thalamus, and the parabrachial nucleus (PBn) of the brainstem. Each animal was recorded for a period of 3 to 5 hours of daily natural sleep. We show that, across vigilance states, global changes in neuronal activity were accompanied by the occurrence of transient, coordinated high-synchrony neural events in the PBn-dLG electrical activity. These episodes displayed electrical characteristics consistent with pontogeniculooccipital (PGO) waves. In line with a previous study in anaesthetized macaques (Ramirez-Villegas et al., Nature 589: 96-102, 2021), we show that the brainstem transiently modulates hippocampal high-synchrony events through PGO waves. These interactions spanned both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep phases, influencing hippocampal ripple episodes and REM-associated phasic theta waves. Crucially, learning of a spatial memory task resulted in an increase in the coupling between PGO waves and hippocampal events, observed during extended post-learning sleep periods. The preliminary results of this investigation indicate that the control of hippocampal ensembles by PGO waves might be a phylogenetically conserved neural mechanism. These episodes may correspond to windows for promoting hippocampal-cortical communication and plasticity during NREM and REM sleep, likely promoting memory consolidation and synaptic homeostasis.

Disclosures: J. Ramirez-Villegas: None. **D.K. Rangel-Guerrero:** None. **P. Baracskay:** None. **J. Csicsvari:** None.

Poster

321. Hippocampal-Subcortical Circuits

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Topic: H.08. Learning and Memory

Support: R01MH111773-01
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R01MH122692-01
RF1AG058160-01
BBRF Grant 29600

Title: Hypothalamic modulation of adult hippocampal neurogenesis regulates memory and anxiety

Authors: *Y. LI[1], Y.-J. LUO[2], J. SONG[3];
[1]Univ. of North Carolina At Chapel Hill, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]UNC, CHAPEL HILL, NC; [3]Univ. of North Carolina, Chapel Hill, NC

Abstract: Adult hippocampal neurogenesis plays a critical role in memory and emotion processing, and this process is dynamically regulated by neural circuit activity. However, whether manipulation of neural circuit activity can achieve sufficient neurogenic effects to modulate behavior remains unknown. We report that chronic patterned optogenetic stimulation of supramammillary nucleus (SuM) neurons in the mouse hypothalamus robustly promotes neurogenesis at multiple stages, leading to increased production of neural stem cells and behaviorally relevant adult-born neurons (ABNs) with enhanced maturity. Functionally, selective manipulation of the activity of these SuM-promoted ABNs modulates memory retrieval and anxiety-like behaviors. We will employ this strategy to rescue memory deficits in Alzheimer's disease.

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Poster

321. Hippocampal-Subcortical Circuits

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Program #/Poster #: 321.05

Topic: H.08. Learning and Memory

Support: NSERC CGS-M
NSERC Discovery MOP 506730
CHIR MOP 507489

Title: Evaluating the role of hippocampal inputs to the mammillary nuclei in spatial memory

Authors: *K. D. MAR[1], J. KIM[1,2];
[1]Dept. of Psychology, [2]Dept. of Cell & Systems Biol., Univ. of Toronto, Toronto, ON, Canada

Abstract: The mammillary bodies (MB) are implicated in processing spatial information however, the role of hippocampal (subicular) inputs to the MB has remained elusive. Projections from the subiculum to the MB are topographically organized and project to distinct nuclei and subnuclei within the structure which may represent a functional differentiation of information processing. Until now, an investigation into parallel projections from the subiculum arriving at distinct MB nuclei was not possible due to the size and location of the structure. The present study is aimed at determining the precise role of hippocampal input to the medial and lateral MB nuclei in spatial memory in mice. Spatial navigation strategies using self-motion cues (egocentric), visible cues (allocentric) and scenarios when self-motion and visible cues are in conflict (cued conflict) were evaluated with three escape assays on the Barnes maze. In the egocentric assay, escape to a previously memorized shelter location was initiated in the dark with success contingent upon the animal's ability to continuously integrate its body position relative to the remembered shelter location. In the allocentric assay, mice were trained to navigate to a shelter based on distal visible cues with success being continent upon the animal's ability to remember the cue configuration relative to the shelter location. In the cued conflict assay, animals were tested in the dark with an LED cue above the shelter location. At escape onset during a subset of trials, the LED cue was shifted to an alternate position on the platform perimeter creating a conflict between the expected location of the shelter based on egocentric cues to that of allocentric cues. The resulting behaviour was determined by the relative contribution of either navigation strategy at the time of escape.

Disclosures: K.D. Mar: None. **J. Kim:** None.

Poster

321. Hippocampal-Subcortical Circuits

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Program #/Poster #: 321.06

Topic: H.08. Learning and Memory

Support: 00115700-URC 2022-23

Title: Neuronal interactions between the amygdala and hippocampus during an affective object-context association task in rats

Authors: *J. L. KRASNEY[1], A. ARUN[2], J. R. MANNS[1];
[1]Emory Univ. Dept. of Psychology, Emory Univ. Neurosci. and Animal Behavior, Atlanta, GA;
[2]Program in Neurosci. and Behavioral Biol., Emory Univ., Atlanta, GA

Abstract: The bidirectional connections between the amygdala and hippocampus support complementary functions. Specifically, the hippocampus is a key node for associating objects with a given spatial context, and the amygdala can modulate those object-context associations. For instance, a previous study showed that optogenetic stimulation of the basolateral complex of the amygdala (BLA) accelerated acquisition of object-context associations. Under naturalistic conditions, the BLA is modulated by affective or social stimuli, with inactivation of the BLA leading to atypical interactions with those types of stimuli. The present study investigated oscillatory interactions between the BLA and hippocampus as rats formed, consolidated, and retrieved affective and nonaffective object-context associations. Female rats learned to associate objects, which were pots filled with scented digging media, with spatial contexts, which were visual-distinct boxes. Specifically, rats learned to retrieve a buried reward in one of two objects given the current spatial context (i.e., Box 1: A+B-; Box 2: A-B+). Local field potentials were recorded from the BLA and ventral hippocampus while rats learned, consolidated, and retrieved the object-context associations of four testing conditions. Each condition included one object scented with a nonaffective plant-based odorant (e.g., lavender) and second object scented with either conspecific male urine, conspecific female urine, fox urine, or a second plant-based odorant. The focus of the study was to determine whether oscillatory interactions between the amygdala and hippocampus differed while forming, consolidating, and retrieving affective versus nonaffective object-context associations. Preliminary results suggested that affective and nonaffective object-context associations differentially modulated amygdala-hippocampus interactions during the task. The results have implications for understanding typical bidirectional interactions between the amygdala and hippocampus that underlie remembering the spatial context where affective events have occurred.

Disclosures: J.L. Krasney: None. **A. Arun:** None. **J.R. Manns:** None.

Poster

321. Hippocampal-Subcortical Circuits

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 321.07

Topic: H.08. Learning and Memory

Support: NIDA Grant T32 DA 43469-4

Title: Dopaminergic inputs signal reward expectation and novelty to dorsal CA1 of the hippocampus

Authors: *C. HEER[1], S. KRISHNAN[2], M. SHEFFIELD[1];
[1]Neurobio., [2]Biol. Sci. Div., Univ. of Chicago, Chicago, IL

Abstract: Dopaminergic activity in the hippocampus impacts synaptic plasticity, alters hippocampal neuron activity, and impacts hippocampal dependent learning and memory processes. These effects have previously been attributed to dopamine release from sparse projections from the ventral tegmental area (VTA) to the hippocampus. However, it has recently been shown that locus coeruleus (LC) inputs to the dorsal hippocampus also release dopamine and impact performance on hippocampal dependent learning and memory tasks. To dissect the impacts of VTA and LC dopamine release on hippocampal activity and memory performance, it is necessary to determine how these inputs are differently active during learning and memory and the mechanisms of action through which they impact hippocampal activity and plasticity. Therefore, using 2-photon microscopy, we functionally imaged the activity of VTA and LC axons in the hippocampus of head fixed mice as they explored linear virtual reality (VR) environments. We observed ramping activity in VTA axons in the hippocampus during approach of a rewarded location. This activity may provide reward expectancy information to the hippocampus as removal of reward lead to a disappearance of this activity, and reintroduction of reward reinstated this ramping activity. On the other hand, LC inputs to the hippocampus were heterogenous exhibiting velocity, pupil diameter, and novelty encoding. Together this reveals the two sources of dopamine in the dorsal hippocampus provide different information to the hippocampus and likely play different roles in modulating hippocampal activity.

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Poster

321. Hippocampal-Subcortical Circuits

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Topic: H.08. Learning and Memory

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NIH Grant R01DA043988
NIH Grant T32DA007237

Title: The role of the nucleus accumbens and the ventral hippocampus in cocaine contextual memories

Authors: *C. CABAN RIVERA[1], C. DO[1], R. PRICE[1], G. M. SMITH[2], E. M. UNTERWALD[1]; [1]Ctr. for Substance Abuse Res., [2]Shriners Hosp. Pediatric Res. Ctr., Lewis Katz Sch. of Med. at Temple Univ., Philadelphia, PA

Abstract: Addiction is characterized by compulsive drug seeking behaviors. Cravings triggered by cues that were once associated with drug use are a main contributor to relapse. Addictive drugs engage molecular pathways of associate learning in which recall of a memory is followed by its reconsolidation, strengthening that memory. The reactivation period is a critical time wherein memories are vulnerable to interference. Here we examine the role of the nucleus

accumbens (NA) in the maintenance of cocaine contextual memories, and explore neuroplasticity involved in reconsolidation. Adult male C57Bl6 mice underwent bilateral intra-NA injections of an inhibitory Designer Receptors Exclusively Activated by Designer Drugs (iDREADD) followed by cocaine conditioned place preference (cCPP). After cocaine place preference was established, cocaine memory was reactivated followed immediately by injection of clozapine N-oxide (CNO) to inhibit NA neuronal activity during reconsolidation. Place preference was re-tested 3 and 7 days later. In a separate cohort of male C57Bl6 mice, we chemogenetically disrupted the ventral hippocampus (vHPC) to NA glutamatergic projections by delivering the iDREADD in a two-vector system. In brief, a retrograde virus carrying a cre-dependent iDREADD was infused into the NA and a second vector containing cre was infused into the vHPC. After 10 days, mice underwent cCPP, after a positive cocaine place preference, mice were re-exposed to the cocaine compartment and receive CNO immediately after to inhibit glutamatergic projections from the vHPC to the NA. Mice were re-tested for place preference 3 and 7 days later. In a third study, fosTRAP2-Ai14 mouse were used to evaluate plasticity in NA medium spiny neurons (MSN) upon reconsolidation of cocaine memories. Male and female fosTRAP2-Ai14 mice underwent cCPP followed by reactivation of cocaine memory and 4-hydroxytamoxifen injection. Dendritic spines of fosTRAP+ cells activated by recall of cocaine context were analyzed for density, surface area, number, and morphology. Results show that inhibition of NA neurons or vHPC to NA projection neurons, following reactivation of cocaine contextual memory, significantly reduced a once established preference for such context. In addition, a significant increase in dendritic spine density in NA MSNs activated by cocaine memory recall was found. These results suggest the NA, specifically the glutamatergic projections from the vHPC to the NA are important for the reconsolidation of cocaine memories. Furthermore, MSNs of the NA exhibit neuroplastic changes upon memory reactivation.

Disclosures: C. Caban Rivera: None. **C. Do:** None. **R. Price:** None. **G.M. Smith:** None. **E.M. Unterwald:** None.

Poster

321. Hippocampal-Subcortical Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 321.09

Topic: H.08. Learning and Memory

Support: CIHR Project Grant 155957

Title: Thalamic head direction cells and hippocampal place cells replay after spatial learning

Authors: *S. ANGELES-DURAN[1], G. R. VITE[1], A. PEYRACHE[2];
[2]Montreal Neurolog. Inst., [1]McGill Univ., Montreal, QC, Canada

Abstract: During exploration, hippocampal place cells form sequences associated with the animal's trajectories, which are replayed during subsequent non-REM sleep, and this

phenomenon is instrumental for learning and memory. In parallel, head-direction cells of the anterodorsal thalamic nucleus, which fire for a specific direction of the animal's head, remain coordinated during sleep. The HD signal is crucial for spatial navigation and the anterior thalamus is necessary for spatial memory, yet whether thalamic HD cells and hippocampal activity are coordinated during sleep and how much this coordination depends on learning remain unknown. To address this question, we recorded neuronal ensembles of HD cells and hippocampal place cells in mice performing a spatial memory task. Animals were trained on a forced alternation task on a Y-maze, during which they had only one path to take on each trial. On the day of the recording, after the forced-choice task, the animal performed a free alternation task in which it was free to choose either one of the two arms and spontaneously alternated between them. Sleep was recorded before and after each task. HD cells fired systematically 50-100 ms before hippocampal neurons. We observed mild reactivation in the hippocampus in the two sleep sessions following the tasks, as expected in a highly familiar environment. HD cells showed no reactivation after the forced alternation but interestingly, showed strong reactivation after the free alternation task. Furthermore, HD and hippocampal ensembles reactivated together after the free alternation task. Hence, during sleep following exposure to a familiar context, the hippocampus potentially reactivates independently of other structures, and spatial learning recruits large-scale thalamocortical networks upstream of the hippocampus. These findings shed new light on the mechanisms of sleep-dependent learning.

Disclosures: S. Angeles-Duran: None. **G.R. Vite:** None. **A. Peyrache:** None.

Poster

321. Hippocampal-Subcortical Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 321.10

Topic: H.08. Learning and Memory

Support: DFG Grant CRC1089
Foundation Deutsche Demenzhilfe Innovative Minds Grant

Title: Decipehring the role of locus coeruleus for hippocampus-dependent learning and its impairment in a mouse model of Alzheimer´s disease

Authors: *H. KHOMYAK[1], M. FUHRMANN[1], S. POLL[2];
[1]German Ctr. for Neurodegenerative Dis. (DZNE), Bonn, Germany; [2]Inst. of Exptl. Epileptology and Cognition Res. (IEECR), Univ. Hosp. Bonn, Bonn, Germany

Abstract: Locus Coeruleus (LC) is a neuromodulatory system that is heavily and early affected during Alzheimer´s Disease (AD). This is observed as the loss of neurons, especially in the part that projects to the hippocampus (HPC). Our understanding about the role of the LC and its catecholaminergic projections to the HPC in memory formation and AD remains incomplete. Therefore, the key goals of this project are to examine the influence of the LC on HPC-

dependent mnemonic processes and to study how such mnemonic processes are impaired in AD. To achieve this, we explored whether the structural and functional connectivity between the LC and the HPC is altered in a mouse model of AD-like pathology.
A structural analysis of LC-HPC projections was performed by establishing an AAV-mediated neural tracing protocol. For the functional analysis of these projections, we will use *in vivo* two-photon Ca^{2+} imaging. Specifically, we will record axonal projections in hippocampal CA1 that originate from the LC while the mouse is moving on a treadmill performing tasks designed to test spatial and working memory. We will use transgenic mouse models that exhibit hallmarks of AD-pathology, namely APPswe/PSEN1dE9 (Plaque-pathology) and P301S mice (Tau-pathology).
First results indicate that employing the AAV-mediated neural tracing protocol allows specific tracing of axonal connections between the LC and CA1. Immunohistochemical analysis of the LC verified that viral expression is restricted to tyrosine hydroxylase (TH)-positive cells. This will enable us to isolate this cell population in further experiments designed to probe its functional significance for LC-HPC connectivity, such as neurotransmitter imaging and optogenetics. Furthermore, another experiment revealed axonal dystrophies of LC-originating neurites in the HPC of APPswe/PSEN1dE9 mice. This result supports our hypothesis that AD-mouse models show morphological impairments of LC-HPC axonal projections. The mice exhibited signs of AD pathology at the time of the experiment. We confirmed this by the presence of amyloid-beta plaques in the HPC which were not present in control mice. Equal numbers of male and female mice were used for this experiment and wild type mice of the APP/PS1 line served as controls.
Our results suggest that defective LC-HPC connectivity might contribute to impaired mnemonic processing in AD. We expect to gain further insights about potential morphological and functional impairments of LC-HPC projections in mouse models of AD-like pathology. This will enable us to pinpoint the functional significance of LC-HPC connectivity for hippocampus-dependent learning and memory.

Disclosures: H. Khomyak: None. **M. Fuhrmann:** None. **S. Poll:** None.

Poster

321. Hippocampal-Subcortical Circuits

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 321.11

Topic: H.08. Learning and Memory

Support: NIH K01MH117444

Title: Hippocampal long-range somatostatin-expressing inhibitory neurons provide top-down regulation of subcortical regions

Authors: *J. KINNEY, M. ZHOU, Y.-Q. JIANG, M. LI, Q. SUN;
Case Western Reserve Univ. Dept. of Neurosciences, Cleveland, OH

Abstract: SFN Abstract – Jessica Kinney

Area CA3 is viewed as a key hippocampal region that is essential for *intra-hippocampal* information processing: linking dentate gyrus to CA1 via the classic entorhinal cortex◊dentate gyrus◊CA3◊CA1 trisynaptic pathway. In comparison, the direct connections between CA3 and *extra-hippocampal* regions are considered to be limited. Additionally, previous studies in CA3 have primarily focused on its excitatory connections, whereas the circuitry and function of inhibitory neurons in CA3 remain relatively understudied. Inhibitory neurons are classically divided based on differences in neurochemical, morphological, and electrophysiological features and are considered *short-range* projecting neurons that suppress the activity of surrounding local neurons. Recent evidence, however, indicates that a variety of inhibitory neurons, including somatostatin-expressing (SOM+) and parvalbumin-expressing (PV+) inhibitory neurons, can send *long-range* projections to distant brain regions to coordinate brain-wide activity and contribute to behavioral tasks. Intriguingly, here, we used cell-type and pathway-specific viral tracing to demonstrate that CA3 SOM+, but not PV+, inhibitory neurons send long-range projections to three subcortical regions: medial septum (MS), lateral hypothalamus (LH), and supramammillary nucleus (SuM). Our retrograde AAV (AAVretro)-based intersectional viral tracing indicates that the same CA3 SOM+ inhibitory neurons likely send long-range projections to all three regions. Furthermore, our ex vivo patch-clamp recordings assisted by channelrhodopsin-2 (ChR2) demonstrated that CA3 SOM+ inhibitory neurons preferentially suppress fast-spiking (presumptive GABAergic) neurons in SuM and LH, and preferentially suppress fast-spiking (presumptive GABAergic) and cluster firing (presumptive glutamatergic) neurons in MS. Thus, a key role of this long-range inhibitory output is to disinhibit these three subcortical regions. We propose that this long-range inhibitory projection is ideally positioned to coordinate and synchronize activity between the hippocampus and multiple subcortical regions simultaneously. We are currently using in vivo optogenetic manipulations in conjunction with behavioral assays to investigate the role of these novel long-range inhibitory neurons in memory formation.

Disclosures: **J. Kinney:** None. **M. Zhou:** None. **Y. Jiang:** None. **M. Li:** None. **Q. Sun:** None.

Poster

321. Hippocampal-Subcortical Circuits

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 321.12

Topic: H.08. Learning and Memory

Support: NIH K01MH117444

Title: Hypothalamic supramammillary nucleus preferentially excites hippocampal CA3 interneurons to suppress CA3 pyramidal neuron activity

Authors: M. LI, J. L. KINNEY, Y.-Q. JIANG, D. LEE, W.-C. XIONG, ***Q. SUN**;
Case Western Reserve Univ., Cleveland, OH

Abstract: The supramammillary nucleus (SuM) in the hypothalamus sends long-range projection to the hippocampus and plays an important role in a number of behaviors, including spatial and contextual memory, social memory, and novelty detection. Previous studies on the SuM-hippocampus circuits have primarily focused on the projections from SuM to CA2 or dentate gyrus (DG). Yet, the functional connectivity between SuM and other hippocampal regions remains largely unexplored. Here we used viral-based retrograde and anterograde tracing and ChannelRhodopsin2 (ChR2)-assisted patch-clamp recording to demonstrate that SuM preferentially excites inhibitory neurons to suppress pyramidal neurons in CA3. Consistent with previous studies, we found that SuM fibers project densely to CA2 and DG, and only sparsely innervate CA3. As expected, optical stimulation of ChR2+ SuM fibers led to very small excitatory postsynaptic currents (EPSC) in a fraction of CA3a pyramidal neurons (near CA2), and no EPSC in CA3b/c (close to DG). Surprisingly, however, optical stimulation resulted in strong feedforward inhibition in all CA3 pyramidal neurons tested along the transverse axis (CA3a = 20 cells; CA3b = 13 cells; CA3c = 7 cells). Using parvalbumin (PV)-tdTomato mouse line, we further showed that optical stimulation of SuM fibers resulted in robust excitatory responses in half of CA3 PV+ neurons (15/30 cells) and was capable of driving spikes in a subset (3/30 cells). Despite apparent denser SuM fibers in CA3a, the light-evoked EPSC in CA3a PV+ neurons (-63.7 ± 19.4 pA, n = 6) was not different from CA3c PV+ neurons (-49.7 ± 12.7 pA, n = 6, p = 0.56). Moreover, the light-evoked EPSC in CA3 PV+ neurons (-56.0 ± 9.6 pA, n = 14) was slightly, but not significantly, greater that in the densely innervated DG granule cells (-37.4 ± 9.2 pA, n = 11, p = 0.18). Taken together, this study, to our knowledge, represents the first demonstration that a long-range glutamatergic input largely evades excitatory neurons, but predominantly innervates inhibitory neurons to suppress excitatory neurons in a brain region. In addition, our findings suggest that SuM is able to regulate the hippocampal activity, independent of its well-studied projections to CA2 and DG.

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Poster

321. Hippocampal-Subcortical Circuits

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Program #/Poster #: 321.13

Topic: H.08. Learning and Memory

Support: NIH K01MH117444

Title: The basolateral amygdala provides a potent glutamatergic input onto hippocampal CA3 pyramidal neurons with distinct topographic and subcellular innervation pattern

Authors: *M. LI, Y. JIANG, K. LIU, J. KINNEY, Q. SUN;
Case Western Reserve Univ., Cleveland, OH

Abstract: The hippocampal CA3 region is crucial for memory formation. CA3 pyramidal neurons are known to receive three major glutamatergic inputs - the mossy fibres from dentate gyrus (DG), the recurrent collaterals from CA3 and the perforant pathway from entorhinal cortex. Although early anterograde tracing has provided anatomical evidence that the basolateral amygdala (BLA), a region that is essential for emotional responses, projects to CA3, the detailed anatomical and functional connectivity patterns and behavioral roles of this circuit remains largely unknown. Here we report that BLA constitutes the forth sizeable glutamatergic input onto CA3 pyramidal neurons with unique topographic and subcellular innervation patterns. Using rabies virus and retrograde AAV tracing, we found that CA3-projecting BLA neurons were primarily clustered in BLA adjacent to the midline, whereas CA1-projecting BLA neurons were relatively uniformly distributed in BLA. Using channelRphodopsin2 (ChR2)-based anterograde tracing, we found that BLA predominantly innervates basal dendrites in CA3a/b (next to CA2) and was nearly completely devoid of CA3c (near DG) in mid-ventral hippocampus. Using ChR2-assisted ex vivo patch clamp recording, we showed that BLA makes glutamatergic connections onto CA3. Remarkably, BLA-CA3 synapses displayed robust temporal summation of postsynaptic potentials (PSPs) in responses to 20Hz light stimulation (the ratio of PSP10/PSP1 = 4.87 ± 0.67; n = 9 cells). By contrast, BLA-CA1 synapses displayed significantly weaker temporal summation with almost no facilitation (the ratio of PSP10/PSP1 = 0.82 ± 0.15; n = 9 cells, $p < 0.001$). Using DREADDs-based chemogenetic approach, we found that suppression of ventral CA3 impaired contextual fear memory and social behavior. As BLA preferentially innervates mid-ventral CA3, we are currently working on whether BLA-CA3 circuit plays a role in contextual fear memory and social behavior.

Disclosures: **M. Li:** None. **Y. Jiang:** None. **K. Liu:** None. **J. Kinney:** None. **Q. Sun:** None.

Poster

322. Hippocampal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 322.01

Topic: H.09. Spatial Navigation

Support: CB SRF Wellcome Grant 548682
Vice Chancellor's Award, Cambridge Trust
Masonic Charitable Foundation Cambridge Scholarship

Title: Specific reduction in the occurrence of replay but preserved trajectory encoding in a mouse model of Alzheimer's disease

Authors: *S. SHIPLEY[1,2], R. HAYMAN[1], G. CASALI[1,3], C. BARRY[1];
[1]UCL, London, United Kingdom; [2]Univ. of Cambridge, Cambridge, United Kingdom; [3]Univ. de Bordeaux, Bordeaux, France

Abstract: The pathological changes that occur in the brain as Alzheimer's disease (AD) progresses are relatively well characterised. However, the functional deficits that accumulate as a result of the advancing pathology are less well understood. Given the salience of the hippocampus in memory and its early inclusion in disease progression, it seems plausible that functional changes in this region may correspond with the symptoms of memory disorder and provide potential new targets for therapies as well as diagnostic tools. With this in mind we hypothesized that replay - the transient reactivation of hippocampal place cell activity - a process that is held to be central for to the formation, consolidation, and retrieval of memories would be adversely affected during AD development.
We performed chronic single cell electrophysiological recordings from the CA1 region of the hippocampus in an APP knock-in mouse model of AD (Chat-cre/APP NL-G-F, n=7) and wild-type controls (n=8). These mice have saturated levels of amyloid plaques by 7 months of age (Saito *et al.* 2014) - we recorded from mice aged 7-18 months. Similar numbers of place cells per mouse were recorded in the two groups (AD mean per mouse=56.0, WT=59.5).
Memory performance was assessed on an 8-arm radial arm maze task with reward configuration changing across days. This paradigm allowed us to assess both working and reference memory performance. We identified mild but significant deficits in memory function in the AD mice when compared to wild type controls, with the AD mice making overall more working memory errors and showing less improvement in reference memory with experience than their wild-type counterparts.
Despite the observed behavioural deficits, we found that the content of replay events was effectively unchanged in AD mice - the proportion of candidate events which contained an identifiable trajectory were equivalent between the groups, as was the quality of the trajectories fit to the decoded position. In contrast, the frequency with which replay events occurred was reduced in the mutant animals, both during active exploration of the maze, and in subsequent sleep recordings. These findings suggest that the amyloid plaques expressed in this model of AD specifically disrupt network components responsible for regulating or possibly initiating replay events, whereas circuit elements necessary for the generation of normal replay sequences are relatively unaffected.

Disclosures: **S. Shipley:** None. **R. Hayman:** None. **G. Casali:** None. **C. Barry:** None.

Poster

322. Hippocampal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 322.02

Topic: H.09. Spatial Navigation

Support: EU-M-GATE 765549

Title: Vectorial coding of spatial goals in the rodent hippocampus during flexible navigation in a large-scale and dynamic environment

Authors: *N. NYBERG[1], C. GAHNSTROM[2], E. DUVELLE[3], C. BARRY[1], H. J. SPIERS[1]; [1]Univ. Col. London, London, United Kingdom; [2]Dept. of Psychology, Univ. of Pennsylvania, Philadephia, PA; [3]Dartmouth Col., Hanover, NH

Abstract: The mammalian hippocampus contains place cells, whose firing patterns represent self-location within a cognitive map. However, to flexibly navigate, animals must also represent their intended goal location relative to this map. While it has remained elusive how and where goal representations are formed, both theoretical and empirical results (Nyberg et al., 2022) have suggested the involvement of the hippocampus. For example, recording from place cells, previous studies have reported the existence of goal-distance and direction tuning (e.g., Sarel et al., 2017), overrepresentation of goals (e.g., Dupret et al.,2010) and 'replay' of future goal-related routes (Pfeiffer and Foster 2013; Widloski and Foster 2022). However, these studies have used tasks which are either not hippocampal-dependent (e.g. stereotyped behaviour or visible goals) or where the goal location relative to the local environment has not been systematically varied. It is therefore unclear to what extent past results have been confounded by biased behaviour or specific task constraints. To remedy these problems, we developed a land version of the hippocampal-dependent Morris water maze task, using a large-scale 2m x 2m maze with 100 possible goal locations. Drop-down barriers can be inserted into the maze to change the local - but not global - environment with minimal perceptual changes. We combined this paradigm with high-density electrophysiological recordings of CA1 place cells from rats (n=5) and investigated i) whether any of the diverse repertoire of previously-reported goal codes emerged in our paradigm, and ii) what the effect of separately manipulating the goal location or the local environment had on these codes. Our results indicate evidence for the presence of vectorial goal-distance and direction coding in the hippocampus, while we find no evidence for the commonly-reported phenomenon of goal overrepresentation. These patterns appear gradually and dynamically adapt when the goal or environment is changed. Our results therefore inform current models of how the hippocampus contributes to truly flexible navigation in dynamic environments and suggests that some previously reported hippocampal goal codes are reflective of biased task constraints.

Disclosures: N. Nyberg: None. **C. Gahnstrom:** None. **E. Duvelle:** None. **C. Barry:** None. **H.J. Spiers:** None.

Poster

322. Hippocampal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 322.03

Topic: H.09. Spatial Navigation

Support: CB SRF Wellcome

Title: Awake replay sequences correspond to theta sequences in a large 2D environment

Authors: *J. M. LEFORT, S. TANNI, C. BARRY;
Univ. Col. London, London, United Kingdom

Abstract: As animals move through an environment hippocampal place cells become successively active, forming sequences at behavioral timescales. In tandem, place cell activity is also organized relative to the ongoing theta-band oscillations (7-10Hz), the resulting 'theta sweeps' represent the past, present, and future positions of the animal on a much shorter physiological timescale (~150ms). Theta sweeps are known to be influenced by cognitive demands placed on the animal, plausibly playing a role in planning and decision making. Conversely, during sleep and pauses in active behavior, sequences of place cells transiently reactivate - 'replay' events typically last 50-300ms and are thought to underlie a range of functions including system-level memory consolidation, trajectory planning, and evaluating potential outcomes.
The generation of replay during sleep is believed to depend on the prior occurrence of normal theta sequences during exploration (Drieu *et al*. 2018) but the mechanistic link between these two classes of phenomena is not fully understood. In part progress has been limited because most studies have been conducted on 1D tracks, limiting the possible trajectories that can be observed during both theta and replay states. Thus, to better explore the relationship between neural sequences generated during these two states, we recorded 687 CA1 place cells from 4 rats as they explored and navigated to reward locations within an extra-large environment (~9m^2). Replay events were identified during brief pauses in motion and decoded with a Bayes-like algorithm - making minimal assumptions about the shape of the trajectories. Comparing the sequences of cells that were active during replay events and theta sweeps recorded from the same recording epoch, we found an experience dependent relationship - indicating that the specific ordering of cells during theta periods influences the subsequent replayed sequences. Since the temporal sequencing of cells experienced during theta states is particularly amenable to Spike-Timing Dependent Plasticity (STDP), we propose that this form of Hebbian Plasticity likely contributes to setting up sequences that are preferentially reactivated during awake replays.

Disclosures: J.M. Lefort: None. **S. Tanni:** None. **C. Barry:** None.

Poster

322. Hippocampal Physiology II

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Program #/Poster #: 322.04

Topic: H.09. Spatial Navigation

Support: Wellcome Grant 555227

Title: Remapping and replay in mouse hippocampal formation following altered spatial transitions

Authors: *T. JAHANS-PRICE[1], M. HORAN[1], H. W. P. DALGLEISH[1], C. BARRY[2], N. BURGESS[3];
[1]Sainsbury Wellcome Ctr., [2]Cell & Developmental Biol., [3]Inst. of Cognitive Neurosci., UCL, London, United Kingdom

Abstract: Flexible behaviours like rerouting and detours in spatial navigation require a model of the environment. Spatial representations found in the hippocampus (HPC) and medial entorhinal cortex (MEC) are candidates for implementing a cognitive map and facilitating flexibility. How updates to such a model are made in a dynamic environment remains unclear. Replay of trajectories in HPC and MEC, occurring during sleep and awake quiescence, is implicated in memory consolidation and is a potential mechanism to propagate updates through a spatial map (Evans and Burgess, 2020).
Rather than encoding a map of pure geometry, HPC and MEC neurons have been suggested to encode state relationships, reflecting a predictive map of future occupancy (Stachenfeld et al., 2017). We test this theoretical work by investigating how manipulations to the transition statistics of a familiar environment are reflected in HPC and MEC representations and examine the role of replay in a dynamic environment. We do so using a task which changes a line state space into a loop.
In our task mice forage for food in a 1m diameter circular linear track with an opaque barrier preventing full transition, creating a line state space. Mice experience multiple habituation pre-sessions. On test day after an initial familiar line session, the barrier is removed, changing the transition statistics and allowing mice to fully navigate the now 'loop' state space. Thus, mice newly experience locations previously on either side of the barrier as being connected. After two sessions of exposure to the loop, the barrier is reinserted and animals run a final session of the original line environment. Rest sessions of 1hr are recorded between track sessions to study offline replay.
We implanted Neuropixels 2.0 in HPC or MEC of 6 mice and recorded spatial cells in this task. Both HPC (362 units) and MEC (384 units) show remapping between sessions, however the greatest remapping occurs in HPC between the two familiar line environments which are perceptually identical except for the intervening experience of transitions around the loop. MEC in contrast showed both less remapping and similar levels of remapping between all sessions. Offline replay in HPC increasingly occurred around and crossed the barrier location, with 67% of replays crossing after the barrier was reinserted.
These results suggest a role for the hippocampus in integrating current and previous transition statistics into a cognitive map of an environment, with replay as a mechanism for updating and maintaining the map. The MEC representations in contrast, may reflect a more stable and generalisable map.

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Poster

322. Hippocampal Physiology II

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Program #/Poster #: 322.05

Topic: H.09. Spatial Navigation

Support: Welcome Grant 548682
BBSRC LiDO Grant

Title: Hippocampal task representations generalize across spatial contexts during transfer learning

Authors: *A. O'LEARY[1], G. CASALI[2], R. BOURBOULOU[1], L. MUESSIG[1], T. WILLS[1], C. BARRY[1];
[1]Univ. Col. London, London, United Kingdom; [2]IINS, Bordeaux, France

Abstract: The hippocampus has long been thought to provide a map of an animal's spatial environment (O'Keefe & Nadel, 1978). However, recent experiments suggest that the same circuits serve a more general function, mapping task-relevant continuous features such as temporal delays (Pastalkova et al., 2008), auditory-frequency space (Aronov et al., 2017), and discrete relations such as odour-place pairings (Wood et al., 1999). Thus, it seems plausible that the hippocampal formation might provide a cognitive map of abstract task space, which can generalize across contexts to allow animals to flexibly solve novel problems (Behrens et al., 2018). Entorhinal grid cells are thought to play a role in facilitating this generalization as they maintain their firing phase offsets across different environments, while hippocampal place cells tend to remap (Fyhn et al., 2007). However, in CA1 a subset of cells maintain their firing fields relative to salient landmarks (Geiller et al., 2017) and reward zones (Gauthier et al., 2018) across environments. The aim of the current experiment is to investigate whether CA1 representations of abstract rules might also generalize across environments.
GCaMP6f mice (n=6) ran laps of a cue-rich 7m virtual linear track with a fixed goal. On each trial two additional cues appeared at pseudorandom locations within a 3m zone. The goal-cue predicted an invisible reward zone, located +70cm from its centre. The distractor-cue was not associated with reward. On each trial animals licked within reward zones associated with the moving goal-cue and fixed goal to receive rewards. After mice were expert in this task (~3 weeks), they were exposed to the same task in 1-2 additional environments, using different goal and distractor cues. The number of sessions required to reach expert performance was significantly decreased in environments 2 and 3, compared to environment 1.
Two-photon imaging was used to longitudinally record activity in CA1 throughout all learning and generalization phases. Alongside typical place cells, a subset of neurons had stable fields locked to the goal- and distractor-cue reference frames. Goal-cue cells were not purely reward-anticipation cells as population vector correlations showed significant remapping between goal-cue and fixed goal reference frames. In addition, while place cells remapped across environments, goal-cue cells generalized their firing, maintaining population vector correlations across environments. Thus, despite previous evidence that spatial context drives complete remapping, when animal's flexibly transfer previous learning to a new spatial context, CA1 representations of task rules are maintained.

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Poster

322. Hippocampal Physiology II

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Program #/Poster #: 322.06

Topic: H.09. Spatial Navigation

Support: Joint Wellcome. Project number: 555227

Title: Updating mouse hippocampal-entorhinal spatial representation following altered transition structure in a virtual 2D environment

Authors: *M. HORAN, H. DALGLEISH, A. CASTEGNARO, T. JAHANS-PRICE, T. MRSIC-FLOGEL, C. BARRY, N. BURGESS;
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Abstract: Cognitive maps have been proposed to underlie flexible behaviors, including spatial navigation. Neural signatures of such maps have been found in hippocampus (HPC) and entorhinal cortex (EC), including place and grid cells. Firing patterns in both regions remap in new environments, but it is less clear if they represent the connectivity of an environment. In models using the successor representation, HPC place cells can be framed as representing transition structures within a task, with grid cells in EC being the eigenvectors of these transitions (Stachenfeld et al., 2017). We set out to test the model prediction of HPC-EC sensitivity to the underlying transition structure of an environment. We used a virtual 2D 60x60cm environment that allowed mice full 360° rotation while head-restrained (Chen et al., 2018). Mice learned to run to virtual pillars for reward, and showed reliable spatial navigation, collecting up to 20 rewards/min. Once familiar with the environments, mice were implanted with high-yield Neuropixels 2.0 probes in either HPC or EC. We recorded 368 HPC and 684 EC neurons across multiple days (recordings: 8; mean cells/recording: 92 in HPC; 171 in EC; including some resampled cells across days) of which >50% were spatially modulated. Finally, to alter the transition structure within the familiar environment, we activated two bidirectional teleport locations which instantly transported the animal from one side of the arena to the other. This linked remote areas of the environment, while keeping all other elements of the task identical, including visual stimuli. The mice learned to use teleportation points to navigate the environment, while other behaviors (running speed, reward rate) were similar before and after activation. Remapping of spatially selective cells in response to this updated transition structure was prevalent: 62% in HPC and 55% in EC (proportion of cells with reduced spatial correlation relative to pre-teleport-activation). This was greater than the remapping rate in sessions before the first-ever activation of the teleports (inactive vs. active sessions: HPC $\chi^2(1,N=87)=11.1$, $p=.0008$; EC $\chi^2(1,N=227)=15.0$, $p=.0001$). We also found that the remapping of spatial cells was greater nearer the teleports, particularly in HPC. Together, this shows that the HPC-EC circuit is sensitive to updates in underlying transition structure of environments in the absence of any other (visual) changes. Our results help couple the spatial and non-spatial functions of the HPC-EC

circuit in a single framework, as the state-prediction-based models propose that this circuit maps transitions in non-spatial domains too (e.g. Whittington et al., 2020).

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Poster

322. Hippocampal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 322.07

Topic: H.09. Spatial Navigation

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Title: Hippocampal circuits underlying spatial memory deficits in Alzheimer's disease

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Abstract: Deficits in spatial navigation are among the early symptoms in AD patients, consistent with the hippocampal formation being necessary for spatial computations and with disease onset in the hippocampal formation. Although it is recognized that early symptoms correspond to brain regions that are affected early in the disease, it is not clear whether further cognitive decline is solely caused by a spreading cellular pathology, or whether a focal pathology can by itself cause aberrant neuronal activity in a larger network. These possibilities cannot be distinguished in standard disease models, which broadly express AD-related proteins across brain regions. We therefore generated a mouse model in which the expression of mutant human APP (hAPP) was limited to hippocampal CA3 cells (CA3-APP mice). We recorded temporal and spatial properties of individual neurons, in freely moving mice, while they performed a hippocampal dependent spatial navigation task (figure-8 maze). Despite the restricted expression of hAPP we found a robust impairment in the performance of CA3-APP mice in the memory task. We also found a large reduction in the theta and gamma oscillation frequencies in all different areas of hippocampus (CA1 and DG/CA3). We then examined the temporal dynamics of cells in relation to ongoing oscillations and found that 20%-30% of principal cells and interneurons in CA3-APP have an intrinsic frequency below the LFP, while the remaining cells have, comparable to control mice, higher intrinsic frequencies than the LFP. By comparing the effects on activity patterns in

different areas, we found that CA1 (where hAPP is not expressed) and CA3 cells are similarly affected, highlighting the importance of network dysfunction. In addition, by testing CA3-APP mice at two different age points we were able to determine that the reduced network frequency precedes the shift in the frequency of individual cells. Surprisingly, even though theta oscillations and the spatial navigation of CA3-APP mice are disrupted, the spatial properties of place cells remain very precise even in aged mice (19 months). So, what could explain the deficits in spatial navigation we observed in aged but not young mice? We found that CA1 place cells of aged CA3-APP mice show reduced theta phase precession and sequential firing during the behavior task. This is particularly relevant since compression of temporal sequences of place fields within individual theta cycles permits the use of synaptic plasticity for learning of sequential structure, thereby providing a temporal dimension to hippocampal memory traces.

Disclosures: **S. Viana da Silva:** None. **M. Haberl:** None. **K. Gaur:** None. **R. Patel:** None. **G. Narayan:** None. **M. Ledakis:** None. **M.L. Fu:** None. **E.H. Koo:** None. **J.K. Leutgeb:** None. **S. Leutgeb:** None.

Poster

322. Hippocampal Physiology II

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Title: The dentate gyrus, but not the medial entorhinal cortex, is required for the temporal order of CA3 spikes during theta states in spatial working memory tasks

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Abstract: The reactivation of neuronal ensembles during hippocampal sharp-wave ripple (SWR) oscillations is critical for memory. The sequential order of reactivation is thought to be established during behavior via mechanisms that govern the precise temporal patterns of spiking over theta oscillation cycles. The dentate gyrus (DG) is necessary for SWR generation in CA3 during spatial working memory, but it remains unclear whether and how it contributes to the temporal organization of CA3 neuronal spiking. In the hippocampus, the phase of spikes relative

to theta oscillations advances over successive theta cycles, thus preserving the behavioral-scale activation order of neurons within each theta cycle, a phenomenon known as theta phase precession (TPP). To investigate the role of DG in support of TPP, we lesioned DG inputs to CA3 in rats during working memory tasks (N = 9 rats) and compared CA3 TPP to control rats (N = 4) and rats with medial entorhinal cortex (MEC) lesions (N = 8, N = 7 matched controls). This allowed for a comparison of the function of both major excitatory theta-modulated input to CA3. By analyzing 411 single CA3 units from all animals, we found that CA3 TPP was reduced in the absence of either DG or MEC inputs. However, examination of the phase profile of first (onset) and last (offset) spikes in each theta cycle revealed a striking difference. In DG lesioned rats, the onset spikes were shifted to earlier phases. The phase of offset spikes was quantitatively shifted as well, albeit to a significantly smaller amount. In contrast, MEC lesions had no effect on the phase of either the onset or offset of CA3 spikes. Without DG, spikes were more numerous early in the theta cycle, while having greater phase variability as the animals moved through the first half of the place fields. These effects were absent in MEC lesioned rats, suggesting DG, but not MEC, inhibits early phase spikes early in the place field to support prospective coding at late theta phases. In control animals, there was a strong relation between the temporal lag of spikes of cell pairs with overlapping place fields and the physical distance between their place fields, as expected from normal theta sequences. Surprisingly, this relationship was abolished only with DG lesions, but not MEC lesions, suggesting that the DG-CA3 circuit autonomously organizes sequential firing order of ensembles. Finally, we propose a phenomenological computational model that accounts for the empirical findings by demonstrating different effects of each of the feedforward pathways on inhibitory subnetworks. Our results thus describe unique roles for DG in the generation of sequence coding in CA3 during behavior and theta states.

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Poster

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Title: Sharp wave ripples do not have a causal role in support of spatial-working memory and the planning of future spatial trajectories.

Authors: Y. ZHANG, B. TAYLOR, M. WANG, A. VARTANIAN, A. KHAN, S. LEUTGEB, *J. LEUTGEB;
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Abstract: Hippocampal sharp wave ripples (SWRs) are prominent network oscillations (150-250 Hz) observed during sleep and periods of immobility in awake-behavior. SWRs have been shown to support learning and memory consolidation. The observation that CA1 SWRs contain neuronal sequences that represent future paths has led to the hypothesis that SWRs during awake-behavior are important for future route planning and ongoing decisions based on information stored in memory. To test whether CA3 neuronal activity may also contribute to sequence coding during SWRs, we used Baysian decoding to show that future sequences of CA3 neurons occur during CA3 SWRs while rats (n = 5) performed a hippocampus dependent spatial working memory task on the 8-arm maze. To then directly test the causal role of awake-SWRs during planning and decision making we used a closed loop SWR detection and disruption system in the same task. After rats (n = 9) were trained to criterion (80 % correct trials) SWRs were detected across hippocampal subregions CA3 and CA1. SWRs were more likely to occur when the animal visited a reward site, but were not always synchronous across CA3 and CA1 during behavior, and also differed in frequency and amplitude from sleep. From these observations, disruption of ripples by inhibition induced by the stimulation of the ventral hippocampal commissural pathway was performed upon detection of SWRs in CA3 only (n = 11 experiments), CA1 only (n = 8), or both (n = 8). Within animal controls included a behavioral design in which blocks of 4 trials with and without stimulation were intermingled for a total of ~20 trials. Control experiments were also performed in which stimulation was delivered 200 msec after SWR detection in order to allow for SWRs to occur despite stimulation (n = 13 experiments), which controlled for the general effects of stimulation delivery. No significant differences were found when comparing the % correct trials between stimulation and non-stimulation trials across all experimental groups. Furthermore, no significant differences were observed when comparing the % correct trials for SWR disruption experiments and the 200 msec delay control experiments (Wilcoxon test, multiple comparisons, Holm Bonferroni correction). We therefore observe that the presence of future sequences in CA1 and CA3 SWRs at reward sites does not reflect a network mechanism critical for future correct choices, as supported by the lack of memory impairment when SWRs were disrupted during behavior. These findings are contrary to the hypothesis that future sequences observed in SWR activity are required for planning upcoming future paths in space and for guiding future choices during working memory.

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Poster

322. Hippocampal Physiology II

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Title: Time cell sequences during delay intervals are not prolonged by ongoing theta oscillations

Authors: L. YUAN[1], J. F. FIGUEROA[1], A. KHAN[1], C. HSU[1], G. NARAYAN[1], J. K. LEUTGEB[1,2], *S. LEUTGEB[1,2];
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Abstract: Working memory (WM) retention over periods of seconds is essential for cognitive tasks. Many studies have reported sequential firing of hippocampal cells over time intervals (time cells) in WM tasks as a possible mechanism for memory maintenance. The sequential firing patterns of time cells are thought to be internally generated, and time compression by phase precession during theta oscillations is a potential mechanism to generate the sequences. However, time cells have also been reported in tasks in which there was no sustained theta during WM retention. We therefore asked whether the emergence of time cells during a delay interval in a WM task depends on ongoing theta oscillations. While recording hippocampal CA1 ensembles in rats (n=5) in a delayed spatial alternation task with delay intervals of 10 s and 30 s, differences in the occurrence of theta oscillations during the delay period were generated by the link between movement and the emergence of hippocampal theta. The task included blocks of trials when rats were either forced to run on a treadmill or were allowed to rest during the delay interval, and theta power was significantly lower during resting compared to running. Despite the substantial differences in theta power, the proportion of time cells in CA1 did not differ between running or resting during delay intervals [Time cell fraction, treadmill on for 10 s: 8.8%; treadmill off for 10 s: 8.5%; treadmill on for 30 s: 9.0%; treadmill off for 30 s: 8.5%]. In both running and resting trials, reliable time cells with a short time field were limited to the first few seconds of the delay regardless of its duration (10 s or 30 s). In addition, a second population of late-firing cells were observed in 30 s delays, which were silent during the first few seconds, but then intermittently active throughout the remainder of the delay period, albeit not at any well-defined time interval. Similar to the spike trains of well-defined early time cells, the intermittently active cells continue to show phase precession during spike trains throughout the delay interval. Taken together, our results show that the persistence of theta oscillations does not prolong the time over which sequentially active hippocampal cells emerge and that time cells are a time-limited network pattern irrespective of the brain state during the delay interval. With WM lasting 30 s in our experiment (percent correct trials: 84.0% for when running during the delay, 86.7% when resting during the delay) and time cell sequences only lasting for the initial seconds of the delay, WM retention likely requires mechanisms other than the sustained activity of hippocampal time cells.

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Poster

322. Hippocampal Physiology II

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Topic: H.08. Learning and Memory

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Title: Impaired associative memory encoding in the lateral entorhinal cortex of amyloid precursor protein knock-in mice

Authors: *T. NAKAGAWA, J. XIE, M. SAVADKOHI, J. Y. LEE, H. JUN, K. M. IGARASHI; Dept. of Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: Abstract: Alzheimer's disease (AD) is the most common cause of dementia. Previous fMRI studies show that the lateral entorhinal cortex (LEC) is the primary site of dysfunction in early-stage AD patients, but circuit mechanisms are unknown. We recently found that the LEC neurons in healthy animals are critically involved in associative memory encoding (Lee et al., Nature 2021). To test if this LEC activity is affected in AD, we recorded LEC neurons in amyloid precursor protein knock-in (APP-KI) mice engaging in an odor cue-reward task. APP-KI mice showed impaired associative memory performance. LEC neurons in APP-KI mice showed disrupted associative memory encoding. Our results suggest that the disruption of encoding in LEC neurons may lead to associative memory deficit in APP-KI mice.
Reference: Lee JY, Jun H, Soma S, Nakazono T, Shiraiwa K, Dasgupta A, Nakagawa T, Xie JL, Chavez J, Romo R, Yungblut Y, Hagihara M, Murata K, and Igarashi KM (2021)Dopamine facilitates associative memory encoding in the entorhinal cortex***Nature,*** 598:321-326
Jun H, Bramian A, Soma S, Saito T, Saido TC, Igarashi KM (2020)Disrupted Place Cell Remapping and Impaired Grid Cells in a Knockin Model of Alzheimer's Disease***Neuron***, 107:1095-1112

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Poster

322. Hippocampal Physiology II

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Program #/Poster #: 322.12

Topic: H.09. Spatial Navigation

Title: Depletion of the gut microbiome disrupts spatial memory, neural dynamics, and brain metabolism

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Abstract: Growing evidence implicates the gut microbiome in brain health and disease. Since worldwide consumption of oral antibiotics continues to rise, it is imperative to understand how antibiotics affect cognition through the gut-brain axis. We hypothesize that the administration of antibiotics to deplete the gut microbiome will lead to impaired spatial cognition as well as abnormalities in neural dynamics, brain metabolism, and blood-brain barrier (BBB) permeability. Male C57BL/6J mice (2-4 months) were given drinking water containing broad-spectrum antibiotics (ABX), including ampicillin, neomycin, gentamicin, metronidazole, and vancomycin. An additional cohort (ABXBA) was supplemented with butyrate, a key microbiome-derived metabolite, together with ABX. Spatial cognition was evaluated with the object-place memory (OPM) and clockmaze tasks (PMC6568215), at 2-3 weeks of treatment. Mice underwent positron emission tomography using [^{18}F]-fluorodeoxyglucose (FDG) and [^{11}C]-aminoisobutyric acid (AIB) to assess brain metabolism and BBB permeability, respectively, at 4 weeks of treatment. Finally, mice were implanted with tetrode arrays to perform electrophysiological recordings of CA1 place cells in the hippocampus. Our results demonstrate that, compared to controls, ABX mice have reduced FDG uptake in the entorhinal cortex, amygdala, and CA1, as well as increased uptake of AIB in the entorhinal cortex. Moreover, ABX mice show significant spatial impairments in the OPM and clockmaze tasks. Analysis of the neural recordings reveals that ABX place cells have larger field sizes, reduced spatial information, and altered remapping. Remarkably, ABXBA mice display normal spatial cognition, place cell dynamics, and BBB integrity. We conclude that depletion of the gut microbiome with antibiotics leads to disruptions within the hippocampus, amygdala, and entorhinal cortex, which are reflected in weaker metabolism, leakier BBB, disrupted spatial cognition, and aberrant place cell properties. Coadministration of butyrate preserves BBB integrity, spatial cognition, and hippocampal dynamics in microbiome-depleted mice, pointing to potential therapeutic approaches.

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Poster

322. Hippocampal Physiology II

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Topic: H.09. Spatial Navigation

Support: Gatsby Charitable Foundation and Wellcome Trust Core Grant 090843/F/09/Z
Wellcome Trust Principal Research Fellowship Wt203020/z/16/z

Title: Hippocampal place cells exhibit goal-oriented vector fields during navigation

Authors: *J. ORMOND, J. O'KEEFE;
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Abstract: The hippocampal cognitive map supports navigation towards, or away from, salient locations in familiar environments. The map is built upon the spatial receptive fields of the hippocampus's principal cells. These place fields are thought to encode the animal's position in allocentric (i.e. world-centred) coordinates. How place cells support flexible navigation, meaning navigation to a goal from different positions and directions, is not well understood, however. We recorded CA1 place cells from 5 rats that were well-trained to navigate to an unmarked goal location on the honeycomb maze. The maze consists of a tessellating pattern of moving platforms. The animal starts each trial from a randomly selected raised platform, and must move towards the goal iteratively by selecting one of two adjacent platforms which are raised into place; after each choice, the previous and unchosen platforms are lowered and new choices are presented until the animal reaches the goal. The strength of the maze lies in the fact that the animal must continually re-evaluate what is the most efficient direction of travel to reach the goal, often when none of the choices are direct. We find that when the animal navigates the maze, a large proportion of place cells develop egocentric tuning towards positions scattered on, and even off, the maze. On average, these "convergence sinks" (ConSinks) tend to be located in front of the animal, so that a given cell will typically fire when the animal is facing towards the sink location, though sinks to one or the other side of the animal, or even behind it, are possible. While we observed some ConSinks during open field navigation as well, significantly more were expressed during maze navigation, suggesting that the navigational constraints imposed by the maze and the unique behaviours (i.e. circular scanning around the platform perimeter) they elicit facilitate the expression of egocentric coding by place cells. At the population level, the distribution of ConSinks was centred near the goal location, and the population vector field converged on the goal, providing a strong navigational signal. Changing the goal location led to the movement of *ConSinks* and vector fields towards the new goal, despite more limited movement of the place fields themselves. The results suggest the hippocampus creates a vector-based model to support flexible navigation, allowing animals to select optimal paths to destinations from any location in the environment.

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Poster

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Title: Changes in the duration and temporal compression of hippocampal replay events as rats learn reward locations in a delayed match-to-sample task

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Abstract: The hippocampus supports spatial memory via the activity of place cells, neurons that fire in specific regions of an environment. During periods of sleep and rest, ensembles of place cells reactivate sequences of firing that occurred during earlier active behaviors, a phenomenon known as "replay". Replay may occur in the same order as occurred during a previous active experience ("forward" events) or in the reverse order ("reverse" events). Here, we examined replay events during rest periods from a previously published dataset (Zheng et al., Nature Communications 2021) in which rats learned a reward location on a circle track across trials of a delayed match-to-sample task. We used a Bayesian decoding algorithm to estimate angular positions on the circle track represented by ensembles of CA1 place cells during replay events. Recent work has shown that replay events increase in duration and become less temporally compressed with experience in novel but not familiar environments (Berners-Lee et al., Neuron 2022). Here, we show that the duration of replay events increased, and the temporal compression of the represented paths during replay events decreased, as rats learned new reward locations in a familiar environment. Further, replay events that occurred in the rest periods before correct trials were longer and less temporally compressed than events that occurred before incorrect trials. Previous work from our lab has shown that a bias for replay events in correct trials to terminate at the reward location develops over learning (Zheng et al. Nature Communications 2021). Here, we show that this termination bias is specific to forward replay events. Additionally, forward replay events that terminate at the reward location are longer and less temporally compressed than forward events that terminate elsewhere on the track. These results suggest that learning a novel reward location in a familiar environment is associated with slowing of replay. These results raise the possibility that slowing of replay may support performance of a spatial memory task. Furthermore, these results lend additional support to the hypothesis that forward and reverse replay serve different functions.

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Poster

322. Hippocampal Physiology II

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Program #/Poster #: 322.15

Topic: H.09. Spatial Navigation

Support: NIH Grant 1U19NS104648

Title: Hippocampal contributions to context-dependent decision-making

Authors: *J. JULIAN[1], M. SCHOTTDORF[1], E. FONSECA[1], D. W. TANK[2], C. D. BRODY[3]; [1]Princeton Neurosci. Inst., Princeton, NJ; [2]Princeton Univ., Princeton, NJ; [3]Princeton Neurosci. Inst., HHMI / Princeton Univ., Princeton, NJ

Abstract: The decisions we make often depend on context: For instance, "which shirt should I wear?" depends on whether you are going to work or a party. What is the neural source of contextual information that guides context-dependent choices? Cell ensembles in the hippocampus alter their responses to changes in context, but their impact on perceptual decision-making remains unknown. To test whether and how this hippocampal context encoding causally contributes to decision-making, we developed a virtual-reality (VR) context-dependent decision-making task for head-fixed mice. Mice were trained to navigate in two different VR T-maze environments distinguished by visual and auditory features. In both contexts, a salient visual turn guide was presented on one side of the end the VR corridor (randomly placed either on the left or right on each trial, balanced across trials). To receive a reward, at the T-intersections mice were trained to turn toward the turn guide in one context ("Pro"), and away from the turn guide in the other context ("Anti"). Within each testing session, the context switched across interleaved blocks of trials. To test whether context-specific hippocampal ensembles are causal to decision-making during Pro/Anti orienting, we labeled the population of neurons expressing the activity-dependent immediate early gene Arc in one context with channelrhodopsin-2, and later reactivated these same neurons in either the same or different context after mice learned context-specific decision rules (i.e., turn toward vs. away). Bilateral optogenetic reactivation of tagged hippocampal populations in (or near) the dorsal dentate gyrus (DG) biased mice to retrieve a context-specific decision rule on a trial-by-trial basis. Consistent with these optogenetic results, acute neuropixel recordings from the hippocampus (CA1 and DG) during Pro/Anti orienting further revealed that place cell remapping predicted the animals' upcoming decision rule on a trial-by-trial basis, and that hippocampal context representations were more strongly predictive of decision rule retrieval than the contextual cues themselves. Together, our observations suggest that the hippocampus causally contributes to flexible context-dependent decision-making.

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Poster

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Support: NSF Grant 1707408

Title: Hippocampal cells encode future states when choices are sensitive to reward outcomes but not during perseverative responding

Authors: *S. WANG, H. T. BLAIR;
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Abstract: The hippocampal place cells encode the animal's position during navigation. Some place cells predictively encode the animal's future trajectory, suggesting that they might contain successor representations that estimate the probability of visiting certain future states from the current state. Here, we analyzed how the coding of future states by hippocampal neurons depended on behavior policy. Rats (n=3) were trained to perform two responses on a linear track: crossing (running from one end of the track to the other) or backtracking (running a minimum distance along the track before turning around and returning to the start end). Before surgery, rats learned to perform backtracking over 14-21 days of training; backtracking from either side earned the same reward, but rats developed individual preferences for backtracking on just one side (henceforth referred to as the start side). After surgery to implant hippocampal tetrodes, rats ran daily sessions (40-60 minutes) during which reward probabilities for crossing and backtracking were adjusted (between 0-100%) to maintain an approximately equal preference for the two responses on the start side. For each session, we derived the relative contribution of past behavioral choice and reward outcomes to current behavioral choice by fitting a logistic regression model that predicts choice with past behavior trace, model-free reward trace, and model-based reward trace. Sessions were classified as either reward-sensitive (responding was better predicted by reward outcomes than past choices) or perseverative (responding was better predicted by past choices than reward outcomes). From these sessions, the single-unit activity of hippocampal neurons was analyzed on a segment of track where pre-choice behavior (position and running speed) did not differ between crossing vs backtracking trials. We found that trial type (crossing vs backtracking) has higher decoding accuracy from pre-choice cell activity during reward-sensitive sessions as compared to perseverative sessions. By contrast, the trial type was decodable with matched accuracy during both session types on the segment of the track where behavior differed by trial type. Based on these findings, we hypothesize that the coding of future states by hippocampal neurons depends on the sensitivity of the animal's choice to reward outcomes.

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Poster

322. Hippocampal Physiology II

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Support: Howard Hughes Medical Institute

Title: Distinct CA1 manifolds in Contextual Remapping are shaped by Behavioral Timescale Synaptic Plasticity

Authors: *S. VAIDYA[1], R. A. CHITWOOD[2], J. C. MAGEE[3];
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Abstract: In rodents, the CA1 area of the hippocampus is known to produce distinct maps or neural representations of the same environment based on reward contingencies, task requirements or subtle changes in background cues, a phenomenon known as contextual remapping. While contextual remapping remains a distinct signature of adaptive behavior in rodents, the emergence of distinct maps and the cellular and network mechanisms that form their basis remain uncharacterized. In this study, we perform longitudinal two-photon calcium imaging of CA1 neurons across 7 days while mice forage for rewards at two distinct locations on a linear treadmill given a contextual cue at the start of the track. We show that alternate reward contingencies lead to the emergence of two distinct orthogonal neural representations as the animal accrues experience at the task. We find that early representations of the two contingencies are inconsistent and noisy but are successively refined into consistent orthogonal representations suggesting the emergence of two distinct neural attractors in the hippocampus. Our longitudinal access to the CA1 neuronal population lets us probe the cellular and network dynamics at play in the separation of the two CA1 manifolds as they diverge across multiple days. We show that individual cells have an increasingly divergent response to the two contingencies across days while increasing the coherence or response consistency for each contingency. These responses can be interpreted as the divergence and settling of the input vectors or synaptic input profiles onto CA1 cells. To understand the cellular mechanisms that are responsible for encoding and consolidating these changes in CA1 cells, we analyzed the Calcium dynamics of place cell emergence and firing across days. We found distinct signatures of Behavioral Timescale Synaptic Plasticity (BTSP), a non-hebbian form of synaptic plasticity driven by dendritic voltage signals, in the formation and reshaping of place fields across days. Pharmacological manipulation of BTSP on Day 1 by inhibiting NMDA receptors severely impaired the CA1 maps of both contingencies suggesting a crucial role for this plasticity in contextual remapping. Our results characterize the formation, divergence and refinement of distinct CA1 manifolds in contextual remapping and show that BTSP is a prominent cellular plasticity mechanism involved in the settling and consolidation of CA1 contextual maps.

Disclosures: S. Vaidya: None. **R.A. Chitwood:** None. **J.C. Magee:** None.

Poster

322. Hippocampal Physiology II

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 322.18

Topic: H.09. Spatial Navigation

Support: NIH Grant R01AG062762

Title: Hyperglycemia leads to lower delay firing rates and increased reward-modulated activity in the hippocampus in an Alzheimer's disease risk factor model.

Authors: *L. A. CREW[1], E. FLORES[1], R. A. WIRT[1], K. N. CALVIN[1], A. A. ORTIZ[2], J. W. KINNEY[3], J. M. HYMAN[4];
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Abstract: Spatial working memory impairments are among the many debilitating symptoms of Alzheimer's disease (AD). The activity of spatially tuned neurons in the hippocampus (HPC) is widely thought to underlie spatial memory processes and is the most significant predictor of spatial memory performance. Not surprisingly, alterations in hippocampal activity and structure have long been observed in both AD patients and rodent models of AD. The vast majority of AD cases can be classified as late onset AD (LOAD). Although the etiology of LOAD is unknown, several risk factors have been identified, and prominent among these is diabetes mellitus (DM), which leads to increased blood glucose levels or hyperglycemia. Patients with DM are 4 times more likely to develop AD and also exhibit similar memory impairments as those found in AD. Chronic neuroinflammation is a pathological symptom of both DM and AD, and it is possible that neuroinflammation itself impacts cognitive performance. Given the links between DM and AD, we examined HPC neuronal firing patterns during a spatial working memory task in both chronically hyperglycemic and control animals, to help elucidate the neural processes underlying AD-related memory impairments. In this study, we hypothesized that neuroinflammation would disrupt neuronal activity within the HPC, thus leading to impaired cognitive function. To test this, we trained rats to perform a spatial working memory task, which is dependent upon proper HPC functioning. Following training, we induced chronic hyperglycemia by injecting experimental animals with multiple low doses of streptozotocin, to induce chronic hyperglycemia. We found delay-dependent spatial working memory impairments in hyperglycemic animals compared to healthy controls. Electrophysiological data revealed hyperglycemic animals had an overall decrease in HPC firing rates during the delay period. Spatial information scores were higher across the hyperglycemic animal group in both the HPC and in the frontal cortex. Furthermore, hippocampal activity was more strongly modulated by reward expectation in hyperglycemia, seen in increased selectivity as animals approached reward locations. Together, these results suggest that the hyperglycemia-driven neuroinflammatory states that are associated with the physiological and cognitive pathologies of AD, can also induce alterations in HPC spatial-related neuronal activity.

Disclosures: L.A. Crew: None. **E. Flores:** None. **R.A. Wirt:** None. **K.N. Calvin:** None. **A.A. Ortiz:** None. **J.W. Kinney:** None. **J.M. Hyman:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.01

Topic: H.08. Learning and Memory

Support: NINDS Grant 15NS118408

Title: An enhanced learning protocol prolongs the duration of long-term feeding suppression in Aplysia

Authors: A. DAURIA, M. WAINWRIGHT, *R. MOZZACHIODI;
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Abstract: Repeated exposure to aversive stimuli induces long-term modifications in defensive and appetitive behaviors, a process that is pertinent to all multicellular organisms (e.g., LeDoux, 2012). In the mollusk *Aplysia*, a standard learning protocol (SLP), consisting of 4 trials of aversive stimuli, delivered at a uniform 30-min inter-trial interval (ITI), is known to induce two behavioral modifications at 24 h, but not at 72 h: long-term sensitization (LTS), which manifests as enhancement of defensive reflexes, and long-term feeding suppression (LTFS; Shields-Johnson et al., 2013). Zhang and colleagues (2012) have previously developed a computational algorithm based on a biochemical model initiated by serotonin (5-HT) designed to improve memory in *Aplysia*. The model generated the optimal protocol that maximizes the interaction between PKA and ERK and effectively produces the highest concentration of an inducer, which regulates known transcription factors to ultimately enhance long-term memory (LTM). This enhanced learning protocol (ELP), which consists of 5 trials delivered at variable ITIs (10, 10, 5, 30 min), induces an augmented LTS lasting for 5 days after training (Zhang et al., 2012). Because the effects of ELP on LTFS remains unknown, this project aimed to determine if this method of training enhances LTM by prolonging not only LTS, but also LTFS. A defensive withdrawal reflex and feeding were measured before and 24, 38, 48, 62, and 72 h after ELP delivery in 15 trained animals (T), while 15 untrained animals (UT) were used as controls. The experimenter performing the behavioral tests was unaware of the training history of the animals. Data were analyzed using repeated measures ANOVA with two factors (training and time), followed by the Fisher LSD pairwise *post-hoc* comparisons to isolate the sources of significance. Results revealed the presence of LTS and LTFS at all 5 time points, indicating that ELP induced behavioral changes both lasting at least 3 days after training. Notably, ELP surpasses previously-used training protocols regarding the duration of the induced LTFS, including a protocol consisting of the SLP repeated for 4 consecutive days, which fails to induce LTFS at 72 h (Mac Leod et al., 2018). Overall, these findings indicate that the ELP effectively prolongs the co-expression of LTS and LTFS. LTFS is known to be mediated by nitric oxide instead of 5-HT (Farruggella et al., 2019). Therefore, the persistence of LTFS induced by the ELP, which was originally modeled using a signaling cascade different than nitric oxide, suggests that this protocol is widely applicable and not limited to 5-HT-dependent mechanisms.

Disclosures: A. Dauria: None. **M. Wainwright:** None. **R. Mozzachiodi:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.02

Topic: H.08. Learning and Memory

Title: Investigating the dopaminergic circuits in mediating electrical shock stress effects on the learning process in Drosophila

Authors: *T.-W. CHANG[1], H.-C. CHIANG[2];
[1]Inst. of Basic Med. Sci., [2]Natl. Cheng-Kung Univ., Tainan City, Taiwan

Abstract: In our daily life, stress causes various effects on animals' behaviors. Although accumulated evidence has demonstrated that short-term, high-intensity stress may damage learning and memory, the underlying neural and molecular mechanisms remain elusive, especially for the effect of acute pre-learning stress on animal learning. To address this question, we used the *Drosophila melanogaster* as a model to study the neural mechanism in mediating electrical shock (ES) stress-induced learning deficit. Flies received ES stress before classical aversive conditioning training; a training paradigm commonly uses odor as the conditioned stimulus and ES as the unconditioned stimulus (Jellies, 1981; Tully and Quinn, 1985; Davis, 1996). We found that the learning-prepended ES stress damaged the aversive learning but not appetitive learning, a training paradigm uses sugar as the unconditioned stimulus. This data suggests the specificity of ES stress's effect on aversive learning. Further, our study demonstrated that the ES stress-induced aversive learning deficit is not simply due to the saturation of the sensory system, as the sense of pain was still preserved. Synaptic output inhibition in different regions of the mushroom body (MB), the brain regions responsible for learning and memory, showed that inhibited α'β' neuron activity in MB eliminated the learning deficit caused by learning-prepended ES stress. Knocked-down the Drosophila dopamine D1-like receptor 2 (Dop1R2) in α'β' neurons prevented the learning impairment induced by the learning-prepended ES. To identify the responsible dopaminergic neurons, we screened several upstream dopaminergic neurons of MB, including the protocerebral posterior laterals neurons (PPL1 and PPL2) and protocerebral posterior medial neurons (PPM1/2, PPM3). We found that PPL2 and PPM neurons played an essential role in modulating learning behavior after ES stress. Together, our data reveal an unconventional neural mechanism responsible for the event-prepended stress effects on the learning process.

Disclosures: T. Chang: None. **H. Chiang:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.03

Topic: H.08. Learning and Memory

Title: A dedicated, non-olfactory mushroom body sub-circuit mediates the interaction between goal-directed actions and habit formation in Drosophila

Authors: *R. LYUTOVA-HRISTOVA, B. BREMBS;
Univ. of Regensburg, Univ. of Regensburg, Regensburg, Germany

Abstract: Goal-directed exploration of the environment allows an animal to learn about the relationships between stimuli and how the environment responds to its actions. In this goal-directed phase, animals can flexibly apply learned relationships to other contexts. However, flexibility usually implies a cost in time, together with higher cognitive and energetic costs. In contrast, the formation of habits ensures fast and efficient behaviors. The learning mechanisms that lead to flexible and efficient behaviors, respectively, interact with each other. During the early, goal-directed phase of such composite operant learning situations, the process that mediates learning about relations in the environment (world-learning) is known to inhibit the process that renders behaviors stereotypic and efficient (self-learning), presumably in order to prevent premature habit-formation. In humans, imbalance between flexible actions and habitual responses can be linked to neuropsychiatric disorders such as obsessive-compulsive disorder or addiction. We use the fruit fly *Drosophila* to study the interactions between world- and self-learning which mediate the transition mechanisms from goal-directed actions to habitual responses. In *Drosophila* goal-directed behavior inhibits habit formation at the level of the mushroom bodies (MB), such that inhibition of the MBs results in premature habit formation. We identified a single MB output neuron (MBON-β2β′2a) controlling the transition from goal-directed actions to habits. Together with the behavioral results, the anatomy of this neuron indicates that non-olfactory MB Kenyon cells of the β2 and β'2-lobes are involved in this transition. These neurons receive input via their dendrites in the little-studied lateral and dorsal accessory calyx regions of the MB.

Disclosures: R. Lyutova-Hristova: None. **B. Brembs:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.04

Topic: H.08. Learning and Memory

Support: HHMI
NIH grant NS113903

Title: Roles of Aplysia neurotrophin isoforms in neuronal morphogenesis, synaptogenesis, and several simple forms of learning

Authors: Q. YANG[1,2], S. KASSABOV[1,2], E. R. KANDEL[1,2], ***R. D. HAWKINS**[1,2];
[1]Columbia Univ., New York, NY; [2]NY State Psychiatric Inst., New York, NY

Abstract: The precise roles of BDNF and other neurotrophins are not well understood because the mammalian brain is very complex and has multiple neurotrophins, which are each processed into pro and mature isoforms that act on multiple receptors. The Aplysia siphon withdrawal

reflex is advantageous for these types of studies because it has a simple neural circuit and a single neurotrophin (ApNT) with an ApNT(+) mature isoform that acts through a single Trk receptor (ApTrk) and an ApNT(-) pro isoform that acts through a putative p75NTR receptor. We had previously found that overexpression of ApNT(+) mature but not ApNT(-) pro in sensory neurons increased both synaptic growth and long-term facilitation in culture (Kassabov et al., 2013) and that ApNT is important for behavioral sensitization in a semi-intact preparation (Yang et al., 2018). We have now used LNA modified gapmers that inhibit selectively each of the two ApNT isoforms and ApTrk to examine their respective roles in neuronal growth and morphology and in several simple forms of learning.
In culture knockdown of ApNT total or ApNT(-) pro led to a severe loss of branching in motor neurons, which preferentially express ApNT(-) pro, but not in sensory neurons which are enriched in ApNT(+) mature. On the other hand, knockdown of ApNT(+) mature or ApTrk had little effect on branching in sensory or motor neurons. In the semi-intact preparation repeated testing produced a gradual decrease or habituation (HAB) of siphon withdrawal that was blocked by knockdown of ApNT(+) mature or ApTrk but not ApNT(-) pro. Tail shock produced a gradual increase or sensitization (SEN) compared to test alone control, which may also be in part dishabituation (DIS). The SEN/DIS was reduced by knockdown of ApNT total, ApNT(+) mature, ApNT(-) pro, or ApTrk suggesting that all of those molecules may be involved. However, in contrast with HAB, knockdown of ApNT(+) mature was least effective. These results suggest that whereas HAB preferentially involves ApNT(+) mature, like synaptic growth and facilitation, SEN/DIS preferentially involves ApNT(-) pro, like motor neuron morphogenesis. Kockdown of ApNT total also revealed a transient inhibition (INHIB) after the tail shock. Previous studies found that inhibiting PKA also reduces HAB and SEN and reveals transient INHIB whereas inhibiting PKC reduces DIS (Antonov et al., 2021), and that PKA and PKC are both up and downstream of ApNT (Jin et al., 2018a,b). Collectively these results suggest that different isoforms of ApNT that may be part of different signaling pathways contribute preferentially to different aspects of morphogenesis, synaptogenesis and types of learning.

Disclosures: **Q. Yang:** None. **S. Kassabov:** None. **E.R. Kandel:** None. **R.D. Hawkins:** None.

Poster

323. Invertebrate Learning & Memory

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Program #/Poster #: 323.05

Topic: H.08. Learning and Memory

Support: NIH grant R01NS114491
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Title: Dynamic Brain-seq: Operation of the Aplysia genome at the single-neuron resolution across the entire CNS revealed convergent evolution of complex brains and plasticity mechanisms

Authors: *L. L. MOROZ[1], E. DABE[1], C. SUN[2], D. Y. ROMANOVA[4,5], P. G. POLIčAR[6], Y. BOBKOVA[3], G. WINTERS[1], C. BOSTWICK[1], K. MUKHERJEE[7], P. WILLIAMS[7], D. O. WU[2], E. EDSINGER[8], A. B. KOHN[7];
[1]Neurosci., [2]Computer Engin., Univ. of Florida, Gainesville, FL; [3]Whitney lab, Univ. of Florida, Saint Augustine, FL; [4]Ocean Genome Atlas Project, San Francisco, CA; [5]Inst. Higher Nervous Activity & Neurophysiol., Moscow, Russian Federation; [6]Univ. of Ljubljana, Ljubljana, Slovenia; [7]Whitney Lab., Univ. of Florida,, Saint Augustine, FL; [8]Univ. of Washington, Friday Harbor, WA

Abstract: For nearly 60 years, the sea slug *Aplysia californica* has been one of the most prominent paradigms for studying cellular bases of behavior, learning, and memory. However, little genomic information is available for this species. Here, using several sequencing approaches, we recovered 17 haploid chromosomes of the *Aplysia* genome (N50 ~ 49 Mb) and compare this organization both within mollusks and beyond. From the neuroscience perspective, *Aplysia* genome assembly and annotation were complemented by 405 deep transcriptomes from all major developmental stages and adult tissues, confirming the expression of ~19,000 protein-coding genes, including isoforms. Deep sequencing (to saturation points) allowed us to quantify the expression of all transcripts in both cytoplasm and nuclei of key individually identified neurons as they learn and remember (9,000-12,000 genes per cell). Thus, we identified >2000 novel molecular players associated with neuronal identity and plasticity as well as reconstructed their phylogeny and 3D structural organization. Next, we sequenced virtually all neurons composing the entire CNS under stationary and neuroplasticity tests (scRNA-seq; >90,000 single cells) - the approach that we named as Dynamic Brain-seq. Combined analyses of scRNA-seq, single-neuron methylomes and epitranscriptomes with machine learning algorithms led to an unbiassed genealogical classification of all neural cell types in the CNS, which was integrated with cell-specific markers' gene orthology groups identified using custom GIGANTIC pipeline. The resulting Neurosystematics (with predicted neuronal genealogies) is remarkably distinct from scRNA-seq data clustering described for *Drosophila*, *C. elegans*, and mammals. The most notable differences were related to dissimilar types of recruitments of transcription factors and the presence of hundreds of lineage-specific signal molecules. These cell-specific expression patterns suggest the existence of distinct barcoding rules for recruitments of master regulators underlying the establishment of major neuronal phenotypes across phyla. We propose that the observed differences in the genomic bases of neuronal identity and plasticity were results of the convergent evolution of complex brains in the lineages leading to molluscs, arthropods, and chordates started more 500 million yeas ago.

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Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.06

Topic: H.08. Learning and Memory

Support: Texas Comprehensive Research Fund

Title: The circadian rhythm modulates long-term suppression of feeding induced by aversive learning in Aplysia

Authors: *R. MUELLER, M. WAINWRIGHT, R. MOZZACHIODI;
Dept. of Life Sci., Texas A&M Univ. - Corpus Christi, Corpus Christi, TX

Abstract: Previous research in *Aplysia* revealed that the circadian rhythm regulates a form of learned fear called long-term sensitization (LTS), which occurs in response to training with repeated aversive stimuli (Kandel 2001; Fernandez et al. 2003). LTS manifests as an increase in the duration of the defensive tail-siphon withdrawal reflex (TSWR) measured 24 h after training. In animals entrained to a 12:12 h light-dark cycle (with zeitgeber time (ZT) 0 being lights-on and ZT 12 being lights-off), aversive training conducted at ZT 9 induces LTS, whereas training conducted at ZT 15 does not induce LTS (Fernandez et al. 2003). In addition to LTS, aversive training also induces a long-term suppression of feeding (LTFS) measured 24 h after training (Mac Leod et al. 2018). However, unlike LTS, it was unknown whether LTFS is also modulated by the circadian rhythm. Therefore, this project investigated whether nocturnal training affects the expression of LTFS similar to LTS by utilizing 4 groups of 15 animals: diurnal trained group (D-T), nocturnal trained group (N-T), diurnal untrained group (D-UT), and nocturnal untrained group (N-UT). Training consisted of 4 trials, each made of 10-s trains of electrical stimuli (500-ms pulses, 1 Hz, 60-mA AC) spaced 30 min apart. Training was delivered to the animal's body wall using a hand-held probe at either ZT 9 in the D-T group or ZT 15 in the N-T group (Fernandez et al. 2003; Mac Leod et al. 2018). Untrained animals were handled as the trained animals but did not receive any stimuli. The experimenter was kept unaware of the animals' training history. For each group, the duration of the TSWR and the number of bites in response to a food stimulus were initially measured (pre-tests) at ZT 4 in the diurnal groups and at ZT 11 in the nocturnal groups. Post-tests for all groups were conducted 18 h after the conclusion of training instead of the standard 24 h to allow for post-test behaviors to be measured during light hours given that they could not be reliably measured in the dark. Assessment of baseline feeding determined that pre-test bites in nocturnal groups were significantly higher compared to diurnal groups. Consequently, statistical analysis could not be conducted across the 4 groups but was instead performed by comparing D-T with D-UT groups and N-T with N-UT groups using Mann-Whitney U tests. Results revealed expected LTS and LTFS in D-T groups compared to D-UT groups. However, nocturnal training did not induce LTS or LTFS compared to the N-UT group at 18 h. These findings indicate that nocturnal training is not conducive for LTM formation in both defensive and appetitive behaviors, which demonstrates that the circadian rhythm modulates both LTS and LTFS in *Aplysia*.

Disclosures: R. Mueller: None. **M. Wainwright:** None. **R. Mozzachiodi:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.07

Topic: H.08. Learning and Memory

Title: A dopaminergic circuit mediates state-dependent flexibility of orienting behavior in response to food odor in Drosophila

Authors: *X. WEN, K. E. CONNORS, A. MARZORATTI, A. D. L. T. SCHUTZ, J. MOSTYN, D. HATTORI;
Physiol., Univ. of Texas Southwestern Med. Ctr., DALLAS, TX

Abstract: A fundamental function of the brain is to identify and orient animal's attention to salient stimuli in current environment. This orienting behavior allows animals to further investigate the precise meaning of the salient stimulus (Bromberg-Martin, et al, 2010). The salience of a stimulus is not fixed. For instance, the salience of reward-predictive cues varies based on whether the reward is currently needed. The neural mechanism of this flexibility in salience determination is so far poorly understood. We employ *D. melanogaster* as the model to investigate this neural mechanism at cellular level, since the central brain connectome and highly specific genetic drivers are available. To approximate the orienting behavior, we established an assay to capture a stereotyped behavioral transition of flies in response to salient odors, which we called "alerting". Using this assay, we investigated the effect of hunger on alerting behavior and found that starvation enhances alerting to food-predictive odors. We hypothesized that the flexibility in alerting is mediated by the mushroom body (MB), one of the two higher olfactory centers in *Drosophila* brain. In the MB, the MB output neurons (MBONs) receive olfactory information from principal neurons, Kenyon cells (KCs), and the activity of MBONs biases behaviors (Aso et al., 2014). Dopaminergic neurons (DANs) modulate KC-MBON synapses, contributing to the flexibility of behavioral outputs. We carried out optogenetic silencing as well as activation and identified a class of MBONs that is necessary and sufficient for alerting to food odors. We performed *in vivo* calcium imaging of the MBON while monitoring behaviors of the fly and found that MBON responds more strongly to food odor when flies exhibit alerting behavior than when flies do not alert. This association between MBON response and alerting behavior only emerges upon starvation. Moreover, we found that the association between MBON response and alerting behavior is eliminated when upstream DANs are silenced. These results suggest that MBON integrates the olfactory information from KCs with internal state variables encoded by DANs to mediate flexibility in alerting behavior to the same food odor. Given the similarity of the Drosophila MB to the mammalian striatum, our study may reveal a common role of dopamine circuits in computing salience of stimuli and generating flexibility of orienting behavior.

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Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.08

Topic: H.08. Learning and Memory

Title: Single Aversive Olfactory Conditioning forms Long-term Memory in Pre-trained Flies.

Authors: *C.-Q. HUANG, H. CHIANG;
The Inst. of Basic Med. Sci., Natl. Cheng Kung Univ. Col. of Med., Tainan City, Taiwan

Abstract: Long-term memory (LTM) is traditionally induced through repetitive training. In Drosophila classical olfactory aversive paradigm, space training is needed to form LTM that can last for a week. However, recent studies on the appetitive olfactory conditioning and aversive conditioning in fasting animal demonstrated that single training can form LTM, questioning the needs of multiple trainings for LTM. The current study used Drosophila melanogaster as the animal model system combined with behavioral approach and live-cell imagining to study underlying cellular and molecular mechanisms. We found, unlike space training, which employed multiple training trials with intervening rest periods to form the LTM, only two separate aversive olfactory conditioning with 8 days interval induce LTM formation. Further analysis showed that both the first and second training is able to induce new protein synthesis to support the LTM formation. Neurons in the mushroom body, main center of learning and memory for fruit flies, KCγ and KCαβ were responsible for retrieving the LTM, KCα'β' was needed during the consolidation phase and KCγ was involved in the acquisition phase. Altogether, our data implies that the first single training triggers protein-synthesis that is needed for the second training to form the LTM and further supports the hypothesis of cellular tagging in the formation of memory.

Disclosures: C. Huang: None. **H. Chiang:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.09

Topic: H.08. Learning and Memory

Title: Decoding the role of synaptically translated RNA binding proteins in associative memory using C. elegans

Authors: *A. HAYDEN[1], R. AREY[2];
[1]Neurosci., [2]Mol. and Cell. Biol., Baylor Col. of Med., Houston, TX

Abstract: RNA binding proteins are master regulators of protein synthesis that control the translation, localization, polyadenylation, and splicing of their target mRNAs. RNA binding proteins are critical for behaviors that require protein synthesis, such as memory formation and consolidation of long-term memories. Importantly, RNA binding protein dysfunction is being associated with a growing number of neurological disorders including Autism and Alzheimer's disease. Therefore, it is critical to understand how RNA binding proteins contribute to cognition. However, few RNA binding proteins have been tested for direct functional roles in learning and memory, and even fewer have been characterized as molecular learning and memory regulators. Recent studies show that mRNAs encoding RNA binding proteins are locally translated in synapses during memory training, suggesting that local translation of RNA binding proteins in synapses is a critical part of plasticity and behavior. Supporting this idea, our lab has previously utilized subcellular sequencing of neuronal somas and synapses in the nematode worm *Caenorhabditis elegans* to show that synaptic areas are significantly enriched for mRNA transcripts encoding RNA binding proteins. Therefore, we hypothesize that locally translated RNA binding proteins may regulate protein synthesis in synapses to regulate learning and memory. To this end, our lab is testing whether evolutionarily conserved RNA binding proteins are required for learning and memory using RNA interference targeting mRNAs encoding RNA binding proteins as well as assays measuring molecularly conserved positive olfactory associative memory in adult *C. elegans*. We have identified several RNA binding proteins that regulate associative memory, including novel memory regulators. To determine the precise molecular mechanisms by which these RNA binding proteins regulate memory, we are using eCLIP, or enhanced cross-linking immunoprecipitation. Our current focus is on the translational suppressor PUF-8, an ortholog of mammalian Pumilio 2 for which we have identified a novel memory suppressing role. Developing eCLIP will allow us to identify memory-regulating pathways that are downstream of not just PUF-8, but several RNA binding proteins to characterize the memory-regulating RNA binding protein network in synapses and provide novel insight into the role of synaptic transcripts in learning and memory. More specifically, this research addresses the mechanisms of RNA binding protein control of downstream mRNAs during learning and memory.

Disclosures: **A. Hayden:** None. **R. Arey:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.10

Topic: H.08. Learning and Memory

Title: Associative Conditioning of Opposing Locomotor Responses is Blocked by Downstream GABAergic and Cholinergic Activation in C. elegans

Authors: L. N. CHIANG[1], M. R. PRIBIC[2], A. D. BEALE[1], K. NELSON[2], A. B. TOKAR FALATAH[3], *J. ROSE[1];
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Abstract: Associative conditioning of opposing locomotor responses (blue light to activate forward locomotion paired with a mechanosensory vibration to produce backward locomotion) produces a novel pause (or "freeze") response to vibration. The current study sought to uncover signaling mechanisms of this conditioning. Although many sensory neurons in *C. elegans* use glutamate to signal to command interneurons, some of the blue-light sensitive neurons (including ASJ, AWB, AVG) are cholinergic. To test if cholinergic signaling may influence this conditioning, worms expressing channelrhodopsin in cholinergic neurons ($_p$*unc-17*::ChR2) were conditioned with a mechanosensory vibration (300 Hz) stimulus paired with a blue-light (~480 nm) stimulus to both drive conditioning and activate cholinergic neurons at the time of conditioning. Interestingly, with activation of cholinergic neurons during conditioning the expected increase in pause response to vibration was not seen at testing. Instead, $_p$*unc-17*::ChR2 animals showed a significant increase in omega turns as well as a transient decrease in forward locomotion time and distance measured at one minute following conditioning that was gone by 10 minutes. Some sensory neurons are putatively GABAergic or express GABA receptors including photosensory neurons PVT, AVG, ASJ and mechanosensory neurons ALM, AVM, PLM. Worms expressing channelrhodopsin in GABAergic neurons ($_p$*unc-47*::ChR2) also underwent conditioning and similarly did not show an increase in pause response to vibration following conditioning but did show a significant increase in forward locomotion time and distance measured at 10 minutes after conditioning. As these ChR2 constructs activate all *unc-17* or *unc-47* expressing neurons, respectively, it is also possible that this learning is affected by channelrhodopsin activation of downstream cholinergic and GABAergic signaling at the time of conditioning. Postsynaptic command interneurons and efferent motor neurons release GABA and/or acetylcholine. To test this, worms expressing channelrhodopsin in body wall muscle ($_p$*myo-3*::ChR2) were conditioned and again, no increase in pause response to vibration was seen. Further, all measures of locomotion were the same as matched naïve worms at both one minute and 10 minutes following conditioning. These results suggest that associative conditioning may rely on coordinated, circuit-wide signaling. Current studies employing activation of halorhodopsin in these neuron subtypes will help elucidate further the neural circuit signaling dynamics of this conditioning.

Disclosures: L.N. Chiang: None. **M.R. Pribic:** None. **A.D. Beale:** None. **K. Nelson:** None. **A.B. Tokar Falatah:** None. **J. Rose:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.11

Topic: H.08. Learning and Memory

Support: NSERC Discovery Grant GR002982

Title: Multiple neuropeptidergic signaling pathways underlying sensitization and dishabituation in C. elegans

Authors: *A. YU[1], C. RANKIN[2];
[1]Djavad Mowafaghian Ctr. for Brain Hlth., [2]Dept. of Psychology, The Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Nonassociative learning, the simplest form of learning, is thought to help animals selectively allocate attention or neural resources to promote survival. Sensitization and dishabituation are two forms of nonassociative plasticity: both sensitization and dishabituation facilitate the likelihood and/or magnitude of responses. Previous Work from *Aplysia* has shown that although sensitization and dishabituation both require the neuromodulator serotonin, they can be differentiated by the developmental stages they emerge, the electrophysiological profiles, and the downstream second messengers involved. Our previous work using *Caenorhabditis elegans* found a FLP-20/FRPR-3 neuropeptidergic pathway specifically mediating sensitization but not dishabituation of a nociceptive response.
In the present work, we demonstrated that sensitization and dishabituation are mediated by multiple neuropeptidergic signaling pathways. Using our previously established paradigms, we found that sensitization of response duration and response speed, two components of the nociceptive response, are regulated by different signaling molecules downstream of FLP-20/FRPR-3. We also found that sensitization and dishabituation of response speed are mediated by the same neuropeptides, however, they appeared to be recruited by different upstream signaling in these two forms of learning. We are currently performing a genetic screen to identify other molecules involved in these signaling pathways.
Taken together, these data show that sensitization and dishabituation can be mediated multiple distinct and shared, underlying molecular mechanisms, and that sensitization may not be a global, organism-wide, phenomenon, rather, sensitization of different aspects of behavior can be differentially regulated by multiple neuromodulatory pathways.

Disclosures: A. Yu: None. **C. Rankin:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.12

Topic: H.08. Learning and Memory

Title: Investigating memory trace formation of aversive olfactory conditioning

Authors: *C. WANG, H. CHIANG;
Natl. Cheng Kung Univ., Tainan, Taiwan

Abstract: Forgetting, fail to recall the previous learned experience, is the nature behavior to the animal that helps brain to reorganize the memory storage by removing outdated or unnecessary information. Although recent studies have shown that it is possible to retrieve previous un-recalled memory, the detailed cellular and molecular mechanism remains elusive. The current study showed that the memory of one-cycle of olfactory aversive conditioning is recallable after a second mild training even 8 days after first training. Previous learned experience during forgetting is not permanently removed but hided in the brain and is retrievable upon certain stimulation. Our data also showed that one-cycle of olfactory aversive conditioning induces protein synthesis to form memory trace in mushroom body α/β neurons and downstream MBON-α3. Further study showed that reduced PPL1-α3 neurons acitivty facilitate the forgotten memory retrieval. The formed tripartite synapse connection among MBNs, PPL1 neurons and MBONs regulates memory formation and forgetting. Furthermore, we found that passive forgetting and active forgetting possess similar way to decline the memory but with different decay rate. Together, our results indicate that the information of one-cycle of olfactory aversive conditioning forms a retrievable long lasting memory trace in the brain with a neural circuit similar to the formation of long-term memory.

Disclosures: **C. Wang:** None. **H. Chiang:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.13

Topic: H.08. Learning and Memory

Title: Airborne sound sensation and short-term memory in C. elegans

Authors: K. R. REUWER, Y. PAN, S. O. CHASE, K. R. WICKUM, A. K. PRATER, *J. R. RAINVILLE;
Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: A recent study demonstrated the novel discovery that Caenorhabditis elegans could sense airborne sound with a notably aversive response. The role of airborne sound in the context of memory and learning of C. elegans has yet to be established, providing an opportunity for further investigation. We hypothesized that sound exposure can affect associative memory formation between a chemoattractant and food in C. elegans. To assess this, we first trained C. elegans to associate Escherichia coli (food source) with either the chemoattractant 2-butanone or the attractant plus sound stimulus (2700 Hz, 85 dB). Then, we performed a novel phono-chemotaxis association assay in which C. elegans could move toward or away from 2-butanone and/or a sound stimulus to evaluate C. elegans' ability to form associative memories in the presence of sound, and to evaluate whether sound itself can be associated with food as an attractant, despite its reported aversive effects. Our initial results indicate the sound exposure during the chemotactic learning reduces the number of nematodes that form an associative

memory between food and 2-butanone, but do not support formation of a positive association between sound and food. These results will be useful in determining the role of sound sensation in learning and memory in C. elegans.

Disclosures: K.R. Reuwer: None. **Y. Pan:** None. **S.O. Chase:** None. **K.R. Wickum:** None. **A.K. Prater:** None. **J.R. Rainville:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

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Program #/Poster #: 323.14

Topic: H.08. Learning and Memory

Support: NIH Grant NS083690
NIH Grant NS113903

Title: Long-term facilitation produced by 5HT at Aplysia sensorimotor synapses builds upon ApNT signaling during intermediate-term facilitation

Authors: *I. JIN[1], E. R. KANDEL[2], R. D. HAWKINS[1];
[1]Systems Neurosci., New York State Psychiatric Inst., New York, NY; [2]Jerome L. Greene Sci. Ctr., Columbia Univ., New York, NY

Abstract: The transition from short-term facilitation (STF) to intermediate-term facilitation (ITF) produced by 5HT (10 min 20 μM) at *Aplysia* sensory neuron (SN) to motor neuron (MN) synapses in isolated culture is mediated by sequential recruitment of positive feedback loops involving the autocrine, anterograde, and retrograde actions of ApNT (an *Aplysia* ortholog of mammalian BDNF) acting on ApTrk (an *Aplysia* ortholog of mammalian TrkB) receptors and the anterograde action of glutamate on mGluR5 receptors (Jin et al., 2018a &2018b). These positive feedback loops act as driving forces with self-amplifying properties to help overcome thresholds during the transition to the next stages of memory and are essential for the consolidation of synaptic memory. We have begun to examine the contribution of these positive feedback loops during the transition from ITF to LTF with the standard induction protocol of 5 x 5min 5HT (Ghirardi et al., 1992). We find that injection into the sensory neuron of an ApTrk antisense oligonucleotide reduced LTF compared with sense control ($t[11] = 2.40$, $p<0.05$), and that overexpression of ApNT in the sensory neuron strengthened the SN-MN EPSP compared with controls ($F[1,16] = 13.40$, $p<0.01$). However, we found that expression of an ApTrk dominant-negative in the motor neuron also reduced LTF compared with control ($t[24] = 2.66$, $p<0.05$), and overexpression of ApNT in the motor neuron strengthened the SN-MN EPSP compared with controls ($F[1,36] = 15.21$, $p<0.001$). Collectively, these results suggested that ApNT and ApTrk signaling in both the SN and the MN are critical for long-term facilitation and that ApNT may play either a presynaptic autocrine (SN to SN), anterograde (SN to MN), retrograde (MN to SN), or postsynaptic autocrine (MN to MN) role at synapses between

individual neurons during the transition from ITF to LTF, similar to the transition from STF to ITF. One possibility is that the self-perpetuating ApNT positive feedback loop in the presynaptic neuron
during the transition to ITF increases the levels of PKA and MAPK (Jin et al., 2018b), which may then translocate into the nucleus during the transition to LTF to activate transcription via modulation of bZIP transcription factors such as CREB1, CREB2, and C/EBP (Martin et al., 1997). More generally, these results support the idea that ApNT signaling mechanisms during the induction of LTF are similar to, and build sequentially upon, those during the induction of ITF (Jin et al., 2019).

Disclosures: I. Jin: None. **E.R. Kandel:** None. **R.D. Hawkins:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.15

Topic: H.08. Learning and Memory

Support: NIH 1R01NS121220

Title: Distributed organization of the memories for sensitization and habituation in the brain of the marine mollusk Tritonia diomedea

Authors: *V. K. MISTRY[1], M. A. BOERSMA[2], E. S. HILL[1], W. N. FROST[1];
[1]Stanson-Toshok Ctr. for Brain Function and Repair, Rosalind Franklin Univ. of Med. and Sci., North Chicago, IL; [2]LFC-RFU Summer Res. Scholars Program, Lake Forest Col., Lake Forest, IL

Abstract: In response to an aversive skin stimulus, such as contact with a predatory sea star, the marine mollusk Tritonia diomedea jumps off the substrate and executes an escape swim consisting of an alternating series of ventral and dorsal whole-body flexions. Repeating this stimulus 10 times at 2 minute intertrial intervals produces an immediately maximal sensitization, characterized by a faster swim onset latency on the second trial, together with a progressively accumulating habituation of the number of cycles per swim that reaches its maximum on the 10th trial. The very different time courses and behavioral features affected by these two forms of learning indicate that they are at least in part encoded by different processes and loci in the swim network. To further characterize the behavioral signatures of these two forms of learning, in the present study we added a third measure, the height the animal reaches as it jumps off the substrate at the start of each swim, which may reflect the propulsive force of the swim flexions. The response profiles for jump height and swim onset latency, when scaled similarly with respect to response vigor, nearly exactly matched one another across all ten trials, suggesting they are driven by a common network mechanism. Prior studies have shown that one class of central pattern generator (CPG) neuron, the serotonergic DSIs, drives sensitization learning.

Modulation by the DSIs induces swim onset latency quickening and produces a rapid enhancement of the synapses made by CPG neurons C2 and VSI-B onto their target neurons within and downstream from the CPG, including those from VSI-B onto the pedal ganglion VFN efferent neurons that drive the jump height. Furthermore, large-scale optical recording studies have shown that applying the DSI transmitter acts to expand the number of pedal ganglion neurons participating in the motor program, mimicking what happens in nerve-evoked sensitization. Here we used optical recordings of pedal ganglion neural activity to find that nerve-evoked sensitization is also accompanied by an increased burst strength of individual VFN neurons across multiple cycles of the sensitized swim motor program. Taken together, these findings suggest that a common mechanism, modulation by the DSI neurons, drives plasticity at different circuit loci within the CPG to produce sensitization of swim onset latency and jump height, and that the plasticity encoding habituation is located elsewhere. Future work will focus on how these different sites of plasticity share space in a small neural network, including the degree to which doing so may lead to interference regarding the information each encodes.

Disclosures: V.K. Mistry: None. **M.A. Boersma:** None. **E.S. Hill:** None. **W.N. Frost:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.16

Topic: H.08. Learning and Memory

Support: Advancing Researcher Experience/University of Tsukuba

Title: The molecular and neural regulation of ultraviolet light phototaxis and its food-associated learning behavioral plasticity in C. elegans

Authors: *K. OZAWA[1], Y. SHINKAI[4], K. KAKO[2,3], A. FUKAMIZU[3], M. DOI[4];
[1]Col. of Agro-Biological Resource Sciences, Sch. of Life and Envrn. Sci., [2]Fac. of Life and Envrn. Sci., [3]Life Sci. Ctr. for Survival Dynamics, Tsukuba Advanced Res. Alliance, Univ. of Tsukuba, Tsukuba, Japan; [4]Biomed. Res. Inst., Natl. Inst. of Advanced Industrial Sci. and Technol. (AIST), Tsukuba, Japan

Abstract: *Caenorhabditis elegans*, which has only 302 neurons, is widely used as a good invertebrate model for the molecular study of behavioral plasticity. Despite the simple neural networks, *C. elegans* represents a broad behavioral plasticity in a lot of learning paradigms. Ultraviolet light (UV) is quite toxic to all the animals and evoke the avoidance behavior of UV. *Caenorhabditis elegans* senses UV and is known to avoid UV by using four sensory neurons (ASJ, ASK, AWB, ASH). However, it is not clear what signaling molecules act for UV avoidance in the neuronal pathway constituted of four sensory neurons. In addition, it is not clear whether this harmful environmental signal can be associated with other benefit signals such as food. In this study, by using newly developed assay system in which worms were exposed to UV

(350 nm, 5μW/mm2) with food and the avoidance behavior of UV after conditioning was examined, we found that conditioning with UV and food significantly reduced excessive avoidance of UV. This means that *C. elegans* can associate UV and food to change behavioral strategy against harmful UV signal. This is the first indication that *C. elegans* shows associate learning with UV and food. Using our assay system, we also found that glutamate is used as a transmitter for both the UV avoidance and UV associative learning, whereas different sets of glutamate receptors seemed to act for UV avoidance and UV associative learning. To further elucidate the mechanism underlying UV associative learning, we characterized the function of four sensory neurons important for phototaxis. Among these sensory neurons, ASH sensory neuron was indispensable for UV associative learning, since worms that lost ASH sensory neuron could not change the avoidance behavior after the conditioning with UV and food. These results suggest that another neuronal control system is operating that rewrites instinctive avoidance behavior from harmful UV light.

Disclosures: K. Ozawa: None. **Y. Shinkai:** None. **K. Kako:** None. **A. Fukamizu:** None. **M. Doi:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.17

Topic: H.08. Learning and Memory

Support: NSERC Grant GR002982

Title: Evidence that habituation at different interstimulus intervals involves dissociable processes

Authors: *N. KOKAN[1], C. H. RANKIN[2];
[1]Univ. of British Columbia Grad. Program In Neurosci., Univ. of British Columbia Grad. Program In Neurosci., Vancouver, BC, Canada; [2]Psychology, Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Habituation is a simple form of learning that occurs when an organism attenuates its response to repeated stimuli that do not predict the arrival of appetitive or aversive stimuli. A property of habituation is that the interstimulus interval (ISI), the time between each stimulus presentation, greatly impacts both the rate and depth of habituation. This research seeks a more detailed understanding of how ISI influences habituation and to discover genes that have an ISI specific effect on habituation. Animals habituate more slowly and to a lesser extent at long ISIs, but the response decrement will persist for longer than when the decrement was induced by short ISIs. This means that at longer ISIs habituation learning is slower, but memory of this learning is more stable. This suggests that habituation at long and short ISIs may utilize dissociable processes. To further investigate this possibility, I used our Multi-Worm Tracker to monitor the

behavior of dozens of *Caenorhabditis elegans* on plates that receive mechanical taps with a 10s, 20s, 30s, 60s and 90s ISI. Our Multi-Worm Tracker software generated detailed morphology and behavioral data for each worm on the plate. I focused on two behavioral response metrics, reverse probability which is the proportion of animals that reversed in response to the tap stimulus and reversal duration which is how long these stimulus evoked reversals lasted. Most animals will reverse in response to the first tap, but as more stimuli are delivered fewer and fewer animals will reverse, and those that do will reverse for a shorter time. As expected, my results indicate that both reversal probability and reversal duration habituated more slowly with longer ISIs. Interestingly, the difference in the habituation depth of reversal probability at 10s and 90s ISI was much greater than for reverse duration. This suggests that ISI may not impact different habituation metrics equally. Furthermore, habituation at 20s and 30s ISI was remarkably similar, which may be indicative of a shift between habituation processes that are being recruited at shorter or longer ISIs. To further investigate the possibility of ISI specific habituation mechanisms, I have shown that mutations in the glutamate gated chloride channel alpha subunit *avr-14* result in altered habituation of reverse duration, but only at long ISI. This was demonstrated in multiple *avr-14* mutant strains. This provides genetic evidence for the presence of distinct habituation processes at different ISI. Next, I will investigate where *avr-14* is functioning during habituation to identify where in the neural circuit this ISI-specific habituation process may be occurring.

Disclosures: **N. Kokan:** None. **C.H. Rankin:** None.

Poster

323. Invertebrate Learning & Memory

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Program #/Poster #: 323.18

Topic: H.08. Learning and Memory

Support: NIH R35GM124883

Title: Untanglingthe web of behaviors used in spider orb-weaving.

Authors: *A. GORDUS;
Biol., Johns Hopkins Univ., Baltimore, MD

Abstract: Many innate behaviors are the result of multiple sensorimotor programs that are dynamically coordinated to produce higher-order behaviors such as courtship or architecture. Extended phenotypes such as architecture are especially useful for ethological study because the structure itself is a physical record of behavioral intent. A particularly elegant and easily quantifiable structure is the spider orb-web. The geometric symmetry and regularity of these webs have long generated interest in their behavioral origin. However, quantitative analyses of this behavior have been sparse due to the difficulty of recording web-making in real-time. To address this, we have developed a novel assay enabling high-resolution tracking of limb

movements and web structure produced by the hackled orb-weaver Uloborus diversus. With a brain the size of a fly's, the spider U. diversus offers a tractable organism for the study of complex behaviors. Using machine vision algorithms for limb tracking, and unsupervised behavioral clustering methods, we have developed an atlas of stereotyped movements used in orb-web construction. The rules for how these movements are coordinated change during different phases of web construction, and we find that we can predict web-building stages based on these rules alone. Thus, the physical structures of the web explicitly represent distinct phases of behavior. In addition to our behavioral efforts, we are also developing biological assays to investigate how this elegant behavior is encoded in the spider's brain.

Disclosures: A. Gordus: None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.19

Topic: H.08. Learning and Memory

Support: Glenn Foundation for Medical Research and AFAR Grants for Junior Faculty

Title: Investigating neuropeptide signals that slow cognitive aging in C. elegans

Authors: *E. J. LEPTICH[1], R. N. AREY[2];
[1]Neurosci., [2]Mol. and Cell. Biol., Baylor Col. of Med., Houston, TX

Abstract: The average life expectancy has nearly doubled in the last century, leading to increased rates of cognitive decline in aged populations. Therefore, it is critical to identify mechanisms that restore memory function with age. One such mechanism is activation of cAMP response element-binding protein (CREB), which is a highly conserved transcriptional regulator of long-term associative memory (LTAM). Across organisms, increased CREB activity is associated with enhanced memory with age, but the mechanisms underlying this phenomenon are not well-understood. This includes the model system *C. elegans*, where positive olfactory associative memory behavior declines with age and is correlated with the decline of CREB levels and activity. Our recent research in *C. elegans* suggests that enhanced neuropeptide signaling from a single sensory neuron (the AWC) promotes CREB-dependent LTAM and slows age-related deficits in olfactory associative memory. Specifically, transgenic *C. elegans* animals that exhibit enhanced neuropeptide release specifically from the AWC maintain LTAM ability at Day 5 of adulthood, an age at which wild-type animals are unable to form LTAM. These findings suggest that increasing neuropeptidergic transmission from this neuron is sufficient to slow cognitive aging. Because increased CREB activity is linked to LTAM performance, we hypothesize that neuropeptide release from the AWC sensory neuron regulates CREB activity required for LTAM behavior and increases cognitive healthspan in *C. elegans*. However, these memory-promoting neuropeptide(s) and whether their mechanism of action regulates CREB

activity necessary for LTAM is unknown. We have curated a list of 24 candidate peptides informed by two previously generated genomics datasets that identify either AWC-expressed peptides or genes upregulated by LTAM-training. Using this list, we are performing an RNAi-based screen of these candidates in neuronal-RNAi sensitized animals to assess their role in age-related memory decline. We have identified peptide signals that promote associative learning and memory in the worm and, in ongoing studies, we are examining other peptides of interest and investigating the regulation of CREB activity by memory-promoting peptides. Overall, this research will provide insight into the molecular underpinnings of age-related cognitive decline, potentially leading to novel therapeutic targets for cognitive impairment in higher organisms.

Disclosures: E.J. Leptich: None. **R.N. Arey:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.20

Topic: H.08. Learning and Memory

Title: Novel Object Recognition in Octopus maya

Authors: *J. F. VERGARA-OVALLE[1], H. SANCHEZ-CASTILLO[2];
[1]UNAM, UNAM, Cd de Mexico, Mexico; [2]Univ. Nacional Autonoma De Mexico. Fac Psicologia, Univ. Nacional Autonoma De Mexico. Fac Psicologia, Mexico City, Mexico

Abstract: The Novel Object Recognition task (NOR) is widely used for the study of memory in vertebrates and has been proposed as a solid candidate for evaluating memory in different taxonomic groups, allowing similar and comparable evaluations between them. Although, in cephalopods, several research reports could indicate that they recognize objects in their environment, so far, it has not been evaluated as an experimental paradigm, which allows evaluating different phases of memory. In this study, we show that *Octopus maya* can differentiate between a new object and a known one, with high accuracy. We observed that to achieve object recognition, octopuses use vision and touch exploration in new objects, while familiar objects only need to be explored in a visual way. To our knowledge, this is the first time showing an invertebrate performing the NOR task, in a similar way to how it is performed in vertebrates. These results raise the opportunity to use a simple, fast and widely used task in other taxonomic groups, to assess memory in octopuses.

Disclosures: J.F. Vergara-Ovalle: None. **H. Sanchez-Castillo:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.21

Topic: H.08. Learning and Memory

Support: DGAPA IN208722

Title: Effects of bright light exposure in the stress response of octopus.

Authors: *D. A. GONZÁLEZ-NAVARRETE[1], F. VERGARA-OVALLE[2], P. GARCÍA-ANDALUZ[2], F. AYALA-GUERRERO[3], D. B. PAZ-TREJO[4], H. SANCHEZ-CASTILLO[5]; [1]Univ. Nacional Autónoma De México, Univ. Nacional Autónoma De México, Ciudad de México, Mexico; [2]Facultad de Psicología, Lab. de Neuropsicofarmacología, [3]Facultad de Psicología, Lab. de Neurociencias, Univ. Nacional Autónoma de México, México, Mexico; [4]Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [5]Psychobiology and Neurosciences, Univ. Nacional Autonoma De Mexico. Fac Psicologia, Mexico City, Mexico

Abstract: Frequently in the environment, the organisms are exposed to stressful stimuli that require behavioral and physiological adaptation. In this way, the stress response becomes extremely important because it's activation allows individuals to increase the probability of survival. Is well known that this response is present in vertebrate organisms, however, the physiological, behavioral, neuroendocrine, and immune system evidences indicate that the mechanisms underlying the stress response are also present in invertebrate organisms. However, they're a lack of studies where the stress response it will be evaluated in cephalopods, such as octopus. In order to study the stress response in octopus, we need to define an appropriate stimulus that triggers the stress response. To get this, we propose the white bright light as environmental stressor. The main goal of this study was to evaluate the aversive light effects in behavior's octopus evaluated with the Place Preference Test (PPT). Three individuals of *Octopus maya* were used and maintained in tanks with a closed circulation seawater system in a 12:12 LD cycle. These individuals were tested in PPT in a tank (60x40x30cm), divided in three equal sized chambers by black acrylic walls with openings that allowing access into each chamber. We evaluated the preference or aversion to the bright light (130 lx). The test was made during three consecutive days and recorded during the light hours (08:00-20:00). We evaluated of latency and frequency of octopus's behavior. The obtained results showed that *O. maya* has aversion to the bright light chamber. The subjects showed minimal resting behaviors and an increase of locomotion behaviors (climbing, jet propulsion, etc). Additionally, we observe the presence of inking and a decrease of grooming in this chamber. Those results suggest that the use of the white bright light as an environmental stressor triggers the stress response and provoke a change in the behavior of the octopus. This is good precedent for future research in this field and encourage to put more attention to different environmental stimuli.

Disclosures: D.A. González-Navarrete: None. **F. Vergara-Ovalle:** None. **P. García-Andaluz:** None. **F. Ayala-Guerrero:** None. **D.B. Paz-Trejo:** None. **H. Sanchez-Castillo:** None.

Poster

323. Invertebrate Learning & Memory

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Program #/Poster #: 323.22

Topic: H.08. Learning and Memory

Support: NIH NS019895
NIH NS102490

Title: Quantitative comparison of dynamics of ERK activation after three different serotonin protocols that induce long-term facilitation in Aplysia

Authors: *Y. ZHANG, R.-Y. LIU, L. J. CLEARY, J. H. BYRNE;
McGovern Med. Sch. of UTHSC At Houston, Houston, TX

Abstract: The induction and stabilization of memories are dependent, at least, in part, on the dynamics of the underlying molecular processes, but details of those dynamics are poorly understood. Long-term synaptic facilitation (LTF) of the *Aplysia* sensorimotor synapse is a well-established model system for studying the molecular mechanisms of long-term memory (LTM). Empirical studies have identified three spaced serotonin (5-HT) protocols can induce LTF. The first, termed the Standard protocol consists of five, 5-min pulses of 5-HT with regular interstimulus intervals (ISIs) of 20 min (Montarolo et al. 1986). A second, termed the Enhanced protocol consists of 5 pulses of 5-HT with computationally designed irregular ISIs. This protocol induces stronger and longer-lasting LTF than the Standard protocol (Zhang et al. 2011). The third, termed the Two-pulse protocol involves just two 5-min pulses of 5-HT with an ISI of 45 min (Phillips et al. 2013). These three protocols have substantial differences in the total amount of 5-HT application and the total duration of training. In this study, we examined the similarities and differences in the molecular mechanisms of the three protocols with a focus on protein kinase A (PKA), mitogen-activated protein kinase (MAPK), neurotrophin/TrkB receptor and transforming growth factor-β (TGF-β) pathways up to 24 h after treatment with these different protocols. Immunofluorescence analysis revealed a complex dynamical pattern of the MAPK isoform ERK activation. All three protocols led to an immediate increase in ERK activation, which decayed within 5 h post treatment. A second wave of increased ERK activation was detected at 18 h post treatment (Two-pulse: 40.7 ± 6.9%, n = 8; Standard: 22.9 ± 7.3%, n = 8; Enhanced: 23.6± 6.8%, n = 7). Statistical analyses (Wilcoxon SRT, Bonferroni correction) revealed that the increases from all three protocols were significant compared to their Veh controls at 18 h (Two-pulse: Z = 2.527, P = 0.048; Standard: Z = 2.521, P = 0.048; Enhanced: Z = 2.366, P = 0.032). This late increase in pERK was blocked by the inhibitor of PKA (RpcAMP), antagonists of TrkB receptor (TrkB Fc chimera) and TGF-β (TGF-β Fc chimera). These results suggest that the complex interactions among kinase pathways and growth factor cascades contribute to the late increase of ERK activity after different LTF-inducing protocols. Interestingly, ERK activity returned to basal levels 24 h after the Standard or Two-pulse protocol, but remained elevated 24 h after the Enhanced protocol. This finding may help explain, in part why the Enhanced protocol is superior to the Standard protocol in inducing long-lasting LTF (Zhang et al. 2011).

Disclosures: **Y. Zhang:** None. **R. Liu:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.23

Topic: H.08. Learning and Memory

Support: NIH Grant NS019895
NIH Grant NS102490

Title: Deficient RSK activation in a model of Coffin-Lowry syndrome is rescued by computational modeling-predicted dual drugs that activate PKA and inhibit p38 MAPK

Authors: *R.-Y. LIU[1], Y. ZHANG[2], P. D. SMOLEN[1], L. J. CLEARY[1], J. H. BYRNE[1]; [1]Dept. of Neurobio. and Anat., [2]McGovern Med. Sch. at the Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX

Abstract: Coffin-Lowry Syndrome (CLS) is a cognitive disorder caused by mutations in the *rsk2* gene. A cellular model of CLS with impaired long-term synaptic facilitation (LTF) can be produced by pharmacological inhibition of p90 ribosomal S6 kinase (RSK) (Liu et al., 2020). The present study used combined computational and pharmacological approaches to examine strategies to rescue defective LTF in this CLS model. Activation of multiple signaling cascades such as the protein kinase A (PKA) and extracellular signal-regulated kinase (ERK) pathways is necessary to induce LTF. Previously, combining an ERK activator, NSC295642 (NSC), and a PKA activator, rolipram (R), enhanced normal LTF to a greater extent than did either drug alone, but the combined effect was only additive (Liu, et al., 2017). However, in the CLS model, the combined drugs exerted synergistic effects on RSK activation and LTF, restoring both to normal (Liu et al, SFN 2021 abstract). Computational modeling indicated that the extent of this synergism appeared to depend on another MAPK isoform, p38 MAPK. To test this model prediction, immunostaining for phosphorylated RSK (pRSK) was performed 1 h after standard serotonin (5-HT) treatment in the presence of the p38 MAPK inhibitor SB203580 (SB). SB significantly enhanced activation of RSK (One-way ANOVA, $F_{3.21} = 18.318$, $p < 0.001$, post-hoc comparisons: SB+5-HT vs. Veh, $q = 7.614$, $p < 0.001$; SB+5-HT vs. 5-HT, $q = 3.212$, $p = 0.034$; SB+5-HT vs. SB, $q = 9.466$, $p < 0.001$), confirming that p38 MAPK activation limits the extent of 5-HT-induced pRSK. Next, we examined the model prediction that co-application of a PKA activator and a p38 MAPK inhibitor would synergistically enhance RSK phosphorylation. One-way ANOVA indicated significant overall differences among groups ($F_{3.23} = 22.13$, $p < 0.001$). Post hoc comparisons revealed the combined drugs induced a significant increase in pRSK, compared to the other 3 groups (SB+R+5-HT vs. 5-HT, $q = 10.93$, $p < 0.001$; SB+R+5-HT vs. SB+5-HT, $q = 8.77$, $p < 0.001$; SB+R+5-HT vs. R+5-HT, $q = 7.89$, $p < 0.001$). Importantly, the interaction between rolipram and SB on pRSK was significant (Two-way RM ANOVA, $F_{1,7} = 13.91$, $p = 0.007$), indicating SB and rolipram synergistically increased 5-HT-induced pRSK. Our results indicate that combined pharmacological activation of PKA and inhibition of p38 MAPK may be a potential approach to rescue impairments in synaptic plasticity associated with

CLS. More generally, this study highlights the utility of using computational modeling to identify candidate drug combinations to treat deficits in learning and memory associated with cognitive disorders.

Disclosures: R. Liu: None. **Y. Zhang:** None. **P.D. Smolen:** None. **L.J. Cleary:** None. **J.H. Byrne:** None.

Poster

323. Invertebrate Learning & Memory

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Program #/Poster #: 323.24

Topic: H.08. Learning and Memory

Support: NIH grant R01 NS101356

Title: Increases in the excitability of individual neurons contribute to a phase shift of neural population activity induced by operant conditioning.

Authors: *Y. MOMOHARA, R. M. COSTA, J. H. BYRNE;
Univ. of Texas Hlth. Sci. Center, Houston, Univ. of Texas Hlth. Sci. Center, Houston, Houston, TX

Abstract: Revealing the mechanisms of plasticity that are responsible for learning is key for understanding brain function. The neural circuit underlying *Aplysia* feeding is well-characterized and can be modified by operant conditioning (OC). Feeding responses consist of two types of buccal motor patterns (BMPs), which can be distinguished by the timing of activity associated with radula closure relative to the two primary phases of BMPs, protraction and retraction. In a recent study (Costa et al., 2022), we used non-negative matrix factorization to identify a low-dimensional signature of OC in the activity of the neuronal population assessed with voltage-sensitive dye recordings of ~100 simultaneously recorded neurons. This signature consisted of a temporal shift in the recruitment of a subpopulation of neurons active during the retraction phase. Plasticity in the biophysical properties of these neurons may underlie the shift and, consequently, constitute a critical piece of the distributed representation of the operant memory. Here, we tested this hypothesis by analyzing the neuronal properties of retraction neurons B3, B6, B9, and B64. We found that the burst threshold of B64, a retraction generator neuron, was strongly reduced after OC (*Paired t-test*, $p = 0.044$, n = 5). In addition, OC increased the excitability of retraction motor neurons B6 and B9, but not B3 (*Paired t-test*, B6: $p = 0.002$, n = 12; B9: $p = 0.035$, n = 7; B3: $p = 0.155$, n = 11). The B64-B3 and B64-B6 electrical couplings were both unchanged after OC (*Paired t-test*: B64-B3: $p = 0.12$, n = 5; B64-B6: $p = 0.129$, n = 5). These data suggest that OC both accelerates initiation of the retraction phase and modulates a specific subset of motor neurons. Together, these changes likely contribute to the shift observed at the population level. The set of changes underlying the low-dimensional OC signature is therefore distributed among several but, importantly, not all sites that participate in retraction.

Disclosures: **Y. Momohara:** None. **R.M. Costa:** None. **J.H. Byrne:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

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Program #/Poster #: 323.25

Topic: H.08. Learning and Memory

Support: R01 NS092716
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Title: Camkii and pkczeta are required for induction and maintenance of heterosynaptic long-term potentiation of non-nociceptive afferents in hirudo verbana

Authors: *A. D. FRANZEN, R. T. PAULSEN, E. J. KABEISEMAN, B. D. BURRELL; Univ. of South Dakota, Vermillion, SD

Abstract: CamKII and PKCzeta Are Required for Induction and Maintenance of Heterosynaptic Long-Term Potentiation of Non-Nociceptive Afferents in *Hirudo verbana*
Avery D. Franzen; Riley T. Paulsen; Emily J. Kabeiseman; Brian D. Burrell
Pain is a powerful motivator for adaptation in the natural world. One common adaptation is for nociceptive stimuli to elicit sensitization to future stimuli. This sensitization can affect not only nociceptive afferent pathways, but non-nociceptive afferent pathways as well (hyperalgesia and allodynia). Our lab has shown that high frequency stimulation (HFS) of nociceptors not only elicits homosynaptic long-term potentiation (LTP) in nociceptive synapses in *Hirudo verbana*, the medicinal leech, but also produces heterosynaptic LTP of non-nociceptive synapses(Yuan and Burrell 2019). This heterosynaptic potentiation is both endocannabinoid-mediated and involves decreased GABAergic tonic inhibition(Paulsen and Burrell 2022). The present study investigates the intracellular kinases involved in this heterosynaptic LTP. First, Hirudo sequences for calmodulin-dependent kinase II (CamKII) and protein kinase C zeta (PKCζ) were identified by BLASTing the human amino acid sequences against *Hirudo* transcriptomes using a transcriptome shotgun assembly. These sequences exhibited adequate homology to human, mouse, and *Aplysia* to provide a reasonable chance that autocamtide-II related inhibitory peptide (AIP) and zeta inhibitory peptide (ZIP) would inhibit their targets, CamKII and PKCζ respectively. Using dual intracellular recording techniques, we recorded changes in the EPSP amplitude between the presynaptic pressure (P) cell and the postsynaptic anterior pagoda (AP) cell prior to nociceptive (N) cell HFS and again after a one-hour consolidation phase. Drugs were given during the HFS for LTP induction, for fifteen minutes following immediately after HFS for LTP maintenance, or for the last fifteen minutes of the consolidation phase for delayed LTP maintenance. When applied during the HFS, AIP prevented LTP while ZIP did not. When applied after HFS during the maintenance phases, both AIP and ZIP disrupted LTP. Interestingly, ZIP also disrupted LTP when applied 45 mins after HFS, indicating a continual role of PKCζ in maintenance. These findings indicate that endocannabinoid-mediated

heterosynaptic LTP of non-nociceptive afferents requires CamKII for induction, CamKII and PKCζ for maintenance, and that PKCζ is required for the duration of LTP maintenance. This data will aid our understanding of the interconnectivity of synaptic plasticity in the nociceptive and non-nociceptive afferents in chronic pain states.

Disclosures: A.D. Franzen: None. **R.T. Paulsen:** None. **E.J. Kabeiseman:** None. **B.D. Burrell:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.26

Topic: H.08. Learning and Memory

Support: NIH Grant R01 NS092716
NIDA Grant R25 DA033674

Title: Two Forms of Pain-Related Associative Learning in Hirudo: Operant Conditioning and Conditioned Place Avoidance

Authors: C. THOMPSON[1], J. DOHN[1], A. FRANZEN[1], ***B. BURRELL**[1,2];
[1]Basic Biomed. Sci., [2]Ctr. for Brain and Behavior Res., Univ. of South Dakota, Vermillion, SD

Abstract: Pain is defined as “an unpleasant sensory and emotional experience” that indicates damage or potential damage to the body (IASP). There is considerable adaptive value in animals having appropriate responses to a painful stimulus over both short and long time periods. Part of this adaptive process involves identifying and learning to avoid painful stimuli in the future. Using comparative approaches to understand conserved behavioral and physiological mechanisms mediating learning and memory related to pain may have applications in understanding the basic biology of pain throughout the animal kingdom. Here we describe two forms of associative learning to painful stimuli in the medicinal leech, *Hirudo verbana*. The first is a form of operant conditioning in which a series of mechanical nociceptive stimuli (needle pokes) are delivered to the *Hirudo* posterior sucker. Initially animals respond with a withdrawal reflex (local or whole body shortening), but with repeated pokes the animals begin to pre-emptively evade subsequent stimuli, either hiding the sucker underneath their body (sucker evasion) or actively locomoting (locomotory evasion). These increases in evasive behaviors and decreases in reflexive shortening are not observed in yoked-control animals. In the second form of learning, conditioned place avoidance, *Hirudo* were placed in a two-chamber apparatus in which one chamber was lined with sandpaper and the other was smooth. During training, *Hirudo* were exposed to a noxious stimulus, capsaicin dissolved in the pond water, in either the smooth or sandpaper compartment for 30 seconds, five times/day, over three days. Animals exposed to capsaicin in the smooth chamber learned to avoid that chamber when tested on day 3. Animals exposed to capsaicin in the sandpapered chamber failed to learn to avoid the sandpaper region.

Additional experiments are ongoing to determine why conditioned place avoidance works in one direction, but not the other. Experiments are also underway to test the potential role of protein kinases, e.g., PKC-zeta and CamKII, during learning given that both types of kinases contribute to synaptic plasticity in *Hirudo* and to synaptic plasticity and learning in other animals. These initial findings demonstrate that *Hirudo* are not only capable of recognizing painful stimuli but are also able to learn about these stimuli in such a way to develop adaptive avoidance behaviors to mitigate/prevent future exposure.

Disclosures: C. Thompson: None. **J. Dohn:** None. **A. Franzen:** None. **B. Burrell:** None.

Poster

323. Invertebrate Learning & Memory

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 323.27

Topic: H.08. Learning and Memory

Support: Invertebrate Neurobiology Fund

Title: Flexible adjustment of behavioral and neural strategies during spatial learning in Drosophila

Authors: *Y. CHEN, R. ALFREDSON, U. STERN, D. MOTEVALLI, C.-H. YANG;
Duke Univ., Durham, NC

Abstract: The ability to return to places of significance based on memory is advantageous for animal survival. Both visual cues and non-visual cues are rich sources of information animals can use for spatial learning. While recent studies have shown that *Drosophila melanogaster* can form visual cues-induced place memory, the neuronal substrates that regulate spatial learning driven by non-visual cues, both idiothetic (internal) and allothetic (external) ones, are little explored. Here we report a new high-throughput system that can administer different tasks for probing how flies use non-visual cues for spatial learning. Aided by the system, we first discovered that flies were able to use both self-motions and environmental borders for spatial learning in a “regular task” where prominent visual cues were unavailable, and that they relied principally on PFN - a group of central complex neurons that sense idiothetic translational velocity and mushroom bodies (MBs), respectively, to process these two signals. Next, we found that flies showed significantly improved spatial learning ability when additional salient non-visual landmarks were provided at some distance away from the spatial goal; interestingly, they switched to exclusively relying on functional MB for learning in such “cues-enriched task”. Lastly, we found that both EPG neurons that signal allothetic heading direction and PFR neurons that signal allothetic translational velocity were dispensable for learning in both of our tasks. Taken together, our findings put forward an efficient system for studying non-visual cues driven spatial learning and demonstrate that flies could adjust their behavioral strategies and recruit

different circuit substrates depending on the quality and the availability of spatial cues they can exploit.

Disclosures: **Y. Chen:** None. **R. Alfredson:** None. **U. Stern:** None. **D. Motevalli:** None. **C. Yang:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

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Program #/Poster #: 324.01

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
CIHR Project Grant #377074
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NSERC Discovery Grant #74105
Canada Research Chairs Program
FRQS Postdoctoral Fellowship # 271812
CIHR Postdoctoral Fellowship #472750

Title: Evaluating models of hippocampal mapping in geometrically morphed environments across protracted experience in freely-behaving mice

Authors: *J. Q. LEE[1], A. T. KEINATH[1], E. CIANFARANO[2], M. P. BRANDON[1];
[1]Psychiatry, McGill Univ., Montreal, QC, Canada; [2]McGill Univ., Montréal, QC, Canada

Abstract: Survival requires that agents learn useful representations to navigate space effectively, including responses to dynamic changes in environments across experience. The hippocampus is known to have an important role in representing the environment, and disruption to hippocampal circuits severely impairs navigational abilities. Such results have stimulated the development of numerous models on cognitive mapping in the hippocampus and its role in cognition and behaviour. Importantly, such models make distinct but often overlapping claims about cognitive maps. Until now, there has been no consensus on how to compare such model-based predictions to empirical observation, and adjudicate between competing views. In the present study, we conducted a series of systematic geometric manipulations of a large open arena while tracking activity in large neural populations in hippocampal area CA1 across protracted experience in freely-behaving mice with single-photon calcium imaging. We show that repeated geometric deformations of environments produces reliable shifts in representational structure observed in neural activity of CA1, and that greater spatial information and organization develops with experience. Importantly, we also replicate and extend recent observations on spatial coding statistics in our paradigm, demonstrating systematic relationships between spatial field location, size, and number that depend on the environment and/or experience. Next, we generate predictions from theoretical models on hippocampal mapping and compare our empirical

observations to model-based predictions using representational similarity analysis (RSA). With this approach, we demonstrate that specific models show measurable improvements in predicting representational structure in CA1 population activity and its response to systematic deformations of environmental geometry and topology. Our findings thus support the use of such datasets to benchmark theoretical developments and demonstrate the utility of RSA-based approaches to perform model-based comparisons.

Disclosures: **J.Q. Lee:** None. **A.T. Keinath:** None. **E. Cianfarano:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 324.02

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
CIHR Project Grant #377074
CIHR Project Grant #463403
NSERC Discovery Grant # 74105
Canada Research Chairs Program
DFG Research Fellowship OU 135/1
Healthy Brains for Healthy Lives Doctoral Fellowship

Title: Understanding the role of superficial layers of the Medial Entorhinal Cortex (MEC) in spatial memory

Authors: *S. BADRINARAYANAN[1], M. OULÉ[2], M. P. BRANDON[2];
[1]Integrated Program in Neurosci., [2]Dept. of Psychiatry, Douglas Hosp. Res. Ctr., McGill Univ., Montreal, QC, Canada

Abstract: The medial entorhinal cortex (MEC) is a region involved in episodic memory and spatial navigation. Through a careful balance of excitation and inhibition (E/I), the MEC microcircuit generates a myriad of spatially modulated cells and their conjunctive activity form a map of the animal's environment. Interestingly, these spatially modulated cells are mostly present in the superficial layers of the MEC, and a fraction of them directly transmit spatial information to the hippocampus and thus form an anatomical pathway essential for memory. Interneurons expressing the Vasoactive Intestinal Peptide (VIP) disinhibit excitatory cells by inhibiting other interneurons and some excitatory cells to a smaller extent. However, compared to other classes of interneurons, VIP cells are not well characterized and their role in MEC physiology and functionality remain elusive. We first sought to better characterize VIP cells in the MEC and have found that these cells are mostly concentrated in MEC superficial layers, suggesting that they could indirectly control the transmission of memory-related information

both to and from the MEC to the hippocampus. For this purpose, we used the novel object location paradigm to assess behaviorally the impact of VIP cells in spatial memory. Using a combination of behavior, immunohistological detection of the cfos protein, and DREADDs, our preliminary analysis indicates that VIP cells are recruited during the novel object location task. Moreover, preliminary observations show that DREADDS-mediated inhibition of VIP cells prior to sampling is associated with poor behavioral performances in the novel object location task, suggesting that VIP cells might have an essential role in spatial memory. We also investigate the areas and cell types that can activate VIP cells in the superficial MEC through retrograde rabies virus tracing to uncover brain-wide inputs to this region.

Disclosures: S. Badrinarayanan: None. **M. Oulé:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

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Program #/Poster #: 324.03

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
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CIHR Project Grant #463403
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Imprinting and recall of threatening memories in the dorsal and ventral hippocampus

Authors: *L. RUNTZ[1], R. R. ROZESKE[2], A. T. KEINATH[1], A. SOSSIN[1], M. P. BRANDON[1]; [1]Psychiatry, McGill Univ., Montreal, QC, Canada; [2]Psychology, McGill Univ., Toronto, ON, Canada

Abstract: To improve an organism's chances of survival, the brain is responsible for contextualizing environmental information to assess imminent threat and generate adaptive behavioral and physiological responses (Stachenfeld et al. 2017). The hippocampal formation plays a critical role in generating these predictions through the creation of unique spatial maps composed of neurons that fire at different 'place field' locations throughout an environment and memory retrieval processes. Discriminating different contexts is therefore a major challenge for the hippocampus. Indeed, an impairment in the context processing can result in persistent re-experiencing (intrusive thoughts, upsetting dreams, dissociative reactions), as found in post-traumatic stress disorders.
Hippocampal-dependent context encoding and retrieval is often assessed by contextual fear discrimination, in which rodents are exposed to two distinct contexts – one neutral and one associated with shock. To better understand the dynamics of this process, we developed a new fear discrimination task. Inspired by previous studies (Jezek et al. 2011, Rozeske et al. 2018), we

constructed an arena consisting of a cylindrical LED screen and manipulated visual and auditory sensory elements to teleport mice between contexts. For two days, mice were trained to associate one context with shock and another context with no shock. During the following days, mice were tested by presenting a succession of the neutral and dangerous contexts at 2 min intervals. Presentation of shock-paired and non-shock-paired contexts produced high and low levels of freezing behavior, respectively. We simultaneously measured hippocampal dynamics of context fear memory acquisition and retrieval using calcium miniscope recording across the dorsal-ventral axis of CA1.
Our preliminary results demonstrate that conditioning induced significant changes to the spatial map of the shock-paired context. Furthermore, 'teleportation' between the threatening and the neutral context was associated with dynamic alterations in the hippocampal spatial map in both dorsal and ventral hippocampus. Additionally, a strong difference between these spatial maps was positively correlated with the strength of context fear discrimination.
Together these findings show that the hippocampus dynamically represents the retrieval of neutral and threatening memories to guide behavior and provide a framework to understand how spatial representations are linked to emotional behaviors.

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Poster

324. Hippocampal/Entorhinal Physiology I

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Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
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CIHR Project Grant #463403
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Inhibition of theta oscillations reveals that a single and reliable time cell sequence is not needed for working memory

Authors: *H. YONG[1], H. CHANG[3], M. P. BRANDON[2];
[1]McGill Univ., Verdun, QC, Canada; [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Neurosci., Canadian Ctr. For Behavioural Neurosci., Lethbridge, AB, Canada

Abstract: Recent work has shown that neurons in the hippocampus are sequentially activated during the delay period of a delayed spatial alternation task. Experimental data and a model proposed in Wang et al. (2015) suggest that the readout of this sequential activity required hippocampal theta oscillations. Further, the readout of this sequence has been proposed to be

necessary for successful performance in the delayed spatial alternation task. However, this prior study used a long-acting GABA receptor agonist to inhibit the activity of all medial septum neurons for the entire testing session, raising the possibilities that 1) septal contributions other than theta generation may support delay encoding, and 2) that memory deficits could be the result of septal inactivation outside of the delay period. To assess these possibilities, we applied an optogenetic strategy to selectively silence the theta-generating GABAergic population in the medial septum (MS) specifically during the delay period as mice performed the delayed spatial alternation task. Mice were trained to run on a treadmill for 10 seconds between each alternation lap on the Tmaze, and were deemed to have learned the task after reaching 80% correct or better for two consecutive days. Once they reached the criteria, septal GABAergic neurons were pseudo-randomly inhibited in 50% of the trials, for the entire 10 second duration of the delay period. We show that inhibition of these neurons significantly reduced the amplitude of hippocampal theta oscillation (mean theta reduction: 73%), and induced remapping in many of hippocampal neurons that encoded the delay period. Thus, we observed a new delay sequence only on trials when the septal GABAergic input to the hippocampus was suppressed. This phenomenon was not observed in laser-on GFP-only expressing control experiments. This result suggests the possibility that region CA1 of the hippocampus receives competing delay sequences from CA3 and the medial entorhinal cortex (MEC), both of which are known to contain delay cells. We suggest the possibility that MS inactivation disrupts the MEC delay sequence (similar to the effect of MS inactivation on MEC grid cells), which then gives the CA3 input more influence over CA1 sequences. Finally, we observed that remapping of the delay sequence had no impact on behavioral performance on the T-maze delayed alternation task. Our data suggests that this is driven by the preserved trajectory-dependent information.

Disclosures: **H. Yong:** None. **H. Chang:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Topic: H.09. Spatial Navigation

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Title: Grid cells align and anchor to environmental borders in a mouse model of early Alzheimer's disease

Authors: *J. YING[1], A. REBOREDA[2], M. YOSHIDA[2], M. P. BRANDON[1];
[1]Psychiatry, McGill Univ., Montreal, QC, Canada; [2]Functional Architecture of Memory, Leibniz Inst. For Neurobio., Magdeburg, Germany

Abstract: Medial entorhinal grid cells encode space with a hexagonal pattern of spatially periodic fields. Compromised grid cell patterns are sensitive markers of early Alzheimer's disease (AD) in human and animal models of pathology, yet it remains unclear how degraded hexagonal symmetry could underlie the widely reported path integration impairments during early pathogenesis in human APOE ε4-carriers and the J20 transgenic amyloid beta (Aβ) mouse model of early AD. These grid cell and grid-like impairments may represent impaired processing of the individual's self-motion, an idea that has yet to be confirmed at the level of neural coding in a pathological model. In support of this theory, both APOE ε4-carriers at early risk of AD and J20 mice preferred to navigate along the environmental borders while avoiding the center during spatial navigation tasks. This thigmotaxic strategy could reflect an inability to properly integrate self-motion cues in the center where landmarks are sparse, thus causing a behavioral dependence on borders to stabilize the grid map. Therefore, we hypothesized that grid cell hexagonal symmetry in J20 mice is not disrupted in a random manner but reflects a spatial code that is less influenced by the animal's self-motion towards the environmental center compared to the borders. To test this possibility, we analyzed spatially-tuned neurons recorded in the medial entorhinal cortex of J20 mice using *in-vivo* electrophysiology between the ages of 3-7 months, timepoints corresponding to the early stages of pathology prior to the expression of widespread Aβ plaques. We show in J20 mice that the loss of grid cell hexagonal symmetry reflected decreased coding of the environmental center. Using a two-dimensional spatial Fourier analysis, we demonstrate that aged J20 grid cells more frequently adopted a suboptimal rectangular pattern of spatially periodic bands aligned to the borders. Across all mouse groups, hexagonal grid cells appeared to be better path integrators as they fired more in the environmental center compared to their rectangular counterparts. Yet, the smaller proportion of aged J20 hexagonal grid cells exhibited overall reduced theta phase precession (distance coding by spike phase) and were spatially unstable towards the center but not near the borders. These results demonstrate that the grid map detaches from the individual's self-motion while aligning and anchoring to the geometric environment during early Aβ pathogenesis, which might underlie thigmotaxic and path integration impairments during early AD.

Disclosures: J. Ying: None. **A. Reboreda:** None. **M. Yoshida:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Topic: H.09. Spatial Navigation

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CIHR Project Grant #463403

Title: Optogenetic silencing of medial septum glutamatergic and GABAergic neurons distort and disrupt grid cell spatial firing

Authors: *J. ROBINSON[1], J. YING[2], M. P. BRANDON[2], M. E. HASSELMO[1];
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Abstract: Grid cells in the medial entorhinal cortex (MEC) exhibit firing at locations that form a repeating hexagonal array throughout an environment. This spatial periodicity of grid cells relies on input from the medial septum (MS). The MS consists of three separate populations: cholinergic, GABAergic and glutamatergic neurons. Here we evaluate the contribution of septal GABAergic and glutamatergic neurons to grid cell firing. We have specifically targeted either GABAergic or glutamatergic neurons in the MS using optogenetic activation of Archaerhodopsin to selectively silence each cell type while recording grid cells in the MEC. For both groups, an optic fiber was placed above the MS for light delivery, and a four-tetrode microdrive was implanted into the MEC for grid cell recordings. We used a stimulation protocol of 30-second inactivation followed by a 30-second recovery period throughout the recording session. Our results show that silencing septal glutamatergic neurons did not affect MEC theta power nor frequency; however, silencing this population resulted in a slight distortion of grid fields. In addition, glutamatergic silencing also altered the neural coding of velocity. Optogenetic silencing of septal GABAergic neurons showed up to a 60% reduction in MEC theta power. Silencing GABAergic neurons resulted in a significant reduction in gridness score and an increase in the percentage of out-of-field firing compared to baseline. Our results suggest that the extent of the distortion to the grid cells may be related to the degree of spatial periodicity during baseline recordings. Cells with a higher gridness score may be more resilient to septal GABAergic inactivation. Together, these data highlight the critical importance of input from the MS in generating the spatial firing pattern of grid cells.

Disclosures: **J. Robinson:** None. **J. Ying:** None. **M.P. Brandon:** None. **M.E. Hasselmo:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Title: WITHDRAWN

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

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Program #/Poster #: 324.08

Topic: H.09. Spatial Navigation

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CIHR Project Grant #377074
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Title: Network dynamics of representational shifts in thalamic HD neurons

Authors: *Z. AJABI[1], A. T. KEINATH[2], X. WEI[3], M. P. BRANDON[2];
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Abstract: The head direction (HD) system underlies the sense of orientation by continuously tracking the direction an animal is facing. While changes in egocentric sensory input (i.e. vestibular) constitute the main drive for updating the internal HD representation, allocentric cues (e.g. visual, olfactory, auditory, etc...) provide an important feedback control signal that stabilizes the internal HD representation and prevents it from drifting. This feedback signal can be strong enough to reorient the HD network, regardless of movement, when local environmental cues rotate, in a phenomenon called 'reset'. Yet, the mechanisms that mediate such resets are unknown and, more generally, the dynamics that govern HD encoding in unstable conditions remain poorly understood. Here, we studied the neural dynamics of the HD network in situations of instability from a large population perspective. We performed calcium imaging of thalamic HD cells in freely behaving mice while manipulating the location of a visual landmark in a familiar environment. We showed that the appearance of the visual cue at shifted locations caused rotations of the decoded HD with the same amount of angular cue displacement (i.e. resets). Throughout the experiments, fluctuations in population activity were observed and could be captured by a variable that we termed 'network gain'. Effectively, the network gain represented a secondary dimension in the internal HD representation along with the angular dimension. We showed that network gain was anti-correlated with reset speed and could be used in a computational model to predict the reset response. Moreover, network gain patterns revealed a non-uniform distribution across internal angles, in darkness, such that peaks around the internal location of a previously displayed visual cue were consistently observed as if the HD system was maintaining a 'memory trace' of the salient landmark. In addition, changes in network gain patterns were dependent on the duration of prior shifted-cue exposure which implies plastic processes at play. Evidence for plasticity involvement in the internal HD representation was further demonstrated by predictable and time-dependent drift patterns that were reported in darkness periods. By incorporating Hebbian learning into our model, we were able to replicate

this drift behavior. Finally, we showed that the HD system flexibly anchored to a visual cue in continuous rotation and maintained a persistent offset even after the cue was removed. A model of vestibular input recalibration could reproduce these results and indicated that the integration of vestibular information within the HD network was experience dependent.

Disclosures: Z. Ajabi: None. **A.T. Keinath:** None. **X. Wei:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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NIH R01 MH120073
NIH R01 MH060013
NIH R01 MH052090
ONR MURI N00014-19-1-2571
ONR MURI N00014-16-1-2832

Title: Spatial memory related interactions between rat retrosplenial and medial entorhinal cortices

Authors: *A. S. ALEXANDER, D. J. SHEEHAN, G. W. CHAPMAN, M. E. HASSELMO; Ctr. for Systems Neuroscience, Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: The retrosplenial cortex (RSC) shows neural responses associated with different spatial coordinate systems including allocentric spatial location, allocentric head direction, and position within the space of a route (Alexander et al. 2015; 2017). RSC populations are also sensitive to the current action state of the animal and the position of boundaries in egocentric spatial coordinates (Alexander et al. 2020). The RSC forms a unique monosynaptic processing loop with the extended hippocampal formation via the entorhinal cortex and subiculum, and the aforementioned activation dynamics of RSC neurons may mediate systems interactions by which spatial representations in entorhinal cortex and hippocampus influence goal-directed behavior. Specifically, RSC may register current heading information and sensorimotor signals with hippocampal computations relating to spatial navigation in order to signal errors in position estimates based on sensory information and path integration. Consistent with this, neurons in retrosplenial cortex show phasic firing relative to hippocampal theta rhythm (Alexander et al., 2018). A primary mode by which RSC may provide feedback to the hippocampal formation for this hypothetical function is via excitatory projections from RSC into deep layers of medial entorhinal cortex (MEC), yet little has been examined with respect to coordination amongst these two regions. In the current work, we use both projection-specific in vivo calcium imaging and in

vivo electrophysiological recordings to characterize co-activation dynamics in the RSC-MEC circuit at the single cell and population levels in freely moving rats performing spatial memory tasks.

Disclosures: A.S. Alexander: None. **D.J. Sheehan:** None. **G.W. Chapman:** None. **M.E. Hasselmo:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Canada Research Chairs Program
FRQS fellowship - BF15 - 320805

Title: Investigating emergent hippocampal dynamics through standardized high-throughput behavior and neuronal imaging using the McGill-Mouse-Miniscope (M3) platform

Authors: *C.-A. MOSSER[1], M. YAGHOUBI[2], A. NIETO-POSADAS[3], T. GISIGER[3], S. WILLIAMS[4], M. P. BRANDON[5];
[1]Psychiatry, McGill University, Douglas Hosp. Res. Ctr., Montréal, QC, Canada; [2]Dept. of Psychiatry, Douglas Res. Center, McGill Univ., Montreal, QC, Canada; [3]Douglas Hosp. Res. Center, McGill Univ., Montreal, QC, Canada; [4]Dept Psychiatry, McGill University, Douglas Res. Center,, Montreal, QC, Canada; [5]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Recent technical advances enable the recording of large neuronal populations during behavior resulting in increasingly complex datasets. Despite bold and important open-science and data-sharing policies, these datasets across laboratories tend to apply unique data acquisition methods, behavior, and file structures. Discrepancies between protocols present key challenges including the comparison of results between brain regions, laboratories, and species. We have established the McGill-Mouse-Miniscope platform (M3, www.m3platform.org) to combine miniscope calcium imaging with standardized touchscreen-based behavioral testing. Our mission is to curate an open-source and standardized framework for acquiring, analyzing, and accessing high-quality data of the neuronal dynamics that underlie behavior and cognition throughout the brain in mice, and in a near future marmosets. Each experiment provides up to 1000 simultaneously recorded neurons from a single brain region over the course of ~3 months as animals initially learn and master the task. We have collected several datasets to highlight the feasibility of this approach, including hippocampal CA1 and CA3 recordings during the delayed

trial-unique nonmatching-to-location (TUNL) task. The TUNL task assesses spatial working memory and consists of an encoding phase, a delay phase and a retrieval phase. In this task, a white square is presented in one of five positions on the touchscreen. A nose poke to this square starts a delay period. Following this delay, two white squares are displayed, and the mouse must choose the nonmatching square to receive reward. As expected, CA1 and CA3 neurons are spatially modulated, however, they are also sensitive to other behavioral and task-related features. We found that 1) ~10% of neurons participate in sequences tiling the delay period, 2) delay sequences are unique to the sample square location, 3) the fidelity of sequence predict performance, and 4) delay sequence cells were reliably activated during reward. We are now examining how this structure emerges in CA1 and CA3 regions during the initial learning of this task, and how network activity restructures when memory load is changed. These data will help to shed light on how large populations of hippocampal neurons encode a complex memory task, and will allow for comparison across laboratories, mouse models, and species.

Disclosures: C. Mosser: None. **M. Yaghoubi:** None. **A. Nieto-Posadas:** None. **T. Gisiger:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Topic: H.09. Spatial Navigation

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CIHR Postdoctoral Fellowship #472750

Title: Examining hippocampal representation of predictive context associations in augmented reality

Authors: *E. R. CIANFARANO, J. Q. LEE, M. P. BRANDON;
Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Growing evidence suggests that the hippocampus encodes behaviorally-relevant task features that extend beyond an agent's physical location. Recent theoretical models also posit that hippocampal coding is predictive in nature, such that the representation of an agent's current state is tied to likely future states in environments and/or task space, which several human neuroimaging and behavioral studies support. Presently, it remains unclear which models best explain the contribution of predictive relationships to single-cell and population activity in the

hippocampus. In the present experiments, we record from large populations of neurons using miniscope calcium imaging in dorsal CA1 while animals navigate for rewards and behaviorally teleport themselves across contexts in augmented reality. We systematically shift predictive relationships between augmented contexts during behaviorally-generated context teleportation, and examine the contributions of predictive coding to single-cell and population-level representation. Next, we leverage representational similarity analysis (RSA) to compare animals' neural representation with computational models using behavior and neural data to generate and evaluate model-based predictions. We suggest that such approaches will be important to benchmark theoretical progress, and compare representational structure both across species and artificial agents.

Disclosures: E.R. Cianfarano: None. **J.Q. Lee:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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NSERC Discovery Grant 74105
Canada Research Chairs Program
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Title: Tracking hippocampal contextual representations during paired-associate learning in mice

Authors: *Z. HAQQEE[1], S. WILLIAMS[3], M. P. BRANDON[2];
[1]Integrated Program in Neurosci., [2]Psychiatry, McGill Univ., Montreal, QC, Canada;
[3]Psychiatry, McGill University, Douglas Res. Center,, Montreal, QC, Canada

Abstract: Conjunctive associations, particularly with spatial variables, depend centrally on the hippocampal system. Numerous studies have demonstrated the existence of cells with conjunctive coding in well-trained subjects. Less is known about how these representations evolve over time as a subject gradually learns these contextual associations over several days of training and subsequent overtraining. Touchscreen operant conditioning chambers offer incredible flexibility in creative task design and standardization across laboratories. Additionally, one-photon calcium imaging with micro-endoscopes (miniscopes) enables continuous simultaneous recording of several hundreds of cells per day for several weeks in freely behaving animals. We combine these approaches to track the cellular and population-level dynamics of object-location contextual representations in dorsal CA1 of the hippocampus while mice gradually learn a complex paired-associate learning (PAL) touchscreen task. Using a

combination of dimensionality reduction techniques and generalized linear models, we identify specific ensembles of cells that dynamically evolve their tuning to spatial and contextual variables over more than a month of daily training on the touchscreen task, from chamber habituation to overtraining phases of learning. Manifold analyses further confirm the emergence of activity dynamics unique to the task context, sometimes existing only transiently during specific bouts of learning. The data suggest that hippocampal systems generate robust conjunctive associations as needed for solving paired-associate learning tasks.

Disclosures: Z. Haqqee: None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Topic: H.09. Spatial Navigation

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NIH Early Independence Awawrd (DP5 OD023106-01)

Title: Exploring the contribution of dentate gyrus to memory guided spatial navigation in mice

Authors: *L. K. WILMERDING[1], I. KONDRATYEV[2], S. RAMIREZ[3], M. E. HASSELMO[3]; [1]Boston Univ., Boston Univ. Grad. Program For Neurosci., Boston, MA; [2]Boston Univ. Undergraduate Program In Neurosci., [3]Boston Univ., Boston Univ., Boston, MA

Abstract: The hippocampus has been studied extensively for its role in navigation and episodic memory, in particular the CA1 region. To understand the influence of upstream regions on spatial computations in CA1, we manipulated ensembles of learning-associated cells in mouse dentate gyrus (DG) using optogenetics. The DG has been manipulated to produce emotionally-valenced behaviors like freezing, and is necessary for memory-guided spatial navigation tasks such as the Delayed-Non-Match-to-Place (DNMP) T-maze task. Each DNMP trial requires a mouse to traverse one route of the T-maze in the sample phase, and after a short delay choose the opposite route during the subsequent test phase, allowing dissection of the encoding and retrieval stages of memory respectively. We trained male and female wild type mice to perform the DNMP task on a T-maze for sucrose reward. Using the fos-tet-tag activity-dependent labeling system, DG cells were tagged with channelrhodopsin2-eYFP across days as mice learned the task and then performed the task in a novel T-maze. Across five days' performance of the DNMP T-maze task, 20Hz pulsatile optogenetic stimulation was delivered bilaterally during

pseudo-randomly chosen sample phases of the task to stimulate DG ensembles tagged across many days. Within-subjects analyses of trial accuracy, speed, and choice point occupancy were used to assess the behavioral outcomes of this 'nonsense' stimulation during ongoing navigation, revealing decreased accuracy during stimulation trials only. Additionally, we tested for correlation between the degree of behavioral impairment and the size of the labeled population. These results extend previous findings on the role of the DG in memory-guided spatial navigation tasks, in particular its contribution to the encoding of spatial memories for subsequent retrieval.

Disclosures: L.K. Wilmerding: None. **I. Kondratyev:** None. **S. Ramirez:** None. **M.E. Hasselmo:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Program #/Poster #: 324.14

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
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CIHR Project Grant #463403
NSERC Discovery Grant # 74105

Title: Long-term dynamics of hippocampal neurons in a memory-dependent navigation task

Authors: *M. YAGHOUBI[1], A. NIETO-POSADAS[2], C.-A. M. MOSSER[2], E. WILSON[2], T. GISIGER[2], S. WILLIAMS[2], M. P. BRANDON[2];
[1]Integrated Program in Neurosci. (IPN), McGill Univ., [2]Dept. of Psychiatry, McGill Univ., Douglas Hosp. Res. Centrer, Montreal, QC, Canada

Abstract: The cognitive map theory of hippocampal function has largely focused on mapping the allocentric spatial dimensions of the external world [O'Keefe and Dostrovsky, 1971]. More recent evidence points to a broader role of the hippocampus in forming maps for other non-spatial aspects of the environment [Aronov et al 2017, Pastalkova et al 2008, Komorowski et al 2009]. In order to study the long-term coding properties of hippocampal neurons, we use one-photon calcium imaging to record the activity of up to one thousand individual neurons when mice perform a touchscreen based memory-dependent navigation task. In this task, a white square is presented in one of five positions on the touchscreen. A nose poke to this square makes it disappear and starts a delay period (increasing from 2 to 8 seconds as the mice learn the task). Following the delay, two white squares are displayed, and the mouse must choose the nonmatching square to receive reward located on the back wall of the touchscreen chamber. For well-trained animals, we observe that, in addition to spatial tuning, hippocampal neurons become directionally tuned and trajectory dependent. We also observe neurons are less modulated by

some of the salient features of the task like sample and choice, and more modulated by mouse behaviour. For instance, when mouse is executing a pattern of behaviour a neuronal response will be observed regardless of the cognitive demand of the task at that moment and during that pattern of behaviour. These observations motivate us to ask whether hippocampal neurons encode animal's behavioral repertoire on top of spatial coding. To address this question, we build upon a recently developed deep-learning-based approach that quantifies egocentric body movements in a latent space of behaviour [Luxem et al 2022]. This pipeline is subtle enough to reveal individual differences between mice. CA1 neurons were spatially tuned yet, our analysis shows a significant variance of hippocampal neuronal response could be explained by animal egocentric behavioral. Tracking cells across days, we observe the internal representation of spatial and behavioural code are drifting over days. In spite of the drift of the representation, the performance is not affected and in fact is positively correlated with sessions-based information content of the internal representation. Together, these results suggest that, in well-trained animals, the hippocampus contains information about behavioral space in addition to allocentric space. This may function to support memory-guided behavior to solve the task.

Disclosures: **M. Yaghoubi:** None. **A. Nieto-Posadas:** None. **C.M. Mosser:** None. **E. Wilson:** None. **T. Gisiger:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Program #/Poster #: 324.15

Topic: H.09. Spatial Navigation

Support: NIH Grant R01NS104917
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NARSAD Young Investigator Fellowship

Title: Geometry of space sculpts hippocampal sequential place cell assemblies during development

Authors: *U. FAROOQ[1], G. DRAGOI[2];
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Abstract: Euclidian space is the fabric of the world we live in. Whether and how experience with Euclidian geometry shapes our spatial-temporal representations of the world, including cognitive maps and memories, is not known. Here, we deprived rats of experience with Euclidian geometry by rearing them inside spheres and compared the activity of large populations of hippocampal neuronal ensembles during spatial navigation and sleep with that of cuboid cage-reared controls. Despite lack of experience with Euclidean geometry, time-compressed place cell

sequences expressed as preplay, theta sequences and replay depicting the first Euclidean linear trajectory in the life of the rats emerged after sphere-rearing, albeit occurring at lower proportions than their cuboid-reared counterparts. On the other hand, early-life experience with non-Euclidean, curved geometry resulted in similar mapping of linear environment ends and corners by neuronal ensembles suggestive of warped representations of linear tracks, and an impairment in neuronal pattern separation of multiple linear environments due to reduced preconfigured network repertoires. Experience with linear, orthogonally oriented environments over 3-4 days reversed these deficits. Thus, early-life experience with Euclidian geometry enriches the hippocampal repertoire of preconfigured neuronal patterns enabling the emergence of unique neuronal representations of multiple linear environments.

Disclosures: **U. Farooq:** None. **G. Dragoi:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Program #/Poster #: 324.16

Topic: H.09. Spatial Navigation

Title: Improved pattern separation of similar contextual memories is associated with increased rate remapping in CA3 of rats raised in an enriched environment

Authors: *S. VENTURA, S. DUNCAN, J. A. AINGE;
Univ. of St Andrews, Univ. of St Andrews, St Andrews, United Kingdom

Abstract: The ability to disambiguate similar past events is fundamental to episodic memory. In the hippocampus, place cells support this ability via remapping, i.e., they change their place field and/or firing rate following changes in the environment. Exposure to complex environments (environmental enrichment, ENR) has been shown to improve memory in several tasks, particularly under conditions of high memory interference when efficient pattern separation may be required. Here, we aimed to investigate the neural bases of ENR-dependent memory improvements by looking at the effect of ENR on contextual memory, hippocampal activity and place cell remapping in rats. We found that young male Lister Hooded rats housed in an enriched environment for 4 months (enriched rats, N=12) significantly outperformed standard-housed rats (N=12) in a context-dependent associative recognition memory task, testing spontaneous recognition of novel object-context associations. These effects were only found for similar, and not dissimilar contexts, pointing to a selective ENR-dependent improvement in discrimination consistent with pattern separation. Next, we found that ENR led to an increase in adult hippocampal neurogenesis, as indicated by a greater number of doublecortin (DCX) positive cells in the dentate gyrus of ERN rats compared to standard-housed rats. Additionally, ERN resulted in significantly fewer activated cells (Fos+ cells) in the supra pyramidal blade of the dentate gyrus, CA1 and proximal CA3 following exposure to novel and familiar associations of object and contexts. Together, these findings point to increased adult hippocampal neurogenesis

and sparser hippocampal coding of complex experiences as potential mechanisms for ENR-dependent memory improvements. Finally, we investigated the effect of ERN on pattern separation in the hippocampus by looking at place cell remapping in response to small changes to the environment. We recorded CA3 place cells from a separate group of enriched (N=3) and standard-housed (N=3) male rats as they explored a morph box whose shape could be altered by small, incremental steps. CA3 place cells in ERN rats showed smaller place fields, and higher measures of spatial tuning, when compared to standard-housed rats. Additionally, ENR led to increased rate remapping in response to alterations in shape to the environment. These findings suggest that ENR leads to functional changes in the way the hippocampus represents, and responds to, changes in the environment.

Disclosures: S. Ventura: None. **S. Duncan:** None. **J.A. Ainge:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Topic: H.09. Spatial Navigation

Support: The Crandall-Cordero Fellowship
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UConn IDEA Grant

Title: Comparing spatial processing in the rat dorsal and intermediate hippocampus

Authors: *S. L. T. LEE[1], R. TROHA[2], I. H. STEVENSON[2], E. J. MARKUS[2];
[1]Emory Univ., Atlanta, GA; [2]Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT

Abstract: Elucidating the fundamental neural mechanisms of hippocampal information processing is necessary for understanding memory formation and brain disorders. There are differences in hippocampal genetics, anatomy, and connectivity across the longitudinal axis suggesting functional heterogeneity. The dorsal pole is suggested to be primarily involved in spatial processing, whereas, the ventral pole is implicated in emotional processing. These functions seem more prominent near their respective poles and weaker toward the intermediate hippocampus. Much is known regarding place cell activity in the dorsal region since these are closer to the brain surface and relatively accessible. Less is known about intermediate hippocampal place cell activity located deeper in the brain. A 64-channel hyperdrive, with 16 independently moveable tetrodes, targeted simultaneously both dorsal and intermediate regions. Rats performed a familiar/well-learned task traversing a linear track. After the hippocampal representation of the familiar task was determined, one of the two arms was rotated to a novel

position. We measured how the spatial tuning of these neurons was modified when rats experienced this novel spatial manipulation. Similarly, we compared the hippocampal representation of the familiar task with novel social (conspecific) and olfactory (female bedding and coyote urine) experiences. Consistent with prior literature, we found differences in the raw characteristics of place field firing between dorsal and intermediate regions. Place fields of intermediate CA1 neurons exhibited lower spatial information content, greater total number of place fields, larger place field size, and higher percentage of activity in the environment. Despite fundamental differences in place field characteristics, both dorsal and intermediate units responded similarly to both spatial and social/odor manipulations, as measured by changes in the number of cells exhibiting place fields across maze sessions, overall firing rate, direct rate map correlations, and relative place field characteristics. Taken together, there is dense redundancy and recurrent processing between the hippocampus and cortical/subcortical areas that may facilitate the integration of new information.

Disclosures: **S.L.T. Lee:** None. **R. Troha:** None. **I.H. Stevenson:** None. **E.J. Markus:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

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Program #/Poster #: 324.18

Title: WITHDRAWN

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 324.19

Topic: H.09. Spatial Navigation

Title: Gateway identity and spatial remapping in a combined grid and place cell attractor

Authors: *T. BAUMANN, H. A. MALLOT;
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Abstract: The representation of space in the rodent brain is generally attributed to place cells located in the hippocampus. These cells cover the environment with location-specific firing fields ("place fields") and the population code uniquely describes each position in space. Place field shape and locations and the relative population code are subject to change if the animal enters a new compartment in the experimental maze. This effect, known as remapping, cannot be explained from grid cell-based path integration and local sensory cues alone but requires

additional knowledge about the context. This is exemplified in two situations: Normally, when the animal returns to a known compartment, the place cells remap to the original pattern associated with that compartment. However, when the environment includes multiple visually identical but otherwise separate compartments, place cells also remap to the same pattern in each room and the rooms are confused. The process necessarily occurs at the gateways but not within compartments, suggesting the reactivation of a stored pattern based on cues at the entrance. We present a model of the hippocampal-entorhinal circuit in which the activity of place and grid cells follows a joint attractor dynamic. Place cells depend on the current grid cell activity but can also reset and change the grid cell activity in the remapping process. Remapping is triggered by the recognition of a gateway. When this happens, the corresponding place cell pattern is reactivated from long-term memory. The joint attractor then reinstates the grid cell pattern that was active when the gateway had first been learned and path integration can proceed from there. In this sense, the pattern of active cells provides both information about the current context and the position of the animal within that context. The model is tested in various mazes from the experimental literature and reproduces the published results. We also make testable predictions for remapping in new maze types. Based on the model, we hypothesize the existence of "gate cells" that drive the place cells and with them the joint hippocampal-entorhinal loop into the corresponding attractor whenever a gate is detected.

Disclosures: **T. Baumann:** None. **H.A. Mallot:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

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Program #/Poster #: 324.20

Topic: H.09. Spatial Navigation

Title: Spatial coding models of hippocampal CA1 reproduce power-law scaling observed under coarse-graining

Authors: *J. BRIGUGLIO[1], J. LEE[1], A. K. LEE[1], V. HAKIM[2], S. ROMANI[1];
[1]HHMI / Janelia Res. Campus, Ashburn, VA; [2]École normale supérieure, Paris, France

Abstract: Connecting large-scale properties of neural systems to the behavior of interacting neurons is a central problem in neuroscience. One hypothesis, inspired by the study of critical phenomena in physical systems via the Renormalization Group, is that the brain operates at a “critical point”. This state is characterized by scale invariance reflected in power-law scaling for many system properties. A data analysis technique devised by analogy to the Renormalization Group was previously presented (Meshulam et al., 2019) to coarse-grain neural activity from the hippocampus of mice exploring a small environment and revealed power-law scaling which could not be reproduced using a naïve model of place cell activity (Meshulam et al., 2019; Morrell et al., 2021). Here, we sought to study this phenomenon by analyzing place cell activity in large environments, where the statistical properties of place field firing can be better

characterized. We examine calcium imaging data recorded from mice exploring large virtual environments (Lee et al., 2020) to measure power-laws reflecting the scaling in probability of silence, variance, characteristic time, and within-cluster eigenvalues of neural activity. We observe that these data can be described by power-laws, but find some exponents that differ significantly from those previously reported. We next modeled calcium data based on independent cells with the observed statistics of spatial coding (Rich et al., 2014; Lee et al., 2020).The model produces power-laws with similar exponents. The model predicts that, unlike a truly scale invariant system, the measured exponents depend on sampled population size and environment size, which was verified in the data. This demonstrates that simple spatial coding models composed of independent neurons account for the scaling properties of the coarse graining procedure, posing a challenge for drawing conclusions about neural circuit dynamics from these scaling properties.

Disclosures: **J. Briguglio:** None. **J. Lee:** None. **A.K. Lee:** None. **V. Hakim:** None. **S. Romani:** None.

Poster

324. Hippocampal/Entorhinal Physiology I

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 324.21

Topic: H.09. Spatial Navigation

Support: JSPS KAKENHI Grant JP20K11974
NEDO Grant JPNP16007

Title: A hippocampal spiking neural network model for replay and place-field deformation in spatial learning

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Abstract: The sequential firing of hippocampal place cells during exploration is spontaneously reactivated during awake rest or sleep after rats perform a maze task. Such hippocampal “replay” has been proposed as a part of mechanisms for memory consolidation, recall, and navigation planning. The formation of feed-forward connections between place cells in hippocampal CA3 potentially leads to hippocampal replay. Simultaneously, the formation of feed-forward connections expands the place fields in the direction opposite of the animal’s movement during spatial learning tasks: a backward shift of place fields. Even though both phenomena are the result of feed-forward networks, different conditions for neuromodulators must be considered for them to coexist. In this study, we investigated the acquisition process of feed-forward connections that generate hippocampal replay and backward shift by simulating spatial learning using a spiking neural network model of the hippocampus and the entorhinal cortex. In the

hippocampus, the balance of the extracellular neuromodulators differs between exploration and resting states. Glutamatergic synaptic transmission is suppressed depending on the extracellular acetylcholine concentration. By assuming that the extracellular acetylcholine concentrations are high during exploration and low during rest, we find that several runs of linear track form feed-forward connections at synapses between place cells by dopamine-dependent synaptic plasticity, causing hippocampal replay in the resting state and backward shift during the task learning. In addition, to learn a reward-dependent navigation task, place cell output was projected to the action selection network. In a Morris water maze-like task (MWM), hippocampal replay and backward shift were acquired simultaneously with the learning of the platform location. The deformation of place cells prevented the fixation of suboptimal pathways to the reward, allowing a virtual rat to find the optimal route in the MWM. Our results suggest that animals switch the dynamics of the hippocampal network to separate the stages of spatial memory encoding and consolidation by modulating the release of such neuromodulators as acetylcholine and dopamine in a behavioral state-dependent manner.

Disclosures: K. Takada: None. **K. Tateno:** None.

Poster

325. Human Spatial Navigation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 325.01

Topic: H.09. Spatial Navigation

Support: EEA and Norway Grants (EEA-RO-NO-2018-0606)

Title: Social representation and collaboration during virtual navigation

Authors: *X. CHEN[1], S. FRAMVIK[1], N. JOHN[1], S. SERBAN[2], C. FACAERU[2], A. BERCEANU[2], C. DOELLER[1], I. CARCEA[2], T. SCHROEDER[1];
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Abstract: Authors:Xiuxian Chen, Stian Framvik, Natalie St. John, Sabin Serban, Ciprian Facaeru, AlexandruBerceanu, Christian Doeller, Ioanna Carcea, Tobias Navarro Schröder
Abstract:Humans are social animals, and extraordinarily good at coordinating actions in groups. Forexample, groups of hunters in hunter-gatherer societies need to communicate and collaborateto be successful at hunting their prey. In our modern society an ever-growing part of our livesand social interactions happen digitally, and in virtual environments. Here we studied howpeople represent the behavior and movement of either a human partner, or a computer-controlled bot, and how they collaborate with the partner or bot to perform an object-location-memory task. To this end, we developed a custom virtual reality, multiplayerexperiment and leveraged ultra-high field 7T fMRI to assess neural representations during thetask. The partner avatars in the human/ social condition and the bot/ non-social conditionwere visually identical.

However, study participants were more likely to behave agreeable inthe social condition and follow suggestions from the partner avatar. Our results indicate thatparticipants successfully collaborated. Preliminary fMRI analyses showed concurrentactivations in areas of the theory-of-mind, and the navigation network, such as occipitalcortex and posterior parietal cortex. These results provide novel insights into the mechanismsof successful collaboration and social representations in virtual environments.

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Poster

325. Human Spatial Navigation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 325.02

Topic: H.09. Spatial Navigation

Support: ONR MURI N00014-16-1- 2832
ONR DURIP N00014-17-1- 2304

Title: The dimensionality of prefrontal representations changes during spatial navigation

Authors: *J. GUO[1], S. S. P. LIAPIS[2,1], K. M. ISENBURG[2,1], T. I. BROWN[4], J. T. MCGUIRE[3,2,1], C. E. STERN[3,2,1];
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Abstract: The prefrontal cortex (PFC) is involved in navigational decision-making and in the disambiguation of overlapping routes (Patai and Spiers, 2021, Brown and Stern, 2013, Brown et al., 2010, 2012). The PFC also supports the ability to focus on goal-relevant information, filtering out irrelevant information, similar to a dimension reduction process (Mack et al., 2020). Given these two roles, we hypothesized that prefrontal regions alter the dimensionality of neural representations when making decisions to disambiguate overlapping navigational routes - compressing redundant information to make differentiation easier. To test this hypothesis, we reanalyzed an fMRI study of healthy adults performing a navigational disambiguation task (Brown, Hasselmo, & Stern, 2014). Participants (N=16) navigated virtual mazes both novel and familiar from prior training over the course of 10 scans. Each maze began with a unique contextual cue period, followed by a critical decision point, a secondary decision point, and finally a unique goal location. Some of the routes overlapped with one another (OL) between the two decision points, whereas others were nonoverlapping (non-OL) throughout. We employed a data-driven approach to examine the dimensionality of neural representations during each of the four trial phases (Mack et al., 2020). Voxelwise activity patterns for each OL and non-OL trial's cue period, decision points, and goal were extracted using beta-series regression (Mumford et al., 2012; Turner et al., 2012) from three regions of interest: dorsolateral PFC (dlPFC), ventrolateral

PFC (vlPFC), and orbitofrontal cortex (OFC). We then derived a dimensionality score for these representations using principal components analysis. This allowed us to compare the dimensionality of OL and non-OL patterns for each trial phase. Consistent with the PFC's role in disambiguating overlapping routes and filtering out task irrelevant features, results showed reduced dimensionality in dlPFC and vlPFC for OL relative to non-OL representations during both decision points. No effects were found for the cue phase. This suggests that the PFC filters out redundant information shared between OL routes when a decision needs to be made. During navigation of the goal hallway however, OL representations were maintained in a higher-dimensional space than non-OL patterns. This could be the result of an error or reward signal derived from the more complex navigation of OL routes. Taken together, these results support a role for the PFC in the disambiguation of overlapping routes by altering the dimensionality of neural representations.

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Poster

325. Human Spatial Navigation

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Program #/Poster #: 325.03

Topic: H.09. Spatial Navigation

Support: Institute for Collaborative Biotechnologies W911NF-19-2-0026
Hellman Family Foundation
California NanoSystems Institute ECCHLG18-19

Title: The relationship between spatial navigation ability during midlife and white matter structural integrity

Authors: *D. M. COSSIO[1], S. YU[2], M. HEGARTY[2], E. G. JACOBS[2], E. R. CHRASTIL[1]; [1]Univ. of California, Irvine, Irvine, CA; [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Spatial navigation is a highly complex behavior that remains critical for survival across many species. Despite its importance, there are large individual differences in navigation ability. One question is the extent to which these individual differences are reflected in differences in brain structure. While this relationship has been examined to some extent in young adults, the relationship between brain structure and individual performance in navigation tasks has not been fully tested in the earliest stages of aging, during midlife. To address this question, we used diffusion spectrum imaging (DSI) to examine the relationship between white matter structural integrity and performance on a virtual maze learning task in midlife participants (ages 45-55, n =32). The navigation task consisted of an exploration and a test phase. During the 16-minute exploration, participants freely explored to find 9 objects located in the environment and were instructed to remember their locations. For each of the 48 test trials, participants started at

one object and were directed to go to another object, without feedback and with limited time. We hypothesized that, similar to the relationships found in younger participants, performance in midlife adults would correlate with better white matter integrity. Specifically, we predicted that performance would be associated with mean diffusivity and fractional anisotropy in the fornix, a major output tract of the hippocampus. DSI data was analyzed using group connectronomy and tract-based spatial statistics analysis. Preliminary results indicate that structural integrity in white matter tracts within the medial temporal lobe and prefrontal cortex corresponded to performance on a maze learning task, but that this relationship differs from that of young participants, such that worse structural integrity was associated with better performance. These results suggest that white matter structure corresponds to spatial navigation performance, but that this relationship changes during midlife. These results provide us with a greater understanding of the changes in brain and behavior that occur during early aging.

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Poster

325. Human Spatial Navigation

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Topic: H.09. Spatial Navigation

Support: ONR DURIP N00014-17-1- 2304
ONR MURI N00014-19-1- 2571
NSF BCS 1625552
NSF BCS 1829398

Title: Exploring egocentric boundary sensitivity in humans using a virtual open field foraging paradigm with fMRI

Authors: *M. F. DUNNE[1], S. LING[1], K. N. MOORE[1], T. M. MORIN[1], E. R. CHRASTIL[3], C. E. STERN[2];
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Abstract: Environmental boundaries encountered while navigating may be represented in either allocentric or egocentric reference frames. In this study, we sought to investigate egocentric boundary sensitivity using fMRI in conjunction with a novel virtual open-field coin foraging task, inspired by food foraging navigation paradigms used in rodent studies. Evidence from rodents suggests that neurons in retrosplenial cortex (RSC) fire when environmental boundaries are a specific orientation and distance relative to the animal and that neurons in dorsomedial striatum display an egocentric coding scheme for boundaries (Alexander et al., 2020; Hinman et al., 2019). Recent work investigating how the brain translates between reference frames suggests

that RSC may be uniquely positioned to carry out this function, as it has connectivity to regions sensitive to both reference frames (Chrastil et al., 2018). Recent single-neuron recordings in humans during navigation have also identified egocentric bearing cells in the medial temporal lobe, predominantly in PHC (Kunz et al., 2021). Our coin foraging paradigm included 325 coins that were hidden throughout a large virtual "open-field" surrounded by four unique walls. 100 coins were randomly distributed and 225 were distributed according to four multivariate Gaussian distributions of varying sizes. Subjects were instructed to freely explore the environment and collect as many coins as possible, naïve to the number and distribution of coins. Subjects completed 10 eight-minute runs over two consecutive days, first as a behavioral task and then during fMRI scanning. Using this paradigm, we explored how brain activity varied as a function of egocentric and allocentric spatial coordinates as a participant moved freely within an environment. Analyses at both the group and single subject level focused on examining changes in brain activity across visual and navigationally sensitive regions that include early visuocortical areas, hippocampus and PHC, RSC, precuneus, thalamus, cortical and subcortical motor regions and the cerebellum. Preliminary group level univariate analyses assessing whether a participant was near a wall, the distance to the wall, and the location of the wall revealed that when there was a wall to the subject's left, there was a corresponding increase in response within the right early visual cortex, left RSC, and bilateral PHC. When the wall was to the participant's right, responses increased in the left early visual cortex, right RSC, and bilateral PHC. These results suggest a sensitivity to egocentric boundaries in RSC, consistent with results demonstrated in animal models.

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Poster

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Topic: H.09. Spatial Navigation

Support: NIH (NINDS) 1R01NS119468-01A1

Title: Graph Metrics and Non-Spatial Navigational Learning

Authors: *T. KAPOGIANIS, E. CHRASTIL, A. BORNSTEIN;
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Abstract: Spatial navigation has long had the idea of a cognitive map, an allocentric representation of euclidean environmental measurements. Recently, the idea of cognitive graphs emerged, in which the topology or connections between locations is the primary representation. Cognitive graphs can also represent non-spatial relationships of learned associations between items or concepts, such as social relationships within a friend group. It is unknown what

properties of graphs affect the ability of individuals to mentally represent and navigate these structures. Primary candidates are the number of states (nodes) contained within a graph, and the number of connections among states (edges). Here, we independently manipulate these quantities and examine how they affect both the ability to identify paths between nodes and the efficiency of paths chosen in non-spatial graphs- associative networks of object pictures that have no overt spatial properties. Participants learned and navigated 4 graphs with distinct node quantities and edge-to-node ratios. Each block, with a unique graph, consisted of a learning and a test phase. During the learning phase, participants were asked to memorize groups of objects, which represented nodes connected to the current state. They then selected one of the objects, after which they would be presented with a new set of objects whose identities depended on the object selected. During the test phase, participants' understanding of the underlying graph structure was tested by asking that they navigate to trial-unique target items, using the same selection procedure as in the learning phase. Consistent with our hypothesis that participants' learning would be affected by graph complexity, we observed that test-phase accuracy and path efficiency declined as the number of nodes in the graph and the edge-to-node ratio increased, independently of each other. Together, these findings indicate that a greater degree of interconnectedness between nodes influences learning. Results suggest that the ability to learn and navigate non-spatial graphs through repeated learned associations involves both the graph size and the interconnectedness or complexity of the graph. Findings demonstrate the influence of graph structure on learning, with implications for both spatial and non-spatial graphs.

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Poster

325. Human Spatial Navigation

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Program #/Poster #: 325.06

Topic: H.09. Spatial Navigation

Support: California NanoSystems Institute
UCSB Academic Senate
Institute for Collaborative Biotechnologies

Title: The relationship between hippocampal subfield volumes and individual differences in navigation

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Dept. of Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Despite the need for successful navigation, humans vary greatly in their abilities to navigate, and these individual differences may relate to differences in brain structure. Studies on navigation experts and older populations have shown a relationship between hippocampal volume and navigation ability, but this relationship in the healthy, young adult population has

recently been called into question. Many of these studies analyzed total hippocampal gray-matter volumes despite there being anatomical differences in hippocampal subfields. This study tested the abilities of young adults to successfully navigate a virtual desktop maze environment, and the participants' high-resolution anatomical brain images were automatically segmented into four hippocampal subfields and three adjacent medial temporal lobe regions. We theorized that successful navigation correlates with the volumes of the Cornu Ammonis 3 (CA3) and dentate gyrus (DG) subfields since CA3 and DG volumes are related to pattern separation ability in humans. With the need to distinguish between similar-looking maze hallways and partially-overlapping routes, young adults who have stronger pattern separation ability may perform better in this task and have larger CA3 and DG volumes. In addition, rodent literature informs us that Cornu Ammonis 1 (CA1) and entorhinal cortex (ERC) are both associated with spatial memory, suggesting a possible relationship between their volumes and navigation performance. Our findings indicate that DG volumes positively correlated with maze performance, consistent with a pattern separation hypothesis, and ERC volumes positively correlated with path efficiency, which align with the ERC's role in updating metric information for path integration. This result is informative to our future understanding of the link between brain and navigation behavior for healthy, young adults.

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Poster

325. Human Spatial Navigation

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Program #/Poster #: 325.07

Topic: H.09. Spatial Navigation

Support: Institute for Collaborative Biotechnologies
Hellman Family Foundation
California Nanosystems Institute

Title: Changes in spatial exploration behavior in early aging

Authors: *V. PUTHUSSERYPPADY[1], D. COSSIO[1], M. HEGARTY[2], E. JACOBS[2], E. CHRASTIL[1];
[1]Univ. of California, Irvine, Irvine, CA; [2]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Recent studies have suggested declines in spatial navigation ability to be seen with age as well as represent an early marker for Alzheimer's Disease (AD). These studies have in large part only studied how individuals use their spatial memory to navigate a learned environment, and have not investigated the navigation patterns individuals exhibit as they explore and learn novel environments. Hence the extent to which spatial exploration patterns might be altered in healthy aging, and in AD, is poorly understood. The aims of this study are

threefold: 1) to investigate how spatial exploration behavior is altered in healthy midlife adults, 2) to assess whether these alterations may mediate age-related declines in their spatial memory, and 3) to explore what extent these alterations may represent an early marker for cognitive aging. 51 healthy young adults (aged 18-28) and 112 healthy midlife adults (aged 43-61) performed a maze learning task using desktop virtual reality. In the exploration phase, participants freely explored the maze using button presses on a keyboard and learned the locations of 9 target objects. The outcome measures for this phase were the number of button press moves, turns made, total distance traveled, number of object visits, evenness of exploration, and pause duration. In the test phase, participants were placed at a target object and asked to navigate to other target objects within a given timeframe. The outcome measure for this phase was navigation accuracy (i.e., % of correct trials). We found that midlife adults made fewer button press moves ($p = 0.03$), travelled less distance ($p = 0.01$), visited target objects more often ($p < 0.001$), had less even exploration ($p < 0.001$), had longer pause durations ($p < 0.001$), and had less navigation accuracy ($p < 0.001$) when compared to the young. We also found that age-related differences in number of target object visits during exploration partially mediated age-related differences in navigation accuracy in the test phase ($p < 0.001$, proportion mediated = 22%). We will next assess the extent to which we can classify our participants into young vs. midlife based purely on their exploration variables. The results of this study will help inform our understanding of how spatial navigation changes with age, as well as provide a platform for future studies to investigate whether changes in spatial exploration behavior could represent a cognitive marker for AD.

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Poster

325. Human Spatial Navigation

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Topic: H.09. Spatial Navigation

Support: NIH Grant T32AG073088
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Title: Domain generality and specificity across egocentric and allocentric distance ratings

Authors: *M. J. STARRETT AMBROSE[1], Y. CHENG[2], R. C. DAVIS[4], B. TRANQUADA-TORRES[1], E. R. CHRASTIL[1,2,3];
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Abstract: The concept of "space" can be applied to a wide range of cognitive processing beyond that of simple physical locations. Humans use figural representations to illustrate spatial (maps), temporal (timelines), and even social (family trees, personality traits, or social media connections) relationships. Indeed, relationships between any two "points" across each of these distinct domains can be visualized in the same fashion: one-dimensional linear distances. Recent research has investigated the relational representations within and across these domains as well as the extent to which similar (domain general) or distinct (domain specific) brain structures and networks support these representations. However, to date, this research has largely focused on relationships relative to the individual making the judgement. The field of spatial cognition has long distinguished between such self-referential (egocentric) representations and those that are observer-independent (allocentric) such as the relationships between landmarks on a map. To provide a more complete understanding of the cognitive and neural mechanisms that support relational processing, it is important to directly compare distance ratings for spatial, temporal, and social relationships across both egocentric and allocentric frames of reference. In a behavioral experiment, we used a similar task to previous egocentric work. On each trial, participants were cued whether they would be making a spatial, temporal, or social judgement. Then participants viewed images of two people: One was a well-known individual (e.g., Barak Obama), and the other was either the participant themself (egocentric condition) or a different well-known person (e.g., Donald Trump). Participants then rated the distance between the two individuals within the cued domain. Critically, the same pairs were used across all domains, thus equating the stimuli while only varying the type of judgment. Participants completed eight blocks of thirty trials and then separately rated their confidence for each of the judgments. We found that participants' distance ratings for each domain were consistent with pre-screening questionnaire responses and available objective data. For all domains, participants utilized the full range of ratings. Considering subjective ratings to be the ground-truth for future work, we will use functional magnetic resonance imaging to provide a neural index for distances across domains and reference frames. The presented work provides a proof of concept for the cognitive basis of these planned neuroimaging studies.

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Poster

325. Human Spatial Navigation

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Topic: H.09. Spatial Navigation

Support: NIH (NINDS) 1R01NS119468

Title: Brain network dynamics for navigational learning and memory

Authors: *E. WARD[1], R. F. WOODRY[3], J. CARLSON[4], E. R. CHRASTIL[2];
[2]Neurobio. & Behavior, [1]Univ. of California, Irvine, Irvine, CA; [3]New York Univ., New York City, NY; [4]UCSB, Santa Barbara, CA

Abstract: Brain network dynamics for navigational learning and memory
Erica Ward, Robert Woodry, Jean Carlson, Elizabeth R. Chrastil
Effective spatial navigation is dependent on several cognitive processes including learning, attention, and memory. But how do people learn and remember new environments?Several studies have explored the brain activity of already known environments, however, comparatively little is known about the acquisition of this knowledge. In the present study, we analyzed fMRI images of humans (n=98) completing a challenging maze learning task. Participants were given 16 minutes to explore a virtual hedge maze and learn the locations of 9 objects. Next, their object location memory was tested in a series of 48 (min) trials, each of which started at one object with instructions to find another object using the paths of the maze (e.g. clock lamp). To reduce feedback in the testing phase, all objects were replaced with red spheres. Accuracy ranged from near 0% to 100% correct, enabling us to quantify brain network and behavioral differences that distinguish between poor, average, and exceptional performers. We used dynamic community detection to identify brain networks changes during the learning and test phases, and to determine whether the network dynamics differentiate between good and poor learners. We parcellated the brain images into ROIs (Schaefer 200 atlas combined with the Harvard-Oxford subcortical atlas) and calculated coherence values between each ROI. Then, by implementing a generalized Louvain method to assign each ROI to an algorithm-determined community in each time window, we determined whether and how each ROI changed networks over time. Specifically, we examined flexibility – the number of times an ROI changed communities – and promiscuity – the number of communities an ROI joins, relative to the total number of communities. Using these metrics, we characterized the dynamic brain networks during the learning and test phases. Preliminary results suggest that average navigators exhibited high flexibility throughout the brain during learning, whereas poor navigators only exhibited high flexibility in the salience network. In addition, we examined behavioral exploration patterns in the learning phase to determine whether they correlate with eventual navigation accuracy in the test phase, finding that better navigators tended to explore more evenly than poor navigators during the middle portion of their learning phase. Together, the brain and behavioral dynamics in this study provide rich insight into navigational learning and memory.
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Poster

325. Human Spatial Navigation

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Topic: H.09. Spatial Navigation

Support: NIH Grant R21AG063131

Title: Cognitive and neural mechanisms involved in strategic spatial navigation across the lifespan

Authors: *P. MAXIM, S. D. MOFFAT, T. I. BROWN;
Sch. of Psychology, Georgia Inst. of Technol., Atlanta, GA

Abstract: How we navigate our world depends on many factors, however not everyone holds equal expertise or ability. Research suggests that aging negatively impacts flexible navigation performance, perhaps in part due to memory integration and route memory deficits, so it is imperative we understand how memory influences navigation strategies and what factors lead to navigation differences as we age. The present study tested young adults (YA, 18-40) and older adults (OA, 65-80) across two days in a virtual navigation task, and used fMRI to examine complementary views of how the medial temporal lobe and prefrontal cortex contribute to route planning. Participants trained on specific paths through six virtual towns, and after 24-hours were tested during fMRI on memory of these now-familiar routes (FR). They were then given novel goals and permitted to reach the goal using any strategy (unbound to the FR). Some trials were designed so that the optimal route (OR, optimal shortcut) followed the same direction as the FR (forward-shortcut), while in others the OR required traveling backward relative to the FR direction (backward-shortcut) - i.e., greater heading conflict between OR and FR. The relationship between strategy (familiar vs. shortcut) and execution (efficiency of strategy) was complex. Analyses show YAs and OAs have similar navigation strategies and efficiency for forward-shortcuts. But during backward-shortcut trials, when given a choice between a) backtracking or shortcutting in a "conflicting" direction with the FR vs. b) traveling along the FR direction, YAs show a significant bias toward more closely following the FR than the OR that, interestingly, the OAs do not. Unlike YAs, the OAs instead showed similar strategies and performance across forward and backward trials, resulting in them tending to take fewer FRs compared to the YAs on backward-shortcut trials. Parallel analyses suggest nearly every OA underperformed in FR memory tests compared to the YAs, indicative of it being a more effortful experience for OAs trying to remember the FR as a strategy on novel goal trials. Together, these findings suggest that route familiarity and directional conflict with prior experiences may both be important moderators of past evidence that aging leads to decreased flexibility/increased route-based navigation. We further examine fMRI correlates of both planning and *en route* behavior in frontal and medial temporal circuitry. These fMRI data help unpack the neural bases of how these individuals execute their navigational strategies and which neural factors play a role in the different strategies and abilities between young and old in our paradigm.

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Poster

325. Human Spatial Navigation

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Keck Award

Title: Virtual reality as a tool to study spatial navigation with deep brain recordings in ambulatory and stationary participants

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Abstract: Episodic memories integrate past experiences with complex stimuli and spatial environments and are integral to day-to-day life. The medial temporal lobe (MTL) supports episodic memories through oscillatory activity. Until recently, studying episodic memories in humans has been limited to stationary formats with screen-based paradigms, due to the constraints of classic neuroimaging approaches. Rodent studies have identified critical local field potential (LFP) and neural mechanisms that support memory processes. However, classic human neuroimaging approaches (fMRI, scalp electroencephalography (EEG)) lack the resolution to draw conclusions about deep brain structures with sub-second temporal resolution and regional specificity. Recent advances have created a clinical opportunity to record intracranial EEG activity from freely moving human participants (Topalovic et al, 2020). Here, we present a method to investigate episodic memories using both intracranial EEG (iEEG) recordings as well as LFP recordings by developing a virtual reality (VR) spatial memory task that can be completed in two complimentary participant cohorts: (1) freely ambulating humans with iEEG recordings and (2) stationary neurosurgical patients with LFP recordings. A spatial navigation memory task was constructed to be completed in a head-mounted VR headset. Six ambulatory patients completed the task and displayed spatial modulation of theta oscillatory power. Interestingly, we found that the virtual environment's boundaries modulated theta oscillation power in the 3-5 Hz range in ambulatory iEEG, similar to prior findings of real-world ambulation (Stangl et al, 2021). We also found that the virtual environment boundaries modulated theta oscillatory power in a similar range (4-5 Hz) in preliminary data from stationary LFP participants, supporting the utility of VR as a tool to investigate spatial navigation in iEEG and LFP data across recording platforms. Prior reports have also highlighted that theta oscillations occur in significant bouts during real-world ambulation in humans (Aghajan et al, 2017). We also found that the prevalence of theta bouts occurred in 10-15% of samples in iEEG recordings in participants ambulating while wearing VR headsets. Altogether, this approach provides the opportunity to investigate similarities and differences in neural mechanisms underlying spatial navigation across immersive VR and ambulatory and stationary paradigms.

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Poster

325. Human Spatial Navigation

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 325.12

Topic: H.09. Spatial Navigation

Title: Single neurons in the human medial temporal lobe engage in distinct aspects of different tasks

Authors: *T. DONOGHUE[1], R. CAO[2], Z. HAN[1], C. HOLMAN[1], N. J. BRANDMEIR[3], S. WANG[4], J. JACOBS[1];
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Abstract: A long history of investigations of the hippocampus and surrounding areas has found single-neuron responses related to navigation, including place and spatial target cells. Other work has emphasized the role of these brain areas in recognition and memory processes, reporting cells that respond to specific concepts and individuals. Given that these different coding properties are typically investigated in separate experiments, the relationship between these different representations in the medial temporal lobe (MTL) remains unclear. Do different cells selectively engage in different tasks, or do the same cells engage in different representations in different contexts? In order to address this question, we recorded neural data from the hippocampus and amygdala of human neurosurgical patients with implanted microwires while they performed a pair of tasks with distinct memory and navigational demands. In the first task, participants did a one-back memory task while viewing a series of images, responding to repeated presentations of the same image. In the second task, participants navigated an open arena, learning and later recalling the location of presented items. Each of these tasks could occur in one of two versions, a “face” version where presented items were famous faces, or an “object” version where items were images of objects or non-human organisms. These tasks were conducted in combined experimental sessions from 5 patients, for a total of 23 task pairings. Spikes were sorted together across paired tasks, allowing the same putative single-neurons to be compared between the different tasks, resulting in the identification of 1271 cells. Examining each task individually, in the one-back task, we found face identity and object-selective cells that responded to specific stimuli. In the navigation task we identified target-location responsive cells whose responses reflected the position of presented items. We then compared the group membership of cells between tasks and found a significant overlap in the number of cells that encode object or facial identity in the one-back task, and target location in the navigation task. We further used the responses from one task to predict the responses from the other. Overall, our

results support a flexible encoding of multiple, distinct aspects of different tasks by single neurons in the human MTL, whereby individual neurons encode distinct features in different contexts. These results help to clarify the specificity of representations in the human medial temporal lobe, and provide a template for future work using paired tasks to continue to investigate the consistency of neural responses across different contexts.

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Poster

325. Human Spatial Navigation

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Topic: H.09. Spatial Navigation

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Title: Conjunctive Encoding in Human Place and Time Cells

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Abstract: In order to navigate through dynamic environments, the brain must keep track of where we are and where we are going, as well as the timing and duration of different actions. Single neurons in the medial temporal lobe of humans and animals encode place and time, and thus reflect a potential mechanism underlying spatial navigation. In animals, neurons have also been found with 'conjunctive encoding', in which cells have a complex representation of multiple features of the task and/or environment. This includes, for example, cells that appear to reflect a conjunction of current position and future trajectory (sometimes called splitter cells). However, it is currently unknown whether these kinds of conjunctive representations exist in humans, and if so, how they may contribute to spatial navigation and memory formation. To investigate this, we recorded single-neuron activity from the medial temporal lobe and surrounding ventral prefrontal cortex in neurosurgical patients who performed a virtual spatial-navigation and memory task. In this task, subjects navigate a fixed-track virtual world and, at a navigation choice point, are instructed to alternate between choosing to go left or right at a

choice point. The task also has a spatial memory component, as patients have to learn and then later retrieve the location of treasure chests along the track. When analyzing single-neuron activity while subjects navigate this virtual world, we find 'place' cells, whose firing rates relate to the subjects' spatial position. When analyzing stationary periods, such as the time at the choice point before subjects make a navigation decision, we find 'time' cells, whose firing rates relate to the amount of time elapsed. In addition to cells that appear to be selective only to place or time, we find a subset of cells with conjunctive encoding which are modulated by the combination of a specific place or time as well as the future direction that the subject will take. Our results demonstrate conjunctive encoding in human single neuron activity by showing how spatial and temporal encoding can relate to both current context and future decisions.

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Poster

325. Human Spatial Navigation

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Topic: H.09. Spatial Navigation

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Swebilius Foundation Grant (to K. N. Graves)

Title: Walking in the real world and in memory: spatial navigation during human neurophysiology

Authors: *K. N. GRAVES[1], T. E. GRAY[2], A. LETROU[1], I. H. QURAISHI[2], N. B. TURK-BROWNE[1,3];
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Abstract: Navigation through the world is a foundational behavior ubiquitous across species. Yet, for decades, much of our understanding of its underlying neural mechanisms in humans has been limited to what can be extrapolated from animal models or from neuroimaging studies in which participants navigate virtually. This work has centrally implicated the hippocampus in navigation, but what role the human hippocampus plays in real-world navigation and how this role interacts with the critical associative and memory functions of the hippocampus remain unknown. We recruited 12 epilepsy patients implanted with Responsive Neurostimulation (RNS) devices to navigate in the real-world as we simultaneously recorded local field potentials (LFP)

from hippocampal contacts. We hypothesized that real-world navigation would evoke hippocampal oscillations in the theta band and that these neural signals could be paired with distinctive auditory cues presented during navigation such that they could be subsequently reactivated by the cues while patients were stationary. The task consisted of three stages: In Stage 1, patients passively listened to two different instrumental songs while seated. In Stage 2, patients alternated between walking and standing still along a linear track in 1-minute intervals for a total of 20 minutes. The songs from Stage 1 served as musical cues that indicated whether to walk or stand (akin to musical chairs), with one song playing during walking epochs and the other song playing during standing epochs. Stage 3 was identical to Stage 1, with patients once again passively listening to the two songs while seated. We found significantly greater oscillatory power and prevalence in the theta and alpha bands at the onset of movement during actual navigation in Stage 2. Amazingly, this same effect occurred again in Stage 3, while the patient was stationary, at the onset of the song that had been associated with movement during Stage 2, compared with the song previously associated with nonmovement in Stage 2. We further showed that a classifier trained on navigation-related oscillations in Stage 2 could decode the cues associated with movement versus nonmovement in Stage 3, demonstrating that the frequencies of neural activity elicited during ambulation were those being subsequently reinstated. Collectively, these findings suggest that neural signals of real-world navigation are subject to binding in the hippocampus to sensory features of the real-world contexts through which we navigate.

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Poster

325. Human Spatial Navigation

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Program #/Poster #: 325.15

Topic: H.09. Spatial Navigation

Support: NSF BCS 1829398
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ONR DURIP N00014-17-1-2304

Title: Virtual Human Foraging Efficiency and Parameter Estimation for Heavy-Tailed Search

Authors: *K. N. MOORE, C. YI, M. F. DUNNE, C. E. STERN, J. T. MCGUIRE; Boston Univ., Boston, MA

Abstract: Heavy-tailed search patterns are well documented in animal studies of foraging. To investigate the extent to which humans engage in heavy-tailed search strategies in a virtual environment, we studied 32 human participants (ages 18-35) as they completed a virtual coin

foraging task. Participants were instructed to freely explore and collect coins in a virtual field (160m x 160m) for 8 minutes at a time across 10 trials (total foraging time: 80 min). In each trial, 100 coins were randomly dispersed and 225 coins were predictably clustered in four Gaussian-distributed patches. Trajectory step lengths were extracted from 8 min foraging bouts by segmenting trajectories where turn angles exceeded 40 degrees. Maximum Likelihood Estimation was used to fit step-length distributions for participants' concatenated foraging trials to probability density functions of common models in foraging literature including power law, lognormal, and exponential distributions. We analyzed frequency distributions for goodness-of-fit using Akaike Information Criterion (AIC). The power law model was the best fit for 84% of concatenated trials, the exponential model was the best fit for 16%, and the lognormal model was the best fit for 0%. Analyses of the best fitting parameters of the power law model suggested that estimated power law exponents for search trajectories fell within the range predicted by studies of animal and naturalistic human foraging: $1<u<3$. Our data demonstrate that larger estimated exponents were associated with successful foraging outcomes. Greater estimated power law exponents were associated with the total number of coins collected by each participant, and this relationship was stronger among high scoring individuals. Across foraging trials, increases in subjects' estimated exponents were associated with greater average coin collection. Additionally, there was a weak association between subjects' average change in estimated power law exponents between trials and the average number of coins they collected. These results suggest that human foraging behavior in a virtual environment is a heavy-tailed search process: the power law model emerged as the best fitting model for participants' search trajectory steps. However, there was relatively little variability in estimated exponents from these data: exponents were within the lower half of the predicted range, yet $u=2$ has been associated with optimally efficient foraging. Additional studies are necessary to better understand the variability of estimated model parameters and their relationship with efficient foraging in different task conditions.

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Poster

325. Human Spatial Navigation

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Program #/Poster #: 325.16

Topic: H.09. Spatial Navigation

Support: NIH R01 AG061355

Title: Sleep supports spatial memory but not navigation in both children and adults using the Minecraft Memory and Navigation Task

Authors: *K. SIMON[1], M. AGUIRRE[1], A. FENGER[2], L. GALUT[1], H. PASCUAL[1], S. POLK[1], L. WARREN[1], M. YAZDZHYAN[1], J. ZHANG[1], D. CLEMENSON[1], F. C. BAKER[3], S. C.

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Abstract: The acquisition and retention of spatial information is uniquely supported by the hippocampus, and sleep is known to support the consolidation of hippocampal-dependent information. However, findings remain mixed in adults as to whether sleep supports spatial cognition and this question has not been investigated in children. We used our new Minecraft Memory and Navigation (MNN) task to disentangle the contributions of sleep for unique locations within a memory compared to the navigation metrics to them and contrasted across age groups. Thirty-one youth (M_{age} = 10.6, SD = 1.56) and twenty-nine adults (M = 22.24, SD = 3.72) were administered the MNN task. Training included two free-explorations of an open-field environment where they learned the locations of 12 unique objects. At test, participants were teleported around the environment and required to navigate to and place the object in its remembered location. All participants were tested twice, immediately after training and 12-hours later, after a period of sleep or wake. Sleep/wake conditions and unique environments were counterbalanced. Navigation was analyzed by the total steps (path length), trajectory angle while walking, and search behaviors. For both adults an children, we found a significant benefit of sleep, compared to wake, on the spatial location accuracy, such that over a period of sleep, the object-location metric accuracy improved. In adults, we found improvement over time for all navigation metrics regardless of condition. However, in adults, we did not find improvement on any navigation metrics. For both children and adults, spatial location memory did not correlate with any navigation metrics, suggesting further disentanglement between the processes. We demonstrate in two age groups similar sleep-dependent benefits for the metric accuracy of spatial locations. However, only in adults do we see navigation improvement, irrespective of sleep. In contrast, children did not improve over a delay. Our findings provide support that sleep supports the maintenance of the spatial relationships within environments, termed cognitive maps, but does not specifically support navigation behaviors. Furthermore, we provide evidence that across development, navigation may require further brain maturation to support improvement over time.

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Poster

325. Human Spatial Navigation

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Program #/Poster #: 325.17

Topic: H.09. Spatial Navigation

Support: NSF Grant No. DGE-1650112
UCSD Office of Research Affairs Center Launch Program

Title: Multi-sensory virtual reality task design to examine the neural basis of context-dependent spatial navigation in people with iEEG implants

Authors: *P. Q. NGO-PHAM[1], A. PATEL[2], R. A. GULLI[3], V. GILJA[2];
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Abstract: While the hippocampus is known as a cognitive map of space, other structures and their role during spatial navigation and recall have not been extensively studied in humans. During the planning of goal-directed spatial navigation, humans and animals use models of their local spatial environment, where salient elements like objects and landmarks are applied to map location. When initiating recall and memory decision processes for navigation, they must do so with respect to navigational context, defined by spatially dependent sensory features (Do, Tien-Thong Nguyen, et al. Sci. Reports 2021). These sensory features include visual landmarks (e.g. buildings and trees) and auditory environmental cues (e.g. birds chirping or people speaking). However, current studies investigating multi-sensory cue integration during spatial navigation are limited. Studies in nonhuman primates have begun to evaluate context-dependent spatial navigation with visual context cues (Gulli, Roberto A., et al, Nature Neuroscience 2020). These studies employ a virtual reality based visual environment with game dynamics designed to interrogate the neural basis of context-dependent spatial navigation. We extend these designs to human subjects in the epilepsy monitoring unit (EMU). Towards this goal, we designed a multi-sensory virtual reality navigation task simulating a town environment as a set of distinct road intersections. This cognitive task is designed for the EMU to study the neural correlates of human behavior as measured by intracranial neurophysiology. While the subject is engaged in the task, we measure simultaneous neurophysiology from stereoelectroencephalography (sEEG) probes which are implanted based on clinical care needs. Implants are typically positioned in the hippocampus and adjacent structures enabling the study of neurophysiology from spatial navigation related areas. The task environment includes specific visual context cues adjacent to the goal landmark used during both learning and recall. The subject is encouraged to learn the map through the environment by playing a game presented as a foraging task, and is evaluated on recall in a later phase with the same context cues. sEEG coverage also includes electrodes in the auditory pathway, including speech processing areas. To study multi-sensory integration we use distinct audio cues composed of English phonemes that can evoke perception related processes at the multiple levels of auditory and language processing. By tailoring the task to the EMU we investigate the neural correlates of behavioral and cognitive states during multi-sensory spatial navigation.

Disclosures: P.Q. Ngo-Pham: None. **A. Patel:** None. **R.A. Gulli:** None. **V. Gilja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); V.G. holds shares in Neuralink, Corp., and Paradromics, Inc. These organizations had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract.. Other; V.G. currently consults for Paradromics, Inc. They had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract..

Poster

325. Human Spatial Navigation

Location: SDCC Halls B-H

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Program #/Poster #: 325.18

Topic: H.09. Spatial Navigation

Title: Brain sources of the theta EEG rhythm underlying inhibitory control and replanning in active navigation in the Virtual House Locomotor Maze

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Abstract: The purpose of this preliminary study is to investigate cognitive brain function approached by the EEG oscillations of inhibitory control and mental flexibility during active navigation in the "Virtual House Locomotor Maze" (VHLM) (Castilla *et al.*, 2021). Concretely, the VHLM tests replanning processes by first asking the participant to repeat five times a self-chosen path to reach a given house in the maze. After having learned it, the path is blocked on the 6th navigation, imposing the subject to inhibit the learned trajectory and to design a new one. Interestingly, it has been recently shown that theta EEG oscillations increase their power spectrum during heading changes in active navigation and that such an effect is localized in the retrosplenial cortex (Do *et al.*, 2021). We hypothesize here that theta EEG oscillation power increase characterizing active navigation will be localized in the prefrontal cortex in the 6th trial underlying intentional inhibition and replanning processes in comparison to the preceding trials. 64 EEG signals are recorded on ten healthy participants (age range 18 - 29 years) with eegoTM sports (EEG system LE-200, ANT Neuro, The Netherlands) while performing the VHLM protocol. Off-line, data treatment, and statistics are performed by means of EEGLAB software (Delorme & Makeig, 2004). Initially, a 200 Hz low pass and a 0.1 Hz high pass filters are applied. Then any artefactual portion of the EEG data is rejected by visual inspection. Synchronous or partially synchronous artefactual activity (mostly blinks) is detected and rejected by independent component analysis (ICA). The baseline-normalized spectrograms or event-related spectral perturbation (ERSP in dB) for each EEG signal and subject are calculated with respect to the house target's event (-1 s. to 2 s. epochs). Source locations of the theta oscillations are estimated with swLORETA modelling (Cebolla *et al.*, 2017) (ASA software, ANT Neuro, The Netherlands). Following non-parametric permutations and Holm post hoc tests the preliminary results confirm that the EEG power spectrum increases in the theta rhythm during active navigation for all the trials and that the dorsolateral prefrontal cortex and also the orbitofrontal cortex are activated in the 6th trial of navigation suggesting the involvement of these areas in the inhibition of the learned trajectory and the replanning of the new one.

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Poster

326. Human Language Processing and Production

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.01

Topic: H.11. Language

Support: NIH Grant R01 NS109367-01A1

Title: Tracking lexical access during sentence production

Authors: *A. M. MORGAN[1], W. DOYLE[2], O. DEVINSKY[2], A. FLINKER[1];
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Abstract: During word production, stages of lexical representation come online in a feed-forward sequence beginning with the conceptual, then grammatical, phonological, and finally articulatory (Levelt, 1989; Indefrey, 2011). However, this model is based primarily on picture naming data, and recent work indicates that the temporal dynamics of lexical access may be fundamentally different during sentence production (Momma & Ferreira, 2019).
Here, we employ direct neural recordings (ECoG) in humans and leverage a machine learning (decoding) approach to track the activation of two stages of lexical representation during sentence production, elucidating (1) the patterns of neural activity that code for discrete stages of lexical representation (conceptual/articulatory), and (2) the temporal dynamics of these stages during various production tasks.
Eight patients undergoing neurosurgery for refractory epilepsy repeatedly produced 6 nouns (dog, ninja, etc.) in response to cartoon images while electrical potentials were measured directly from cortex. In picture naming blocks, patients saw an image of a cartoon character and responded overtly (“dog”). In scene description blocks, patients saw cartoon images of the same characters embedded in static scenes and produced corresponding sentences (e.g. “The dog tickled the ninja”).
We were able to predict above chance (p < 0.05 permutation test, accuracy level ~30%) which of the 6 nouns a subject was about to produce during sentence production using multi-class classifiers trained on data from the picture naming block, thereby tracking lexical activation during sentence production. We separately tracked the activation of words’ articulatory and conceptual representations in two analyses. To track articulatory representations, we trained the classifier on neural data collected just before production onset during picture naming trials, and used this to predict word identity during sentence production. This analysis accurately predicted word identity ~200ms prior to each word’s articulation onset in sentences.
To track conceptual representations, we trained a classifier on neural data from picture naming at the time point when word-specific activity patterns were detected, and used this classifier to predict word identity during sentence production. This analysis revealed different temporal patterns for the first noun in a sentence (subject) and the second (object). Our results constitute an important step toward understanding how the brain accesses words during fluent speech, and toward linking neural spatiotemporal codes to theoretical models of sentence production.

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Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.02

Topic: H.11. Language

Support: TL1TR001431
MCGSO SRGP2203

Title: Architecture of Speech Production and Speech Perception Pathways

Authors: ***P. NIKOLOV**[1], S. DAMERA[1], J. LIN[1], C. CORNELL[1], S. KIM[1], K. YOON[1], N. ZUR[1], E. ZSIGA[2], J. RAUSCHECKER[1], M. RIESENHUBER[1];
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Abstract: Introduction: Spoken language processing can be broken down into two components: speech recognition and speech production. Speech recognition deals with the process of extracting meaning from what one hears (“sound-to-meaning”) whereas speech production involves generating a word from an idea (“sound-to-articulation”). In current “dual-stream” models of language processing in the brain, these two processes are thought to be subserved by the anteroventral and dorsal streams, respectively. Computational models of speech processing (“internal forward and inverse models”) propose dynamic interactions of neural representations involved in speech perception and speech production. Yet, despite their importance for speech processing and acquisition, these interactions are still poorly understood Using a computational model (internal model) framework, we used multivariate imaging analyses of EEG data (EEG-RSA) to probe the nature (perceptual or articulatory) and dynamical interactions of neural representations in the speech processing pathways during speech perception and overt production tasks as a means of elucidating the architecture and interaction of the speech processing pathways.
Methods: Our preliminary analyses are based on a sample of so far 10 healthy L1 English speaking adults who completed 10 runs of a speech perception and speech production task while recording their neural activity using a 64 channel Biosemi Active2 EEG system. A novel aspect of our experiment was the design of a stimulus sets optimized to differentiate perceptual and articulatory neural representations. Overt production onset times were determined using PRAAT and MATLAB scripts, and EEG data were preprocessed using EEGLAB. RSA analyses were done using MATLAB2022a, with a 30ms sliding window and a cross-validated squared Euclidean distance metric with multivariate noise normalization.
Results and Discussion: Preliminary RSA results for the neural representations are shown over three neural “searchlights” over the frontal, temporal, and parietal regions. We found evidence for neural selectivity for speech sounds in temporal sensors following perception of the stimulus.

During overt production, we also found evidence of increased neural selectivity with a latency of approximately 100ms *prior* to speech production, which allowed us to probe the nature of these representations during motor planning as well as during overt production. These preliminary results provide initial evidence for the sensorimotor transformations between articulatory (motor-based) and perceptual (sound-based) neural representations in key speech processing regions.

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Poster

326. Human Language Processing and Production

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Topic: H.11. Language

Title: Real-time assessment of respiratory parameters during speech production in verbal tasks with varying degree of cognitive load

Authors: *M. GULLSVÅG, C. RODRÍGUEZ-ARANDA;
Dept. of psychology, UiT Arctic Univ. of Norway, Tromsø, Norway

Abstract: Background. Respiratory function is linked to sensory, affective, and cognitive processes and it is affected by environmental constraints such as cognitive load. It has been suggested that specific cognitive processes such as working memory or executive functioning may impact breathing. **Aim.** The present investigation aims to evaluate whether respiratory outcomes vary as a function of verbal tasks assessing different cognitive processes and with different difficulty levels. To our knowledge, no previous investigation has attempted to address this issue during actual speech production of verbal tasks. Additionally, the study implements a novel methodology for the evaluation of respiratory parameters during execution of verbal tasks in real time. **Method.** Thirty (16 women) healthy young adults, (age M = 25.37 years), participated in the study. The association lung-cognitive functioning was explored during execution of 4 verbal tasks presented in a computer monitor: reading, naming, and 2 verbal fluency tests (semantic/phonemic). The Phonatory Aerodynamic System (PAS) consisting of a spirometer, microphone and facemask was used to acquire simultaneously, verbal responses and respiratory measurements (e.g., peak expiratory/inspiratory airflow, expiratory/inspiratory volume, expiratory/inspiratory airflow duration). Data were analyzed with one-way repeated measures MANOVA. **Results**. Preliminary analyses showed significant differences in respiratory parameters specifically for the verbal tasks with higher demands of cognitive load, i.e., verbal fluency tasks. Of interest is the difference observed in peak expiratory airflow, which has been found to be a sensitive measure of physical and cognitive function in populations at risk of cognitive decline. **Conclusions.** The data suggest that respiratory measures during speech production vary as a function of cognitive load in healthy younger adults, which is line with

earlier studies using silent conditions. Our findings contribute to this line of research by assessing respiratory measures during actual verbal production (i.e., speech breathing), which is still an issue little explored in the literature.

Disclosures: **M. Gullsvåg:** None. **C. Rodríguez-Aranda:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.04

Topic: H.11. Language

Title: Assistive completion of agrammatic aphasic sentences: a transfer learning approach using neurolinguistics-based synthetic dataset

Authors: *R. MISRA, S. S. MISHRA, T. K. GANDHI;
Electrical Engin., Indian Inst. of Technol. Delhi, New Delhi, India

Abstract: Damage to the inferior frontal gyrus (Broca's area) can cause agrammatic aphasia wherein patients, although able to comprehend, lack the ability to form complete sentences. Difficulties arising due to this communication gap can be alleviated by developing assistive technology that reads aphasic sentences and suggests complete sentences to the patient. However, computational research on language disorders is limited due to the lack of large datasets of affected speech. In this study, we propose a solution by generating a synthetic dataset with sentences modeled after neurolinguistic studies on agrammatic aphasia. For dataset creation, the C4 corpus was cleaned by eliminating sentences longer than 15 words and ones containing questions or symbols due to the lack of corresponding quantitative studies. Further, part-of-speech tagging was used to omit determiners, prepositions, and copulas with a probability of 0.9 and adjectives/adverbs with a probability of 0.5. Tree parsing of sentences was done to classify them as complex if the noun phrase/verb phrase ratio was > 2. These complex sentences were then rejected with a probability of 0.8 to match the simple/complex sentence (S/C) ratio of aphasic speech. Finally, a training dataset of 20,000 aphasic-target sentence pairs was generated which had mean length of utterance (L) = 6.99 words, S/C = 9.08, noun/verb (N/V) ratio = 2.41. The testing dataset had 10,000 pairs with L = 7.01 words, S/C = 9.11, N/V = 2.44. The training dataset was used to fine-tune the T5 model (pre-trained on the mC4 corpus). The training paradigm was as follows: learning rate = 4e-5, Adam optimizer, batch size = 16, epochs = 3. The trained model yields 5 possible reconstructed sentences given an aphasic sentence. The performance of the model on the testing dataset was evaluated using the BLEU score and cosine semantic similarity of predicted and target sentences. Affirming results were obtained with an average BLEU score = 0.827/1.00 and semantic similarity = 0.904/1.00. These results offer proof of the concept that a synthetic dataset based on the linguistic features of agrammatic aphasia can be used as an effective proxy for clinically collected data of smaller sizes. The present study highlights our attempt to bridge the research gap between neuroscience, linguistics, and natural

language processing. Detailed quantitative characterization of aphasic speech is required to build more accurate linguistic models. While disorders like Broca's aphasia offer a small sample size of patients and data, synthetic linguistic models like ours offer extensive scope for developing assistive technology and rehabilitation monitoring.

Disclosures: R. Misra: None. **S.S. Mishra:** None. **T.K. Gandhi:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.05

Topic: H.11. Language

Title: Brain plasticity and figurative thinking

Authors: *M. ORKODASHVILI;
Vanderbilt Univ., Tbilisi, Georgia

Abstract: The paper looks into the influence of the so-called *figurative thinking* on the development of neuroplasticity in monolingual, bilingual and multilingual brains. *Figurative thinking* involves understanding of linguistic units and linguo-pragmatic means with non-literal meanings such as certain abstract notions (often of polysemantic nature), metaphors, and humor. The paper analyzes the effects of the recognition of abstract notions, metaphors, and humor in monolingual, bilingual and multilingual individuals on the development of their cognitive capacities. It is hypothesized that transferring, explaining and understanding abstract notions, metaphors, and humor across languages is more difficult than transferring, explaining and understanding simple and concrete concepts or actions (e.g. concrete nouns and verbs denoting simple actions). As a result of systematic mental exercise, subjects develop three important advantageous features of cognitive capacity: 1) quicker response time to understanding expressions and statements with non-literal meanings; 2) multitasking ability of understanding and reacting (or proacting) to simultaneous multiple tasks such as simultaneous translation and interpretation; and 3) quicker context anticipation. All three advantages indicate higher brain plasticity. The study so far has come to the following findings:
a) bilinguals and multilinguals are 85-95 milliseconds quicker in recognizing and understanding non-literal meanings as opposed to monolinguals; b) monolinguals are 45-55 milliseconds quicker understanding complex texts after being exposed to the metaphoric expressions for a while; c) subjects who have been exposed to metaphoric expressions and texts report the ability of performing two tasks at a time such as simultaneous translation in multiple languages more often than those who have not been exposed to such non-literal expressions; d) furthermore, in the process of reading a new text, the subjects who have been exposed to metaphoric expressions, anticipate the subsequent words, actions or concepts from the context with 78 % precision, as compared to those who have not been exposed to such expressions and who reveal only 22 % precision of anticipation.

Disclosures: M. Orkodashvili: None.

Poster

326. Human Language Processing and Production

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.06

Topic: H.11. Language

Support: Alexander Humboldt Foundation (Postdoctoral Fellowships)
DFG BR 5908/1 -1
DFG NI 618/11 -1

Title: Crows can produce center-embedded recursive sequences

Authors: *D. A. LIAO, K. F. BRECHT, M. J. JOHNSTON, A. NIEDER;
Univ. of Tübingen, Tübingen, Germany

Abstract: Recursion, the cognitive capacity to embed an element structure within similar structures, is often considered a foundation of symbolic competence and a uniquely human capability. To understand its evolution, we can study the recursive aptitudes of our extant relatives via the comparative approach. We adopted the behavioral protocol of a recent study (Ferrigno et al., 2020) demonstrating that humans and non-human primates can grasp recursive sequences. We presented the same training lists of bracket pair stimuli (e.g., [ ], { }) to crows - corvid songbirds - who were instructed to peck in a specified order. The crows were then tested for their ability to spontaneously transfer center-embedded structure to novel pairings of brackets. These crows, birds phylogenetically distant from primates, also possess recursive capacities; they outperformed macaques and were on par with young children. Furthermore, the crows continued exhibiting high performance after extension to deeper embeddings (i.e. longer sequence lengths). These results demonstrate that recursive capabilities are not limited to the primate genealogy and may have evolved separately from, or prior to, human symbolic competence in different taxa.

Disclosures: D.A. Liao: None. **K.F. Brecht:** None. **M.J. Johnston:** None. **A. Nieder:** None.

Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.07

Topic: H.11. Language

Support: Whitehall foundation
NIDCD, NIH (CRCNS)
Burroughs Wellcome Fund

Title: Is the semantic system organized based on semantics or timescales?

Authors: *S. JAIN[1], A. G. HUTH[2];
[1]Computer Sci., [2]Computer Science, Neurosci., The Univ. of Texas At Austin, Austin, TX

Abstract: The semantic system comprises a group of regions in the cerebral cortex that represent the meaning of language. Currently, its functional organization is viewed through two disparate axes: the timescale at which regions integrate information, or the semantic domains those regions represent. Here, we jointly analyzed these properties to understand how the brain represents meaning at multiple timescales. To do this, we built voxelwise models that simulated cortical responses to multi-word sequences. Our fMRI experiment comprised 7 participants listening to 5 hours of English stories. Models were built by first extracting features of each 11-word sequence in the stimuli from GPT, a neural language model, and then using ridge regression to map features to elicited BOLD responses in each voxel. We measured performance on a held-out set and found significant predictions throughout the semantic system. To characterize semantic selectivity, we used the models to predict each voxel's response to 104,205 diverse sequences. The top sequences per voxel revealed nuanced descriptions of semantic properties. We then characterized how each voxel integrates information across a top sequence by measuring its sensitivity to different constituent words. We did this by individually removing each word in the sequence, predicting the response to the ablated sequence, and comparing it to the original response. This showed that some voxels are lexical—sensitive to only one word in a sequence—while others are combinatorial, relying on multiple words. Finally, we investigated the relationship between these two functional properties and their anatomical organization. We found diverse integration behavior within each semantic concept network, with some regions capturing combinatorial semantics and others representing lexical information. However, concrete concepts were, on average, less combinatorial than abstract, suggesting diversity across networks. We then investigated if these global differences prevailed locally such that the integration behavior of voxels was determined by their semantic selectivity and not anatomical location. Although true for many regions, we found that relative integration behavior across regions was the same for each network. For example, emotion voxels in a small patch of prefrontal cortex combined information over more words than numeric voxels in the same region, but each group was more combinatorial than its semantic counterpart in middle temporal gyrus. This suggests that the semantic system is organized hierarchically based on timescales, but the absolute scale at which different semantic concepts are represented greatly varies.

Disclosures: S. Jain: None. **A.G. Huth:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

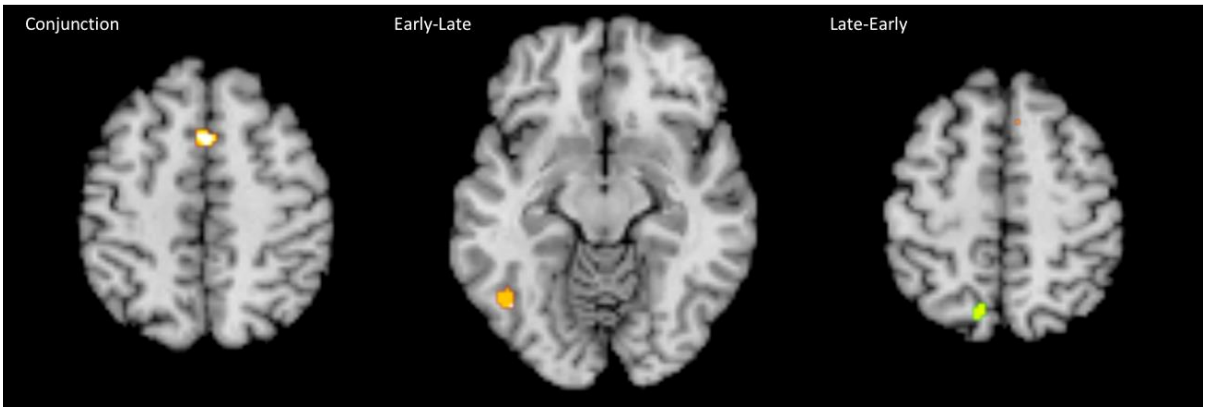
Program #/Poster #: 326.08

Topic: H.11. Language

Title: Functional task-switching networks in early and late bilinguals: an ALE meta-analysis

Authors: *A. KRAFNICK[1], K. KRAJ[1], D. SHARRAH[1], A. DANYLKIV[1,2];
[1]Dominican Univ., River Forest, IL; [2]Univ. of Illinois Chicago, Chicago, IL

Abstract: The potential cognitive and neural benefits of speaking multiple languages continues to be debated and discussed (Bialystok, 2017; Antoniou, 2019), and recent meta-analyses have examined the language and task-switching networks in bilingualism finding both language task specific and domain general areas involved in cognitive control (Tao et al., 2021; Jiao et al, 2022). However, heterogeneity in individual studies goes beyond task and includes factors such as L2 age of acquisition and proficiency. Here we performed a meta-analysis on language and task-switching whole-brain fMRI studies of bilingual/multilingual individuals. Using Pubmed and Google Scholar, 2,340 studies were identified in searches, and selected down to 10 studies with eligible contrasts in early bilinguals (L2 acquisition at kindergarten age or younger) and 14 studies with eligible contrasts in late bilinguals (L2 acquisition after the start of formal schooling). Using GingerALE we performed meta-analyses on each group ($p<0.001$ uncorrected voxelwise threshold, minimum cluster size 150mm^3 in line with the recent meta-analyses cited above); and conjunction/disjunction analyses to look for overlap and distinct clusters for each group ($p<0.05$, 1000 permutations, minimum cluster size 50mm^3). The conjunction analysis found 1 cluster (464mm^3) spanning left superior and medial frontal gyrus. The Early-Late comparison found 2 clusters. One in left fusiform gyrus (504mm^3) and one in right superior frontal gyrus (72mm^3). Finally, the Late-Early comparison also found 2 clusters, both primarily in the left precuneus (264 and 144mm^3), with the second cluster extending into the cuneus. While the number of eligible studies in each group is still relatively low, the results here suggest that there may be differences in the cognitive control network for early and late bilinguals. Continued understanding of how factors like age of acquisition, proficiency, and characteristics of the L2 itself interact with age of acquisition will be helpful in determining the overall profile of cognitive control in bilingualism.



Disclosures: **A. Krafnick:** None. **K. Kraj:** None. **D. Sharrah:** None. **A. Danylkiv:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.09

Topic: H.11. Language

Title: EEG functional connectivity analyses before and after one-month English training using Cloze Test

Authors: *N. NAGAIGUCHI[1], Y. TANAKA[2], S. YAZAWA[3], K. HIRAKI[3], S. SHIMADA[2]; [1]Grad. Sch. of Sci. and Technol., [2]Meiji Univ., Kawasaki, Japan; [3]Grad. Sch. of Arts and Sci., The Univ. of Tokyo, Tokyo, Japan

Abstract: A Cloze Test is a type of fill-in-the-blank test that measures a subject's language ability by guessing a word from the preceding and following sentences. Cloze Test is available in two answer formats: the multiple-choice format, in which the examinee selects the appropriate answer from a list of choices, and the fill-in-the-blank format, in which the examinee writes their answer. While research on the learning effects of the Cloze Test has been conducted, there has been little research focusing on the answer format of the Cloze Test. In this study, we examine the effects of learning methods with the Cloze Test in terms of changes in brain activity while answering English questions.The subjects were twenty healthy male university students (mean age 20.5 ± SD 1.69). Tests were administered before and after a training period of approximately one month. The training content included the Cloze Test, in which blanks were provided for long sentences, and the Cartoon Cloze Test, in which blanks were provided for cartoon dialogues. For the two types of Cloze Tests, 10 subjects were trained in the multiple-choice format, and the other 10 subjects were trained in the fill-in-the-blank format, during the one-month training period. In the pre- and post-tests, all subjects were given two types of questions: "multiple-choice" questions in which they had to answer the words that fit the blanks in the sentences from the choices, and "rearrangement" questions in which they had to rearrange the words in the sentences in the correct order and then answer the words in the specified order. Functional connectivity analysis was conducted based on the measured Electroencephalography (EEG) data. The power spectra values for each channel were also calculated for the subjects' EEG data. The calculated connectivity and power spectra values were compared between pre- and post-tests for each group.Comparing the connectivity between the fill-in-the-blank and the multiple-choice training groups during the post-test administration of multiple-choice and rearrangement questions, the fill-in-the-blank group showed significantly higher connectivity in the left frontal lobe than the multiple-choice group. When the mean power spectra values at post-test were compared between groups, the fill-in-the-blank group was significantly larger in the left frontal channel in the alpha and theta bands for both multiple-choice and rearrangement questions. These findings suggest that more brain activity is occurring in the fill-in-the-blank group than in the multiple-choice group in brain regions related to parsing and semantic decision making, especially in the left frontal lobe.

Disclosures: **N. Nagaiguchi:** None. **Y. Tanaka:** None. **S. Yazawa:** None. **K. Hiraki:** None. **S. Shimada:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.10

Topic: H.11. Language

Title: Eeg functional connectivity analyses while solving different types of second-language cloze test

Authors: *W. ZHAO[1], Y. TANAKA[2], F. HASHIKAWA[1], S. SHIMADA[3];
[1]Electrical Engin., Meiji Grad. School, Sci. and Technol., [2]Sch. of Sci. and Technol., [3]Electronics and Bioinformatics, Sci. and Technol., Meiji Univ., Tokyo, Japan

Abstract: Cloze test is a type of fill-in-the-blank test that measures a subject's second-language ability by guessing a word from the preceding and following sentences, used especially in the field of educational technology. Cloze test has been studied mainly from the perspective of its measurement capability, but few studies have focused on the effect of different response formats on learning performance. In this study, we examined the effects of different response formats of the Cloze Test on changes in brain activity and functional connectivity among brain regions. We measured electroencephalography (EEG) data from non-native English speakers when answering two types of Cloze test formats: the "multiple-choice" format, in which the subjects select the appropriate answer from a list of choices, and the "fill-in-the-blank" format, in which the subjects enter their answer in a blank. Sixteen male subjects participated in the experiment (mean age 21.9 ± SD 0.8). The multiple-choice question condition and the fill-in-the-blank question condition were conducted twice, alternating. Half of the subjects took the multiple-choice question first, while the others took the fill-in-the-blank question first. We used Total Interdependence (TI) as a measure of EEG functional connectivity. TI values were estimated by calculating amplitude squared coherence using the Welch method. The obtained TI values were examined for significant differences between the two conditions of the task by conducting t-tests. Comparing the functional connectivity between the multiple-choice and the fill-in-the-blank question, we found significantly higher connectivity between the right frontal lobe and the occipital lobe during the multiple-choice question. Connectivity between the right inferior frontal gyrus and other components of the executive control network is known to be associated with individual differences in the amount of vocabulary. On the other hand, the left inferior frontal gyrus showed significantly higher connectivity with the left parietal and right frontal lobes during the fill-in-the-blank question. The left frontal lobe is the brain region related to parsing and semantic decision making. Taken together, our study showed that there were differences in brain connectivity depending on the test format, such that multiple-choice format was associated with lexical processing and fill-in-the-blank with parsing and semantic decision making.

Disclosures: W. Zhao: None. **Y. Tanaka:** None. **F. Hashikawa:** None. **S. Shimada:** None.

Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.11

Topic: H.11. Language

Support: NIH Grant U01NS113339

Title: A long-lasting aftereffect of the McGurk Effect

Authors: A. LADO[1], Y. ZHANG[1], J. F. MAGNOTTI[1], A. NATH[2], A. MAASØ[3], *M. S. BEAUCHAMP[1];
[1]Dept. of Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [2]2Department of Neurosurg., Univ. of Texas Med. Br., Galveston, TX; [3]Dept. of Media and Communications, Univ. of Oslo, Oslo, Norway

Abstract: Auditory information from the voice of the talker and visual information from the face of the talker provide independent cues about the contents of speech. Cue conflict resulting from audiovisual incongruency can produce unexpected percepts. In the McGurk effect, integration of auditory "ba" and visual "ga" can produce a "da" percept. We report the surprising finding that repeatedly experiencing the McGurk effect results in a long-lasting aftereffect influencing auditory-only speech perception. Thirty online participants completed a 16-day experimental protocol. On day 1 ("pre-test") participants reported their perception of 27 auditory-only recordings of "ba", "da" and "ga" syllables from eighteen different talkers, including talkers AM and AN. On days 2 to 15 ("training") participants viewed one minute of McGurk syllables (auditory "ba"+visual "ga") spoken by talkers AM and AN. On day 16 ("post-test") participants reported their perception of the same 27 auditory-only recordings presented during the pre-test.In the pre-test, accuracy was uniformly high, indicating that participants were attentive to the stimuli; "ba" recordings from talkers AM and AN were always perceived as "ba". During training, McGurk stimuli were perceived as "da" on 35% of presentations, with considerable variability across participants (range 0% to 100%). In the post-test, participants accurately reported the identity of the auditory-only syllables with two exceptions. Surprisingly, many participants (12/30; 40%) reported at least one "da" percept in response to the auditory-only "ba" recordings from talkers AM and AN, even though no visual information was presented. For these 12 participants, the rate of McGurk effect during training was four-fold higher than for other participants (66% vs. 14%, p=0.0000042). Participants who did not experience the McGurk effect showed no training effect, suggesting that repeatedly experiencing the McGurk effect remapped participants' representation of the auditory component of the McGurk stimulus, so that what was initially perceived as a "ba" became mapped to the "da" region of perceptual space, minimizing incongruity between the encoded auditory and visual cues. The phenomenon was specific to individual talkers; the "ba" recordings of talkers AM and AN were perceived as "da", while "ba" recordings from other talkers were unaffected. The remapping was unsupervised, since no experimenter feedback was given at any time. Importantly, the perceptual change was long-lasting and endured in additional testing sessions conducted weeks after training.

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Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.12

Topic: H.11. Language

Support: 'Chen Guang' project supported by Shanghai Municipal Education Commission and Shanghai Education Development Foundation (WBH4307002)

Title: Disambiguation and semantic drifting of arbitrary reference of the "shenme…dou" structure: from experimental evidence

Authors: *Z. WANG[1], K. XU[2];
[1]Dept. of Chinese Languages and Literature,, Fudan Univ., Shanghai, China; [2]Fudan Univ., Inst. of Modern Languages and Linguistics, Shanghai, China

Abstract: The Chinese word *shenme* (roughly corresponding to English 'what') has been much surveyed for its double roles as both interrogative in questions and non-interrogative in statements when combined with *dou* (roughly corresponding to English 'all'), indicating the meaning of the arbitrary reference. However, some studies claimed that the semantic content of '*shenme + dou*' structure often leaded to the ambiguity and the possible effect of syntactic structure on the interpretation of this structure has been rarely investigated. Therefore, this study conducted four behavioral experiments adopted from Zhou and Crain (2011)'s Question-Statement Task to examine how mature speakers of Mandarin interpret *shenme* in sentences containing *dou* in written and spoken forms with topic or non-topic constructions. In the Question-Statement task, test sentences were presented following a story background acted out by puppet characters. The subjects were required to determine the truth value of the sentence if they perceived it as a statement, or make their assertion to a possible question. Furthermore, an eye-tracker study was designed to verify when and how the ambiguity of the '*shenme + dou*' structure arises. The behavioral data showed that *shenme* was more likely to be interpreted as non-interrogative in a topic structure. In topic sentences (e.g., *shenme shuiguo tuzi Dou meiyou chi*. 'The rabbits ate none of the fruit.'), *shenme* at the object position was more accepted as non-interrogative in written sentences (82.35%) than in spoken sentences (50%). While in non-topic sentences (e.g., *shenme ma Dou meiyou mai bingqilin*. 'None of the horses bought ice creams.'), *shenme* at the subject position was recognized as non-interrogative also in more written sentences (55.88%) than spoken sentences (14.71%). Afterwards, the eye-tracking data indicated that the readers fixated significantly longer time at *dou* when *shenme* at the object position to realize topicalization, implying that topicalization could to some extent highlight the follow-up cues and the meaning of *dou*. However, when *shenme* was at the subject position, *dou* achieved the shortest time compared to other words. More attention seems to be shifted on the recognition

of the negation word, thus the participants tend to recognize the sentence as a question. Together, the results reveal that different comprehension of sentences with *shenme* is significantly affected by the interaction between syntactic structures and sentence representations, providing evidence for the interface between syntax and semantics. Moreover, this study provides a good example for the experimental application on the linguistic debate.

Disclosures: **Z. Wang:** None. **K. Xu:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.13

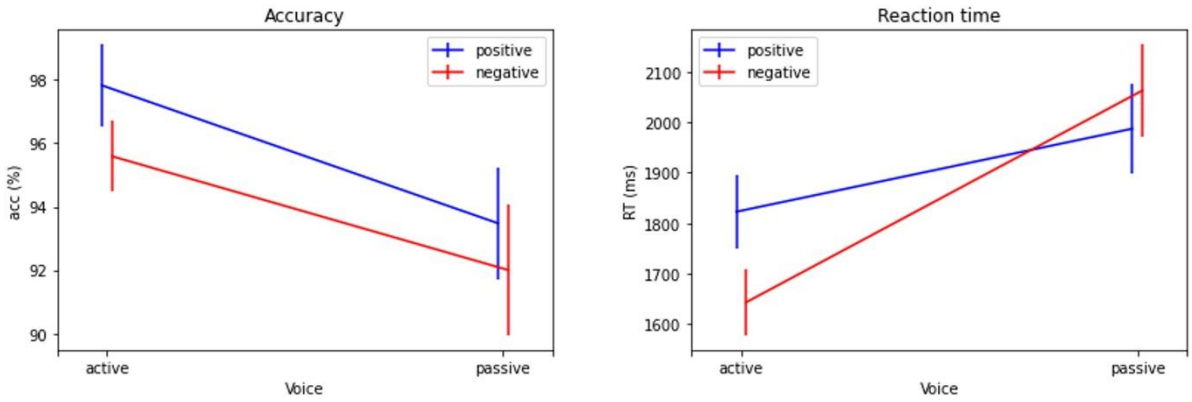
Topic: H.11. Language

Title: Negativity bias suppression in passives: an interaction between voice and verb connotation in Thai pragmatic sentences

Authors: *K. JONGMEKWAMSUK[1], W. SIRIBOONPIPATTANA[1], K. BOONLERT[1], S. CHUNAMCHAI[1], C. CHUNHARAS[2];
[1]Cognitive Clin. & Computat. Neurosci. Lab, Fac. of Med., Chulalongkorn Univ., Bangkok, Thailand; [2]Chula Neurosci. Ctr., King Chulalongkorn Mem. Hospital, Thai Red Cross Society, Bangkok, Thailand

Abstract: Past studies have shown that passive sentences revealed poorer performance than active sentences. Verb annotation, on the contrary, was inconsistent though the majority reported that negative verbs were found to be more difficult than positive verbs; this is also known as a positivity bias. The interaction between voice and verb connotation is scarcely studied across various language groups. Thai language has a unique syntactic structure and contains higher usages of passive sentences. Since passive-negative sentences are commonly seen in Thai, we thus hypothesized that there will be effects of voice on verb connotation. 22 healthy individuals ages 20 to 40 were asked to perform a binary sentence-picture matching task. Each individual participated in 48 trials. Each trial consisted of either one of four conditions: active-positive sentence, active-negative sentence, passive-positive sentence, and passive-negative sentence. Results showed that accuracy in the active voice was greater than that in the passive voice ($p = 0.0076$) without differences in verb connotation. Active voice, on the other hand, revealed a faster response than in the passive voice ($p = 0.0004$). In the active verb tasks, participants who heard negative sentences responded faster than those who heard positive sentences ($p = 0.0174$). This result may suggest the existence of a negativity bias when participants hear the sentences in the active voice. However, no differences in reaction time were observed in sentences made in the passive voice. The interaction between voice and verb connotation was tested with 2-way ANOVA ($p = 0.043$). There was asymmetry within the main effect of voice which possibly manifested as suppression of negativity bias in sentences with passive form. These findings can be described as influences of both culture and language that altered language comprehension.

Therefore, we will explore further in the clinical population, specifically patients with aphasia, to get more insight into the interaction between voice and verb connotation and in cultural aspects.



Disclosures: **K. Jongmekwamsuk:** None. **W. Siriboonpipattana:** None. **K. Boonlert:** None. **S. Chunamchai:** None. **C. Chunharas:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.14

Title: WITHDRAWN

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.15

Topic: H.11. Language

Title: An unconscious effect of Chinese classifiers on object categorization

Authors: *C. Y. LI, K. J. HOLMES, E. CANSECO-GONZALEZ;
Reed Col., Portland, OR

Abstract: Does the grammatical system of our native language influence how we perceive and categorize objects? We assessed object categorization and its neural correlates among speakers of Chinese, in which all nouns are modified by classifiers—morphemes that group objects based on semantic features like shape, function, or animacy. In Experiment 1, Chinese-English

bilinguals and English monolinguals rated pairs of pictures based on their similarity. Both groups rated the objects as more similar when they came from the same taxonomic category (e.g., vegetables) than from different categories, but also when their Chinese labels shared the same classifier than when they did not. In Experiment 2, Chinese-English bilinguals and English monolinguals completed a taxonomic categorization task using triads of pictures, while their electrophysiological response (ERPs) to the third picture was recorded. Unbeknownst to the participants, the third object either shared or did not share the same Chinese classifier as the first two. No effect of taxonomic relatedness or classifier congruency was observed in the behavioral results for either group. However, the ERP data revealed a taxonomic relatedness effect in both groups, and notably, a negativity on different-classifier trials only in the bilingual group. Together, these results suggest that speakers of non-classifier languages are sensitive to the semantic features that underlie classifier categories, but that only speakers of classifier languages unconsciously access these features when they are task-irrelevant. Our findings provide insight into how and when grammatical knowledge shapes object categorization.

Disclosures: C.Y. Li: None. **K.J. Holmes:** None. **E. Canseco-Gonzalez:** None.

Poster

326. Human Language Processing and Production

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 326.16

Topic: H.11. Language

Support: NIH R01 NS109367
NSF 1912286
1R01NS115929

Title: Two distinct prefrontal networks for semantic integration and articulatory planning

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Abstract: The spatiotemporal neural dynamics in frontal cortex underlying speech production and word retrieval remain poorly understood. Traditionally, the inferior frontal gyrus (IFG) has been implicated in articulatory, syntactic and semantic processes. Growing evidence has implicated the posterior MFG (area 55b) and dorsal precentral gyrus as critical for language. We hypothesized that articulatory preparation, lexical retrieval, and semantic integration may not be localized to one region but rather may be distributed in nature. We leveraged a battery of language production tasks including picture naming, word reading, auditory naming, auditory word repetition, and auditory sentence completion with 24 neurosurgical patients undergoing

Electrocorticographic monitoring. Focusing on high gamma broadband (70-150 Hz), we reported unique profiles of task-related neural recruitment per region during perception and production across all 1035 significant electrodes (sustained activity for over 100ms compared to baseline, t-test $p<0.05$). We found specific enhancement in IFG and MFG related to semantic load (ANOVA, post hoc t-test $p < 0.01$). In order to test how distributed these networks were, we employed an unsupervised clustering approach in a data-driven manner without an anatomical bias. This approach revealed two new networks in frontal cortex with differentiated articulatory and semantic specificity. One cluster of electrodes, centered in IFG and precentral cortices, showed comparable activity prior to speech onset across all tasks. The second cluster, centered on the border of IFG and MFG, showed strong task-selectivity prior to articulation with significantly greater (t-test, $p<0.001$) activity for tasks requiring semantic access. In order to investigate the nature of these two clusters, we performed representational similarity analysis across the auditory naming and sentence completion tasks. Neural covariance across time-locked to perception in the semantic cluster highly correlated with the semantic embeddings of the changing auditory stimuli (based on the last 3 layers of a deep neural network GPT-2 model, spearman correlation $p<0.001$ rho= 0.53, 0.27, respectively for each task) which quickly diminished as speech production onset approached. The articulatory cluster, however, was strongly correlated with phonetic information. Our results suggest two distinct language production components distributed across frontal cortices, a preparatory motor-related component agnostic to task, and a component recruited specifically as semantic integration is required.

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Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.17

Topic: H.11. Language

Support: NIH DP1HD091948

Title: Language models mediate speaker-to-listener coupling in natural conversations

Authors: *Z. ZADA[1], S. A. NASTASE[2], A. Y. GOLDSTEIN[2], U. HASSON[2];
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Abstract: How does language mediate brain-to-brain coupling during face-to-face communication between speakers and listeners? People rely on the shared meaning of words to successfully communicate. Most studies of the neural basis of language processing, however, have been constrained to studying single speakers in isolation, and cannot speak to the spontaneous, contextual, and communicative nature of real-world dialogue. The challenge of

developing formal models that can accommodate the complexity of real-world language has hindered our ability to capture the brain-to-brain linguistic coupling between interlocutors. Here, we explore whether the linguistic embedding space learned by deep language models (DLMs) can serve as a common intermediary model for aligning brains during communication. In five dyadic pairs of epilepsy patients, we recorded electocortigraphic (ECoG) brain activity during free-form, face-to-face conversations. We used time-resolved transcriptions to extract context-sensitive word embeddings from the high-performing DLM GPT-2. We estimated encoding models for the speaker and listener to predict the neural activity of each word at varying lags from the word embeddings using 10-fold cross-validation. To assess speaker-to-listener linguistic coupling, we tested whether the speaker's model predictions for a given region of interest (ROI) correlated with the listener's neural signal at each ROI. We also performed a control analysis using random embeddings that contain no linguistic information. We found widespread inter-regional speaker-listener coupling between the speaker's language production system and the listener's comprehension systems; i.e., inferior frontal gyrus (IFG), precentral cortex (PREC), and postcentral cortex (PSTC), and superior temporal gyrus (STG, r=.365, r=.3, r=.195, respectively). We investigated the temporal profile of coupling between the speaker's PREC and the listener's STG areas across lags and found that the linguistic features of the speaker's pre-word-onset activity (-0.438 ms) best modeled the listener's post-word-onset responses (+125 ms). Our work is an early attempt to model context-dependent, word-level neural activity during free, spontaneous conversations. We develop a method that leverages a linguistic embedding space to capture shared meaning in brain-to-brain coupling. Our results reveal inter-regional, time-resolved linguistic coupling between speaker and listener. Moreover, our findings suggest that a shared linguistic space aligns brains and translates internal states between interlocutors in conversation.

Disclosures: **Z. Zada:** None. **S.A. Nastase:** None. **A.Y. Goldstein:** None. **U. Hasson:** None.

Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.18

Topic: H.11. Language

Support: NCCR Evolving Language, Swiss National Science Foundation Agreement #51NF40_180888"

Title: Prosodic cues enhance the encoding of syntactic information during naturalistic speech perception

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Main, Germany; [3]Neurolog. Surgery, New York Univ., San Francisco, CA; [4]Univ. of Vienna, Vienna, Austria

Abstract: Recent work has tried to shed light on the cortical encoding of the different levels of the linguistic hierarchy in ecologically-valid scenarios, in order to drive a more natural exploration of our brain dynamics. This approach allows researchers to better assess the location and time windows at which abstract concepts like syntactic operations are localized in the cortex, together with the representations of lower-level features of human speech, such as prosody. Given that prosody can serve as a cue to guide interpretations and predictions of syntactic structure, a crucial knowledge gap is how these two neurally encoded features interact to shape the predictability and robustness of speech processing. Here we specifically test whether the cortical encoding of syntactic representations can be enhanced by acoustic cues such as those carried by prosody.We analyzed a previously published MEG dataset recorded from 11 participants while they listened to 4 different TED talks. Two sets of features were extracted from the stimuli. The first was the strength of the prosodic boundaries for each word in the sentences. The distribution of this feature was modelled via two gamma mixture models, in order to assign words to two groups with low or high prosodic boundary strength (PBS). The second was the syntactic feature. This was characterized by the presence or absence of left-sided dependencies (LD) per word, as obtained from a transition-based dependency parser. This putatively represents the incremental grouping of word relations that the human brain computes over time. Subsequently, we trained a logistic classifier to learn the mapping between LD and brain activity. Crucially, this model was trained only on words with low PBS, in order to avoid any bias regarding the syntax-prosody interaction. We then tested the model's classification accuracy on the set of words that presented high PBS and LD, as well as high PBS and no LD. These generalizations allowed us to understand the presence and specificity of prosodic enhancement on syntactic operations by (1) contrasting the two levels of prosodic content on words with LD, and (2) contrasting the impact of prosodic boosting in words with versus ones without LD, respectively.Both contrasts revealed that prosodic content (i.e. presence of high PBS) improved the decoding performance for the classification of words with LD, suggesting that prosodic cues enhance our neural processing of higher-level, more abstract linguistic features. This prosodic ‘boosting’ has likely evolved to assist the processing of specific components of syntactic structure over time, thus facilitating language comprehension.

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Poster

326. Human Language Processing and Production

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Program #/Poster #: 326.19

Topic: H.11. Language

Support: FWO Grant G0D8520N

Title: Electroencephalography reveals emphasized scale-free network properties in aphasia compared to the healthy condition during natural speech listening

Authors: *R. MEHRARAM, J. KRIES, P. DE CLERCQ, T. FRANCART, M. VANDERMOSTEN;
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Abstract: Aphasia is a language disorder commonly caused by a stroke in the left hemisphere, which affects communication abilities. Recent fMRI works have reported altered functional brain network features associated with aphasia (Siegel et al., 2018; Chen et al., 2021). Despite the diagnostic potential of EEG network analysis for diverse pathological conditions, there is a lack of EEG-based network studies on aphasia. In this work we investigated EEG-based network alterations associated with aphasia.
Our sample comprised 25 persons with post-stroke aphasia (PWA) and 22 age-matched healthy control (HC) participants. High density EEG was recorded while the participants listened to a 25-minute-long story. Artifact removal was performed using an automated pipeline, and connectivity was measured at delta (1-4 Hz), theta (4.5-7 Hz), alpha (7.5-14 Hz), beta (15-30 Hz) and low-gamma (31-49 Hz) bands using the weighted phase lag index (Vinck et al., 2011). Differential subnetworks were obtained through the Network Based Statistics (NBS), and network graphs in the affected frequency bands were thresholded between 3-40% of the strongest connections. Graph measures were compared between groups with two-tailed Mann-Whitney U tests ($P < 0.05$). Robustness of the network was assessed with 1) iterative removal of nodes with the highest node-strength, 2) measurement of the average characteristic path length at each step, and 3) comparison between groups of the percentage of removed nodes at which the maximal peak of the path length occurred.
The NBS revealed a stronger subnetwork in PWA compared to HC within the theta-band between left temporo-parietal regions and right frontal and posterior regions ($P < 0.005$). In the low-gamma band, we found a hypo-connected left posterior-anterior network in PWA ($P < 0.03$). Node strength and clustering coefficient in PWA were higher over the left parietal regions, whilst path length and small-worldness were lower within the theta-band network. This latter was less robust to targeted node-removal in PWA, as path length showed an earlier peak with respect to percentage of removed nodes compared to HC.
Our results showed increased interhemispheric EEG theta-band functional synchronization and left hypoconnectivity in the low-gamma-band network in PWA. The graph theory analysis showed a more pronounced scale-free and reduced small-world organization of the theta-band network in aphasia, i.e. higher hubness within the left language region and lower robustness to targeted damage. Although a scale-free highly connected network is typical of a healthy brain, it may reflect here a compensatory mechanism to preserve language processing in aphasia.

Disclosures: R. Mehraram: None. **J. Kries:** None. **P. De Clercq:** None. **T. Francart:** None. **M. Vandermosten:** None.

Poster

326. Human Language Processing and Production

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Support: FWO Grant 1S40122N
FWO Grant G0D8520N

Title: Decreased Neural Envelope Tracking in Persons with Aphasia

Authors: *P. DE CLERCQ, J. KRIES, R. MEHRARAM, T. FRANCART, M. VANDERMOSTEN;
Katholieke Univ. Leuven, Katholieke Univ. Leuven, Leuven, Belgium

Abstract: Introduction Aphasia is a language disorder impairing the ability to communicate, commonly caused by a stroke in the left hemisphere. EEG-based research currently relies on event-related potential paradigms which involve artificial stimuli that require many repetitions. A potential new avenue is to use natural speech stimuli. The envelope of continuous speech is tracked by the human brain and contains important cues for speech understanding, including acoustic processing cues and boundaries of lexical units (syllables and words). Here, we investigated, for the first time, whether EEG-based neural tracking of the envelope can be used as a neural marker for speech understanding in persons with post-stroke aphasia (PWA). **Methods** Our sample included 27 PWA in the chronic phase (> 6 months post-stroke) and 22 healthy age-matched controls (HC). PWA suffered a stroke in the left hemisphere or bilaterally and had to score below a cut-off threshold on an aphasia screening test in the acute phase for inclusion. Hearing ability was not different between groups as assessed with participant audiograms. Participants listened to a 24-minutes long story while their EEG was recorded. Neural envelope tracking was analyzed using mutual information (MI) as a measure of statistical dependency between the envelope and the EEG, with higher values reflecting higher neural envelope tracking. At behavioral level, a language and an acoustic processing task were administered, i.e., the Dutch naming test (NBT) and the rise time task (RTT), respectively. In the latter task, a higher score reflects worse performance, as it represents the threshold in ms at which the participant can detect a difference in the rate of change in amplitude at sound onset. **Results** PWA had worse performance on both the NBT (Wilcoxon signed-rank, $W=489$, $p<.001$) and the RTT ($W=94$, $p=.001$) compared to HC. Concerning the EEG data, the mean MI was significantly lower in PWA ($W=454$, $p<.001$). Within the aphasia group, MI was not significantly correlated with the NBT (Spearman $r=.24$, $p=.29$), whilst it negatively correlated with the RTT ($r=-.71$, $p<.001$). A partial correlation correcting for age, hearing ability and NBT remained significant ($r=-.69$, $p=.001$). **Conclusion** Neural envelope tracking of natural speech is decreased in PWA compared to HC. Envelope tracking was strongly associated with the RTT, meaning that higher neural tracking correlated with better acoustic processing skills. Effects were not attributed to age, hearing loss or productive language deficits. These results pave the way towards an objective diagnostic test for acoustic processing in PWA, a deficit often being overlooked in aphasia research.

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Poster

326. Human Language Processing and Production

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Topic: H.11. Language

Support: NIH R01DC016345
Department of Veterans Affairs R&D I01CX001290-01A1

Title: Behavioral Cluster Lesion-Symptom Mapping in Chronic Stroke

Authors: T. J. HERRON[1], K. SCHENDEL[2], B. CURRAN[2], N. F. DRONKERS[3], M. IVANOVA[3], *J. BALDO[2];
[1]Neurology, Res., [2]VA Northern California Hlth. Care Syst., Martinez, CA; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Missing data in cognitive test batteries is a barrier to clustering patients with similar behavioral profiles and relating those profiles to distinct stroke lesion patterns. In the current study, we used multiple imputation and clustering to generate associated lesion-behavior maps in a large cohort of well-characterized stroke patients using behavioral cluster lesion-symptom mapping (BCLSM). Retrospective data were sampled from 197 chronic left hemisphere stroke survivors (mean age 62 years, SD=11) who completed a battery consisting of 39 individual behavioral tests. A subset of 177 patients had scores on more than 25% of tests and were kept for further analysis. A Bayesian imputation model using chained equations generated 100 complete datasets (Fig A). The imputed datasets' mean and standard deviations were used to generate a patient-paired distance metric that incorporates uncertainty due to the imputed values. UPGMA clustering was used on the distance matrix in order to identify clusters of size k=4 and 7 (Fig C). The behavioral patterns were mapped to brain areas for 160 of the patients who had lesion masks (Fig D) generated from MRI or CT scans. An approximate binomial distribution statistic was computed to identify voxels that were lesioned disproportionately in patients inside a behavioral cluster. A cluster size-based permutation test generated significant results for several clusters. Behavioral clustering results (Fig B) resulted in 2 clusters that identified patients with severe or mild deficits across most tests in the battery. A third cluster identified patients who performed poorly on general cognitive tests but not language-specific tests. There were also two significant BCLSM results (Fig E&F). One was a large left lateral temporal lobe area associated with poor performance on all tests. The other cluster map was a large left parieto-frontal area associated with the general cognitive deficit behavioral cluster. These findings show that distinct language-cognitive profiles can be associated with specific stroke lesion patterns in chronic patients using BCLSM.

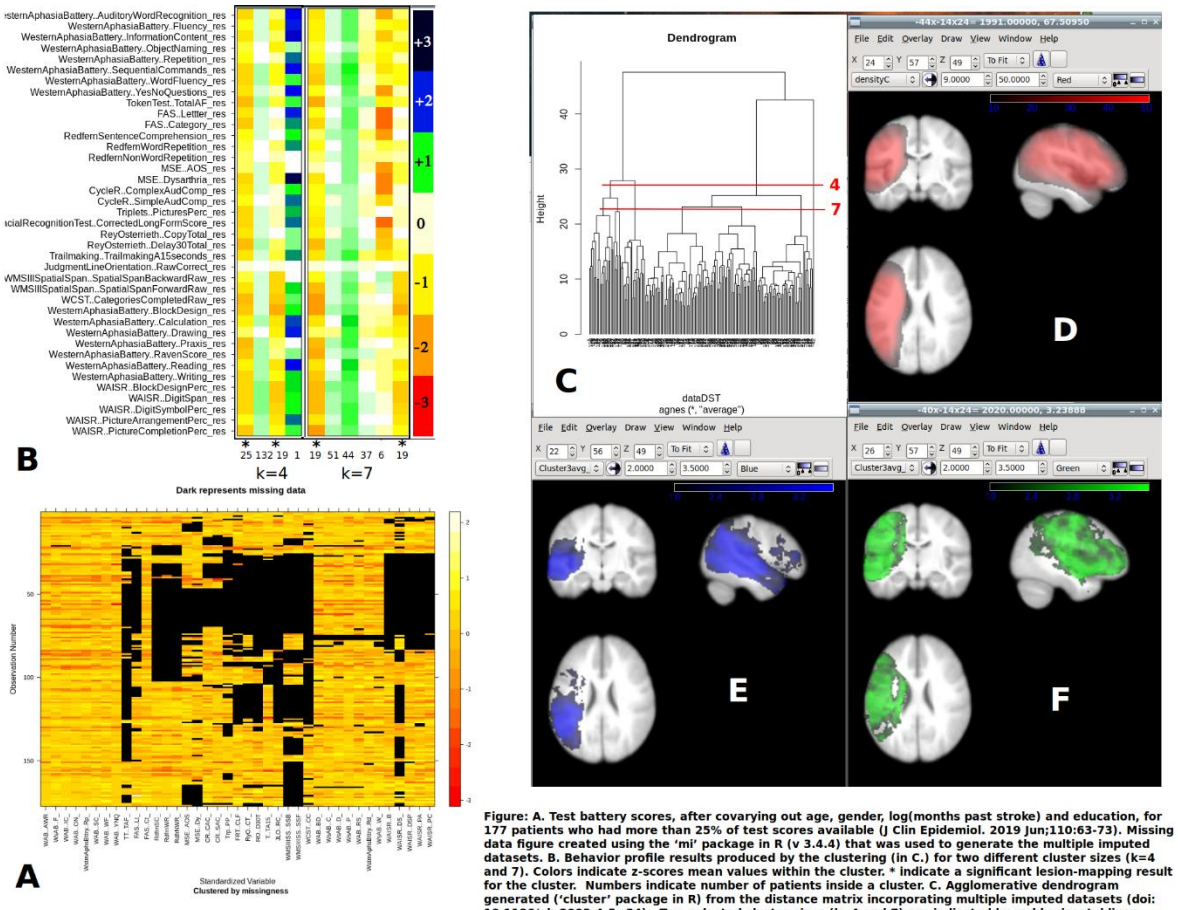

Figure: A. Test battery scores, after covarying out age, gender, log(months past stroke) and education, for 177 patients who had more than 25% of test scores available (J Clin Epidemiol. 2019 Jun;110:63-73). Missing data figure created using the 'mi' package in R (v 3.4.4) that was used to generate the multiple imputed datasets. B. Behavior profile results produced by the clustering (in C.) for two different cluster sizes (k=4 and 7). Colors indicate z-scores mean values within the cluster. * indicate a significant lesion-mapping result for the cluster. Numbers indicate number of patients inside a cluster. C. Agglomerative dendrogram generated ('cluster' package in R) from the distance matrix incorporating multiple imputed datasets (doi: 10.1186/gb-2003-4-5-r34). Two selected cluster sizes (k=4 and 7) are indicated by red horizontal lines. D. Lesion mask of the 160 patients included in the BCLSM analysis. Only voxels with lesions in at last 9 patients were used in BCLSM analysis. E. BCLSM mapped areas indicated in blue for the first k=4 cluster (and 1st k=7 cluster) associated with poor test scores across the board. E. BCLSM mapped areas indicated in green for the third k=4 cluster (and 6th k=7 cluster) associated with poor general cognitive test scores more than poor language-related scores.

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Poster

326. Human Language Processing and Production

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Support: NIH (NIDCD) Grant K01DC016904, Comprehensive pre-surgical identification of the critical language network in tumor patients
the Spanish Association against Cancer, Grant PROYE20005CARR
Spanish Ministry of Economy and Competitiveness, Grant RTI2018 093547 B I00, (LANGCONN) MCIU/AEI/FEDER, UE

Title: How does a brain tumor within a language network mediate grey matter volume?

Authors: *M. M. POłCZYńSKA[1], L. MANSO-ORTEGA[3], L. DE FRUTO[3], S. GISBERT-MUÑOZ[3], N. SALAMON[2], J. QIAO[2], P. WALSHAW[1], I. QUIÑONES[3];
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Abstract: A brain tumor in the left hemisphere can decrease language laterality as assessed with functional magnetic resonance imaging (fMRI) (Połczyńska et al. 2021). It remains unclear whether the decreased laterality is associated with structural changes in the left or right hemisphere, particularly within the language network. This study uses voxel-based morphometry to examine how tumor laterality affects language structures and its right hemisphere homologues before brain surgery. High-resolution MR T1-weighted MPRAGE images were obtained from thirty-one adult patients (19 females, mean age = 47.6 years, SD = 13.9 years; 7 left-handed). Eighteen subjects had brain tumors in the left language-dominant hemisphere, and 13 had tumors in the right hemisphere. Language dominance was confirmed with pre-operative fMRI and neuropsychological evaluations. Using the Automated Anatomical Labeling atlas (Rolls et al. 2020), we defined 11 regions of interest (ROIs) per hemisphere known to subserve language function (Dronkers et al. 2004). A 3D reconstruction of the tumor was created for each patient with the MRIcroGL software. Voxels within the lesions were excluded from the segmented tissues using SPM12. Total intracranial volume was calculated for each participant to normalize ROI volume values. We conducted a repeated-measures ANOVA with the volume per region as the dependent variable. Age, gender, handedness, tumor type, and lesion size were nuisance covariates. Two within-subject factors were included: (1) ROI lateralization with the ROIs being ipsilateral or contralateral to the language dominant hemisphere, and (2) the 11 ROIs. Tumor laterality (left versus right) served as a between-subject factor. We report significant double and triple interactions: ROI lateralization x tumor lateralization ($F=25.927$, $p<0.001$), and region lateralization x ROI x tumor lateralization ($F=6.299$, $p<0.001$). Importantly, patients with tumors in the left hemisphere had more volume in the right ROIs relative to the same ROIs in the left hemisphere (e.g., pars opercularis, $p<0.002$, Bonferroni corrected). Individuals with tumors in the right hemisphere had similar volume values for their left and right ROIs, with three larger structures on the left. To conclude, we found that patients with tumors in the left (but not right) hemisphere had larger ROI volumes in the right hemisphere compared to the left. These results demonstrate that in patients with left hemisphere tumors, there may be an association between decreased functional laterality for language and increased structural volume in the right hemisphere.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.01

Topic: H.13. Schizophrenia

Support: CIHR PJT-153175
CAMH foundation discovery fund
CAMH foundation Krembil startup fund
Ontario graduate scholarship

Title: Cell type-specific changes in abundance and transcriptomic regulation of cortical neurons across psychiatric disorders

Authors: *K. ARBABI[1,2], D. F. NEWTON[3], H. OH[1], D. A. LEWIS[4], M. DAVIE[1], S. TRIPATHY[1,2], E. SIBILLE[1,2];
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Abstract: Psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SCZ) are characterized by altered cognition and mood: brain functions that depend on information processing by cortical cell microcircuits. We hypothesize that disease-associated changes in psychiatric disorders are mediated by cell type-specific changes in the abundance and transcriptional regulation of neuronal cells forming these microcircuits, specifically glutamatergic pyramidal (PYR) neurons and vasoactive intestinal peptide- (VIP), somatostatin- (SST), and parvalbumin- (PV) expressing GABAergic interneurons. Here, we surveyed postmortem samples from subgenual anterior cingulate cortex in 19 tetrads (N = 76 total subjects) of control, MDD, BD, and SCZ subjects matched on age, sex, and tissue quality (University of Pittsburgh Brain Tissue Donation Program). RNAscope staining targeting *Slc17a7 + Sst* or *Pvalb + Vip* was performed to label specific neuronal subpopulations and determine changes in cell density. Subsequent laser-capture microdissection isolated 130 labeled cells per subject for each of two excitatory (L2/3 and L5/6 PYR) and three inhibitory (SST, VIP, PV) neuronal subpopulations, which were independently pooled prior to RNA sequencing. We identified trending and significant loss of SST (Wilcoxon, $p = 0.097$), PV ($p = 0.085$), and VIP ($p = 0.033$) interneuron cell density for SCZ subjects relative to controls, following correction of age, sex, and PMI. Deconvolution of prefrontal cortex bulk tissue transcriptomes from the broader subject cohort (N = 63 overlapping, Pitt CommonMind) corroborated significantly reduced cell type proportions for all interneurons in BD and SCZ cases compared to controls. Lastly, differential expression analysis revealed shared and distinct disease-associated transcriptomic signatures in each neuronal subpopulation, with the greatest amount of differentially expressed genes observed in SST cells in SCZ, and highlighted biological processes such as cellular bioenergetics and mRNA processing. Taken together, our results implicate considerable neuron type-specific changes underlying the pathophysiology of psychiatric illness.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.02

Topic: H.13. Schizophrenia

Support: Grant from Takeda Pharmaceuticals Ltd. to Cardiff University

Title: A non-synonymous variant within SLC39A8 is associated with poorer premorbid IQ in patients with schizophrenia and generalised cognitive function in the UKBB

Authors: *S. E. SMART[1], S. E. LEGGE[1], E. FENNER[1], L. HUBBARD[1], A. F. PARDIÑAS[1], G. E. WOOLWAY[1], R. HODGSON[2], P. MAYCOX[2], E. N. SMITH[2], J. HALL[1], P. A. HOLMANS[1], I. JONES[1], M. C. O'DONOVAN[1], M. J. OWEN[1], L. S. WILKINSON[1], J. T. R. WALTERS[1];
[1]Cardiff Univ., Cardiff, United Kingdom; [2]Takeda Pharmaceuticals Ltd., San Diego, CA

Abstract: rs13107325 is a non-synonymous single nucleotide polymorphism within the gene SLC39A8, which encodes a ZIP8 transporter. The minor allele at rs13107325 has been associated with poorer cognitive performance and lower IQ in large genome-wide association studies. It has also been associated with schizophrenia, a psychiatric disorder marked by hallucinations, delusions, and impaired cognitive ability. The aim of this study was to test whether rs13107325 is associated with cognition in a sample of schizophrenia cases.
Participants with schizophrenia or schizoaffective disorder (depressed type) were included from a cross-sectional multi-ancestry clinical sample. The National Adult Reading Test was used as an estimate of premorbid IQ. Confirmatory factor analysis was used to estimate symptom dimensions (SAPS, SANS, MATRICS) - the current cognitive symptom dimension was composed of all MATRICS domains except social cognition. In regression analyses, adjusted for age at interview (AAI), gender, genetic principal components, and ancestry, rs13107325 was not associated with current cognitive ability, but carrying more schizophrenia-risk alleles was associated with poorer premorbid IQ (N=595, beta = -0.22 (95%CI: -0.41, -0.04), P-value = 0.018, R^2_{ADJ} = 4.23%).
Given these findings we estimated ‘premorbid’ cognition in a non-psychiatric population of European participants from the UK Biobank. We excluded participants with an intellectual disability and used principal component analysis to calculate generalised cognitive function (‘g’) - using tests of numeric memory, reaction time, pair matching, and trail making. We regressed ‘g’ against rs13107325, while adjusting for performance IQ (a test of fluid intelligence), to estimate ‘premorbid’ cognition. This model was also adjusted for the covariates listed above and AAI[2]. Carrying more schizophrenia-risk alleles at rs13107325 was associated with poorer ‘g’

(N=61,578, beta = -0.02 (95%CI: -0.04, -5.70), P-value = 0.010, R^2_{ADJ} = 31.09%). rs13107325 appears to have specificity for 'g' or premorbid IQ, over and above performance IQ. Future work will need to test whether generalised cognition, not impaired by psychiatric illness, accounts for the many pleiotropic effects of rs13107325 observed in the literature.

Disclosures: **S.E. Smart:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd.. **S.E. Legge:** None. **E. Fenner:** None. **L. Hubbard:** None. **A.F. Pardiñas:** None. **G.E. Woolway:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **R. Hodgson:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals Ltd. **P. Maycox:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals Ltd. **E.N. Smith:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals Ltd. **J. Hall:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **P.A. Holmans:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **I. Jones:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **M.C. O'Donovan:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **M.J. Owen:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **L.S. Wilkinson:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd. **J.T.R. Walters:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Takeda Pharmaceuticals Ltd..

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.03

Topic: H.13. Schizophrenia

Support: Silvio O. Conte Center P50 MH103222

Title: Prolonged kynurenic acid elevation during the prenatal period elicits electrophysiological and behavioral changes in adult mice

Authors: *S. MILOSAVLJEVIC[1], S. BEGGIATO[2], P. L. BROWN[3], M. A. R. THOMAS[3], M. V. PIROLI[1], K. V. SATHYASAIKUMAR[3], F. M. NOTARANGELO[3], R. SCHWARCZ[3], A. POCIVAVSEK[1];
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Abstract: Neuroactive metabolites of the kynurenine pathway (KP) of tryptophan degradation are believed to be involved in the pathophysiology of several psychiatric diseases, including schizophrenia (SZ) and bipolar disorder (BPD). Kynurenic acid (KYNA) has received special attention in this regard since its levels are elevated in the brain and CSF of persons with SZ and may be causally related to the cognitive dysfunctions seen in the disease. Previous studies in rats had indicated that abnormally high KYNA levels in the fetal brain may play a pathophysiologically significant role in this context ("EKyn model"; Notarangelo and Pocivavsek, 2017; Beggiato et al., 2018). In anticipation of future studies with genetically altered animals, we now fed pregnant C57Bl/6J mice either with the immediate KYNA precursor kynurenine (10 mg or 30 mg/day; EKyn) or with control mash (ECon) from embryonic day (ED) 11 to ED 18. Separate cohorts of adult male and female offspring were then assessed postnatally in electrophysiological, behavioral, and biochemical studies. Ex vivo recording of evoked local field potentials in coronal brain slices obtained in adulthood (postnatal days 56-70) revealed that prenatal treatment with 30 mg kynurenine/day promoted a longer contralateral response latency in EKyn compared to ECon mice ($P<0.05$), which likely reflects a decline in white matter function. Adult male EKyn mice also showed significantly longer latency ($P<0.05$) and lower speed to enter the escape box during acquisition training when spatial learning was tested in the Barnes maze (BM). During subsequent reversal learning, the latency to escape into the goal box and the number of re-visits to the previous escape location were higher in male EKyn offspring compared to controls ($P<0.05$). Of note, female EKyn mice were not impaired in the BM. Measured in the cortex or hippocampus at PD 21 (pre-puberty), PD 35 (adolescence), and PD 56-70 (adulthood), the levels of the KP metabolites 3-hydroxykynurenine and KYNA did not differ between EKyn and ECon groups. Separately, in preparation of experiments designed to investigate the role of KYNA in the adverse long-term effects seen in EKyn mice, we verified by in vivo microdialysis that inhibition of KYNA neosynthesis with the potent kynurenine aminotransferase inhibitor PF-04859989 (30 mg/kg, s.c.) induces a rapid decrease in extracellular KYNA in the prefrontal cortex of adult EKyn animals. Taken together, our results indicate that prolonged exposure to kynurenine during embryonic development can be envisioned to provide a useful mouse model for studying the role of KYNA and other KP metabolites in the etiology of neuropsychiatric illnesses.

Disclosures: S. Milosavljevic: None. **S. Beggiato:** None. **P.L. Brown:** None. **M.A.R. Thomas:** None. **M.V. Piroli:** None. **K.V. Sathyasaikumar:** None. **F.M. Notarangelo:** None. **R. Schwarcz:** None. **A. Pocivavsek:** None.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.04

Topic: H.13. Schizophrenia

Title: Pre-gestational exposure to T. gondii produced maternal pathogenic antibodies that recognize fetal brain proteins as antigenic targets and reduces glutamate levels during adulthood in the rat brain cortex.

Authors: *M. MARTINEZ DAMAS[1], T. BLANCO AYALA[1], E. ROMERO NUÑEZ[1], G. VÁZQUEZ CERVANTES[1], D. GONZÁLEZ ESQUIVEL[1], B. CASTILLO CRUZ[1], S. MUÑIZ HERNÁNDEZ[2], B. PINEDA OLVERA[3], V. PEREZ DE LA CRUZ[1];
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Abstract: Toxoplasmosis is a parasitic infection caused by the protozoan Toxoplasma gondii (T. gondii) whose prevalence reaches around 30% of the world's population. There are studies that have described the serological profiles of anti-Toxoplasma immunoglobulins M and G (IgM and IgG) in patients with different neurodevelopmental disorders. In addition, prospective epidemiological studies indicate that toxoplasmosis is a risk factor for schizophrenia. However, the physical absence of the parasite and the high levels of anti-T.gondii IgG in these patients suggest an important role of maternal seroprevalence against this parasite during the neurodevelopment of the offspring. Previously, we showed that the progeny of mothers immunized prior gestation (PMIPG) with *T. gondii* lysates had behavioral and social impairments during adulthood possibly because of immune activation by T. gondii antigens. In this line, the aim of this study was to demonstrate that maternal anti-T.gondii IgG from mothers immunized pre-gestationally (MIPG) recognize antigenic targets in neonatal brains and also to determine the neurotransmitter levels in the offspring. Subcutaneous immunization of MIPG with T. gondii lysate (100 μg protein) or PBS was performed 3 times (once per week) prior gestation. First, IgG anti-T. gondii were determined in the rat serum of MIPG. Next, the anti-T.gondii IgG reactivity in the fetal brain was evaluated by immunofluorescent labeling using two strategies: 1) Histological sections of fetal brains (PD18) from MIPG-immunized dams were obtained to identify the presence of IgG and 2) Control fetal brains (non-immunized) were exposed to MIPG serum to test for reactivity. Additionally, glutamate levels were quantified in the brain cortex of the PMIPG (PD60). We detected IgG antibodies specific to T. gondii in the rat serum of MIPG. Our data showed reactivity between the serum of MIPG and fetal brain structures; in addition, IgG were found in the fetal brain from the progeny of MIPG. Parallelly, glutamate levels were found reduced in the brain cortex of PIMG young adults (PD60). Therefore, these results showed that maternal pathogenic antibodies from MIPG can recognize brain proteins as antigenic targets -possibly through molecular mimicry- during the

neurodevelopment, contributing to neurotransmitter alterations that could be related to the behavioral impairments previously observed in PMIPG.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.05

Topic: H.13. Schizophrenia

Support: Some funding has been provided by Takeda PLC, as part of a collaboration between Cardiff University and Takeda PLC.

Title: Assessing the validity of methods to report a schizophrenia diagnosis

Authors: *G. WOOLWAY[1], S. LEGGE[1], A. LYNHAM[1], S. SMART[1], L. HUBBARD[1], M. O'DONOVAN[1], M. OWEN[1], E. SMITH[2], R. HODGSON[2], P. MAYCOX[2], I. JONES[1], J. WALTERS[1];
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Abstract: Diagnoses in psychiatric research can be derived from various sources, including self-report, electronic health records (eHR), diagnostic research interviews and note reviews. Diagnostic interviews are considered the gold standard but are resource intensive and likely associated with bias. The impact of diagnosis source on research studies of schizophrenia has not been examined. This study investigates (i) the validity of self-reported schizophrenia against gold standard research diagnosis and (ii) differences in polygenic risk scores (PRS) and phenotypes between schizophrenia cases defined by self-report vs research interview and vs eHR diagnosis. Participants with schizophrenia or related psychotic disorders were included from the National Centre for Mental Health and CardiffCOGS (3,029) and UK Biobank (1,438). In our clinical sample, we assessed the positive predictive values (PPV) of self-reported diagnosis against a research diagnosis. In both samples, we compared schizophrenia and intelligence PRS and phenotypes using logistic regressions across the diagnosis groups. PRS were calculated using the latest GWAS. Liability-based r^2 was calculated to compare the variance explained to PGC studies. The PPV of self-reported schizophrenia to a research diagnosis of schizophrenia ranged from 0.70-0.75, which increased to 0.81-0.85 when including both schizophrenia and schizoaffective depressed research diagnoses. There were no significant differences in schizophrenia PRS when comparing self-report to research diagnosis in our sample (OR=0.98; p=0.71) or eHR diagnosis in UK Biobank (OR=1.01; p=0.91). Individuals with an eHR diagnosis had a slightly lower intelligence PRS (OR=0.85; p=0.05). Both samples liability r^2 fell within the range of the distribution of r^2 of PGC cohorts. Compared to self-report groups, participants with

a research diagnosis were younger (OR=0.77; p=$9.10x10^{-5}$), more likely to have an academic qualification (OR=1.48; p=0.018) and less likely to be employed (OR=0.59; p=0.012) and participants with an eHR diagnosis were less likely to be employed (OR= 0.56; p=0.0006) and have an academic qualification (OR=0.71; p=0.025). Most people with a self-reported diagnosis of schizophrenia received a schizophrenia or schizoaffective depressed research diagnosis. The variance explained in the self-report groups were comparable to research interview and eHR diagnosis groups, but there were phenotypic differences, indicating potential bias in both clinical and population samples. These findings indicate that less stringent methods of assessing a schizophrenia diagnosis may demonstrate a more representative sample.

Disclosures: **G. Woolway:** A. Employment/Salary (full or part-time):; Cardiff University. **S. Legge:** A. Employment/Salary (full or part-time):; Cardiff University. **A. Lynham:** A. Employment/Salary (full or part-time):; Cardiff University. **S. Smart:** A. Employment/Salary (full or part-time):; Cardiff University. **L. Hubbard:** A. Employment/Salary (full or part-time):; Cardiff University. **M. O'Donovan:** A. Employment/Salary (full or part-time):; Cardiff University. **M. Owen:** A. Employment/Salary (full or part-time):; Cardiff University. **E. Smith:** A. Employment/Salary (full or part-time):; Takeda PLC. **R. Hodgson:** A. Employment/Salary (full or part-time):; Takeda PLC. **P. Maycox:** A. Employment/Salary (full or part-time):; Takeda PLC. **I. Jones:** A. Employment/Salary (full or part-time):; Cardiff University. **J. Walters:** A. Employment/Salary (full or part-time):; Cardiff University.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.06

Topic: H.13. Schizophrenia

Support: NIH T32 GM007752
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Stein Institute for Research on Aging at UCSD

Title: Illuminating accelerated aging in schizophrenia by human plasma multi-omics

Authors: *L.-A. ROSSITTO[1,2], A. CAMPEAU[1,2], R. H. MILLS[1,2], T. STEVENS[6], M. MEEHAN[2], P. DORRESTEIN[2,1], R. DALY[3,4,5], T. T. NGUYEN[3,4,5], D. J. GONZALEZ[2,1], D. V. JESTE[3,4,5], V. HOOK[2,1,5];
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Abstract: Schizophrenia is a severe psychiatric disorder characterized by diverse psychopathic symptoms including delusions, hallucinations, psychosis, apathy, and cognitive impairment. A leading cause of disability worldwide, approximately 1% of the global population are afflicted with schizophrenia, underscoring the need for better understanding of and treatments for this disorder. Previous studies have identified altered aging in schizophrenia, including associated early morbidity, although they have failed to assess systemic markers and assess covariates. To address this gap, we leveraged state-of-the-art mass spectrometry technology to perform global, unbiased proteomic and metabolomic profiling of plasma from schizophrenia patients (n=54) and non-psychiatric controls (n=51). We assess molecular changes over six decades of life and across biological sex, identifying dysregulated pathways associated with accelerated aging in schizophrenia. We reveal a compendium of age-defined molecular factors associated with heightened disease risk in individuals with schizophrenia, including increased broad-scale inflammatory factors and metabolic dysfunction. Overall, this study advances our understanding of the molecular etiology of accelerated aging in schizophrenia and presents targetable determinants of schizophrenia-associated comorbidities and early morbidity.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 327.07

Topic: H.13. Schizophrenia

Support: National Institute of Mental Health Grant (2R01MH097879) award to J.A.G
Columbia University Translational Therapeutics Pilot award to J.A.G

Title: Emc10 reduction in human neurons and adult mouse brain rescues cellular and behavioral deficits linked to 22q11.2 deletion

Authors: *M. LACKINGER[1], P. THAKUR[1], A. DIAMANTOPOULOU[1], S. RAO[1], Y. CHEN[1], A. FERNG[3], C. MAZUR[3], H. KORDASIEWICZ[3], R. J. SHPRINTZEN[4], S. MARKX[2], B. XU[2], J. A. GOGOS[1];
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Abstract: Schizophrenia (SCZ) is a heterogeneous disease which makes it quite difficult to understand its pathophysiology, and makes the development of new treatments extremely challenging. Up to 1% of patients with SCZ carry 22q11.2 deletions, which increase disease risk

by ~30 fold, making this genomic lesion one of the strongest genetic risk factors for SCZ. Up-regulation of *Mirta22/Emc10* is a major transcriptional effect of the 22q11.2-associated microRNA dysregulation and underlies key cellular as well as behavioral deficits. EMC10 is a component of the ER membrane complex, which promotes membrane insertion of a subset of polytopic and tail-anchored membrane proteins. Here we show that *EMC10* expression is elevated in hiPSC-derived neurons from 22q11.2 deletion carriers and that reduction of *EMC10 levels* restores defects in neurite outgrowth and calcium signaling. Moreover, using injection of Antisense Oligonucleotides, we demonstrate that normalization of *Emc10* levels in adult mouse brain rescues social memory deficits. The observations that elevated *EMC10* expression is deleterious in 22q11.2 deletion carriers and that sustained elevation of *EMC10* throughout the adult life can interfere with neural processes point to manipulations of *EMC10* levels and downstream targets as a specific venue to ameliorate or even prevent disease progression in 22q11.2 deletion syndrome.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 327.08

Topic: H.13. Schizophrenia

Title: Probing isoform and cell-specific function of Akt in the brain in regards to cognition and behavior

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Abstract: AKT is a central protein kinase essential to several neuronal processes. AKT is present in most cell types in the brain, with three structurally similar isoforms (AKT1, AKT2, AKT3) encoded by different genes. Of these, AKT1 and AKT3 are expressed in excitatory neurons, with only AKT1 expressed in interneurons. Significantly, recent work from our lab demonstrated these isoforms have isoform and cell-specific roles in synaptic plasticity and sex-dependent effects on behavior. Additionally, Akt is implicated in many neurological disorders such as Alzheimer's disease (AD), autism spectrum disorder (ASD), and schizophrenia. Because AKT function plays a role in memory formation and the expression of anxiety-modeled behavior in rodents, it is important that we study the effect of AKT isoform deficiencies using a comprehensive battery of behavior tests and gene expression analysis. Interneuronal dysfunction has been proposed to contribute to the pathophysiology of neuropsychiatric disorders. Due to AKT's role in neurological disease and AKT1 is the only isoform found in interneurons, we

sought to elucidate AKT1's function in interneuronal regulation linked to cognition. Using mouse reagents that selectively remove AKT1 from interneurons, our studies revealed AKT1 interneuron-specific and sex dependent effects on anxiety-modeled behavior, spatial and contextual memory, and fear memory extinction. Because AKT is involved in numerous signaling pathways, we used an unbiased method to identify AKT1 regulated pathway interneurons that may contribute to the isoform- and sex-specific behaviors we observe. To do this, we isolated interneurons from our *Akt1* interneuronal specific knockout(KO) models and then performed RNASeq to identify gene expression differences between sexes and WT and *Akt1* KO interneurons. To confirm our RNASeq results, we used Q-PCR and western blot analyses. These genetic mouse models allow us to interrogate the role of interneuronal AKT1 in models of human neurological disorders. This may allow us to identify new therapeutic targets for treating and diagnosing diseases such as schizophrenia and AD. These data will also contribute valuable information about AKT1 function in the context of biological sex, a variable that plays a significant role in the manifestation of mental health disorders.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 327.09

Topic: H.13. Schizophrenia

Support: NIH Grant MH077851
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Title: Patterns of polygenic risk for schizophrenia in developmentally based subgroups of psychosis spectrum disorder

Authors: *M. Y. GOTRA[1], J. R. BISHOP[2], S. K. KEEDY[3], C. A. TAMMINGA[4], E. I. IVLEVA[4], G. D. PEARLSON[5,6], M. S. KESHAVAN[7], J. A. SWEENEY[8], E. S. GERSHON[3], B. A. CLEMENTZ[9], S. K. HILL[1];
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Abstract: Background: Psychotic disorders are characterized by cognitive impairments that vary considerably and may be associated with divergent biological and etiological mechanisms. Prior work has addressed heterogeneity of cognitive deficits by characterizing patients into cognitively similar groups based on the relationship between premorbid and current cognitive ability. Patients characterized by both low premorbid and current cognitive abilities may have greater genetic liability; however, differences in genetic risk have not been well examined in these cognitive subgroups. The present study examined differences in schizophrenia polygenic risk score (PRS) across three developmentally based cognitive subgroups in a sample of psychosis spectrum disorder patients. **Methods:** Participants were 330 psychosis spectrum patients (44% bipolar with psychotic features, 33% schizophrenia, and 23% schizoaffective disorder; mean age 36; 54% male) and 190 healthy controls (mean age 39; 54% male) of European ancestry from the Bipolar Schizophrenia Network on Intermediate Phenotypes 1 study who completed cognitive tests estimating premorbid intellect and current general cognitive functioning. Patients were categorized into groups using a regression-based discrepancy approach modeled on the healthy control sample. The groups included a 'preserved' group with average premorbid and current cognitive abilities, a 'deteriorated' group with average premorbid and low current cognitive abilities, and a 'compromised' group with low premorbid and current cognitive abilities. Analysis of covariance with the top principal components of ancestry was used to examine differences in 13 schizophrenia PRS p-value thresholds across these cognitive subgroups, with false discovery rate correction for multiple comparisons. **Results:** Only the deteriorated group had a higher PRS than healthy controls for all p-value thresholds. The compromised group had a higher PRS than healthy controls only for the most stringent threshold, while the preserved group had a higher PRS than healthy controls for less stringent thresholds. **Conclusions:** These findings suggest that the compromised group may be characterized by genetic risk that captures genes with the strongest and most significant relationship with schizophrenia, while the deteriorated group has the broadest polygenic association. In contrast, the preserved group may represent genetic risk characterized by influence from highly pleiotropic genes and potential interaction with factors that are not associated with genetic risk, such as perinatal complications or other non-genetic factors.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.10

Topic: H.13. Schizophrenia

Support: Support from the Stanley Center for Psychiatric Research

Title: Genomic and behavioral characterization of schizophrenia risk gene Xpo7 knockout mice

Authors: *A. J. LAWLER[1], M. T. W. KIM[1,2], N. NADAF[1], V. GAZESTANI[1], D. KWON[1], R. A. SZETO[1,2], C. VANDERBURG[1], N. SACHDEV[1], E. Z. MACOSKO[1,3];
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Abstract: Recent genetic studies identified that loss-of-function variants in the *Exportin 7* (*XPO7*) gene are strongly associated with schizophrenia risk (SCHEMA project; odds ratio 28.1, p = 2e-8). XPO7 is a broad-spectrum nuclear import and export receptor that is widely expressed throughout the body; the brain-specific functions of XPO7 and their connection to schizophrenia are largely unknown. We generated an *Xpo7*-deficient transgenic mouse strain to gain insight into the molecular activities of XPO7 in the brain and their phenotypic consequences. We explored differences between *Xpo7*-deficient mice and their wild type littermates using unbiased transcriptomics, proteomics, and behavioral phenotyping. Heterozygous and homozygous *Xpo7* knockout mice appeared generally healthy and exhibited hyperactive behaviors during open field assessment. Single nucleus RNA-seq from adult male and female mice revealed diverse coordinated and cell type-specific transcriptomic effects in the prefrontal cortex, midbrain, and cerebellum. The most affected cell types in *Xpo7*-deficient mice were excitatory neurons and astrocytic lineage cells, with particular emphasis in the cerebellum. Differentially expressed genes were enriched for functions in glutamatergic signaling (neurons) and sterol metabolism (astrocytes) across multiple brain regions, consistent with impaired glutamate homeostasis in the *Xpo7*-deficient brain. This transgenic mouse strain, informed by human genetics, provides a valuable new resource for investigating potential underlying mechanisms of schizophrenia in an intact, tractable nervous system.

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Poster

327. Psychosis: Cellular Mechanisms

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.11

Topic: H.13. Schizophrenia

Support: R01MH117431

Title: Blood Biomarkers for Psychotic Disorders: Biology and Practical Applications

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Abstract: Psychosis, an alteration of cognition, occurs inside the brain, but may have manifestations (imaging, electroencephalography, behaviors, peripheral molecular biomarkers) that can be objectively and quantitatively measured. Blood biomarkers that track core psychotic manifestations such as hallucinations and delusions could provide a window into the biology of psychosis as well as help with diagnosis and treatment. We endeavored to identify objective blood gene expression biomarkers for hallucinations and delusions, using a stepwise discovery, prioritization, validation, and testing in independent cohorts design. We were successful in identifying biomarkers that were predictive of high hallucinations and high delusions states, and of future psychiatric hospitalizations related to them, more so in women and when personalized by gender and diagnosis. Some of these biomarkers are increased in expression in high psychosis states (being putative risk genes), and others are decreased in expression (being putative protective/resilience genes). Top biomarkers for hallucinations that survived discovery, prioritization, validation and testing include PPP3CB, DLG1, ENPP2, ZEB2, and RTN4, which was genome-wide significant in previous GWAS studies and serves as a de facto positive control. Top biomarkers for delusions include AUTS2, NR4A2, MACROD2, PDP1, RORA, PDE4D, and TCF4, another genome-wide significant previous GWAS finding. Predictive biomarkers without prior genetic evidence include DHX36 and ACYP2 for hallucinations, and IL6ST for delusions. The top biological pathway involved in psychosis uncovered by our work is Rap1 signaling. The majority of the top biomarkers for hallucinations and delusions also had prior evidence of involvement in suicide, and all of them had evidence in other psychiatric disorders, providing a molecular underpinning for the co-morbidity with psychosis in those disorders. Some of the biomarkers are targets of existing drugs, of potential utility in patient stratification and pharmacogenomics approaches. Moreover, the biomarkers gene expression signatures yielded new drug candidates and natural compounds upon bioinformatics drug repurposing analyses. Our work may lead to improved diagnosis and treatment for transient, or chronic psychotic disorders, such as schizophrenia, that result in decreased quality of life and adverse outcomes including violence and suicide.

Disclosures: **A.B. Niculescu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindX Sciences. **M. Hill:** None. **O. MacKie:** None. **H. Le-Niculescu:** None. **K. Roseberry:** None. **S. Gill:** A. Employment/Salary (full or part-time):; MindX Sciences. **S. Kurian:** A. Employment/Salary (full or part-time):; MindX Sciences. **A. Shekhar:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MindX Sciences.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.12

Topic: H.13. Schizophrenia

Support: The Pritzker Neuropsychiatric Disorder Research Consortium

Title: Somatostatin fluorescent in situ hybridization in the frontal pole cortex (ba10): application for psychiatric disorders

Authors: *D. M. KROLEWSKI[1], V. KUMAR[1], A. MEDINA[1], M. FOLTZ[1], R. MYERS[2], F. S. LEE[3], J. D. BARCHAS[3], A. SCHATZBERG[4], B. BUNNEY[5], W. E. BUNNEY[5], H. AKIL[1], S. J. WATSON[1];
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Abstract: Background: Somatostatin (SST) peptide is produced by a specific subpopulation of inhibitory GABA-ergic interneurons which exhibit pronounced synaptic connectivity with distal dendrites of pyramidal neurons. Previous studies indicate SST participates in cognitive processes as SST mRNA is one of the more downregulated interneuron markers the dorsolateral prefrontal cortex in schizophrenia (SZ). Our laboratory recently employed qPCR to investigate SST gene expression in the frontal pole cortex (BA10); a region linked to multiple functions including those related to emotion, nociception, and pain. The latter study also revealed frontal pole SST mRNA to be amongst the most reduced transcripts in SZ. (Medina et al., 2022). To potentially uncover a more specific neuroanatomical foundation to these findings, we look to perform a BA10 cortical layer-based SST+ neuron analysis in SZ and bipolar disorder (BPD) subjects using fluorescent in situ hybridization. **Methods:** Frontal pole cortical tissue was obtained from the Brain Donor Program, University of California, Irvine. All samples had zero agonal factors and met stringent criteria for pH and PMI. Hybridization chain-reaction (HCR) fluorescent in situ hybridization (FISH) was performed on 30µm and 10µm-thick fresh-frozen cryosection. Images were captured on an Olympus Fluoview 3000 and SST+ cells quantified in stitched z-plane stacks with Amira and ImageJ software. **Results:** Using control subjects, we were able to achieve FISH methods that demonstrate clear visualization and quantification of frontal pole layer-specific SST+ neurons while eliminating lipofuscin autofluorescence. During testing, as expected, 30µm-thick sections displayed more SST+ cells versus 10µm, but neurons also appeared more fully labeled superficially and visually more uniform morphologically. SST+ neurons were widely present in layers II-VI with larger neurons in layers II/III and V versus those smaller in layer IV. Given this successful methodology, we are currently processing HCR-FISH for BA10 control, SZ, and BPD subjects for comparison (n=12/group). **Conclusion:** We have completed visual and quantitative methods for frontal pole cortex SST+ interneuron analyses in thicker cryosections. These results will allow for determining possible BA10 cortical layer-based neuropeptide differences in our ongoing study investigating psychiatric disorders.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.13

Topic: H.13. Schizophrenia

Support: NIH Grant MH077851

Title: Using aBSNIP biomarker fingerprinttostratify participants forclinical trials: testing the kynurenic acid hypothesis of cognitive impairment

Authors: *C. A. TAMMINGA[1], M. HUDGENS-HANEY[2], B. CLEMENTZ[3], E. GERSHON[4], S. KEEDY[4], G. PEARLSON[5], M. KESHAVAN[6], S. HILL[7], J. LUND[8], C. KOHLER[8], R. SCHWARCZ[9];
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Abstract: Using a BSNIP biomarker fingerprint to stratify participants for clinical trials: testing the kynurenic acid hypothesis of cognitive impairment C.A. Tamminga. M. Hudgens-Haney, B. Clementz, E. Gershon, S. Keedy, G. Pearlson, M. Keshavan, S. Hill, J. Lund, C. Kohler, R. Schwarcz The brain concentration of kynurenic acid (KYNA), on antagonist of both NMDA and α7 nicotinic acetylcholine receptors, is elevated in the prefrontal cortex of individuals with schizophrenia (SZ). Hypofunction of these receptors is implicated in the pathophysiology and cognitive deficits of the disease.Kynurenine 3-monooxygenase (KMO) and kynurenine aminotransferase II (KATII) regulate brain KYNA levels. Thus, a reduction in KMO activity, as seen in the brains of people with SZ, increases brain KYNA, and inhibition of KAT II, which produces most KYNA in the brain, could lead to a new approach to treat cognitive impairments.
In SZ cases (BA6 region), the KMO SNP *rs2275163 CC* (but not KMOSNP *rs2275163 CT/TT*) is associated with reduced KMO mRNA expression and elevated KYNA levels. Moreover, and unrelated to a SZ diagnosis, this genotype reduces predictive pursuit function and worsens visuospatial working memory (Wonodi et al., 2011).Based on these results, we now compared a "biomarker fingerprint" of KMO SNP *rs2275163 CC*(the "high KYNA" phenotype) versus *rs2275163 CT/TT* in a large cohort (N=200/genotype) of SZ individuals belonging to Biotypes 1 and 2, as defined by the (B-SNIP) consortium. For this analysis, we conducted four separate canonical discriminant analyses with the biotype cases (each bootstrapped 1000x with 95% sampling), evaluating (i) cognition (BACS and Stop Signal Task),(ii) EEG (evoked and intrinsic activity), (iii) oculomotor function (pro-/anti-saccades and smooth pursuit), (iv) cortical thickness, (v) cortical surface area, and (vi) cortical and subcortical regional volumes. The results revealed a 0.7 SD separation between *rs2275163 CC* and *rs2275163CT/TT* probands. Notably, probands with z-scores >0.5 had a >95% likelihood of carrying the*rs2275163 CC* genotype. The CC biomarker profile defines "high KYNA" and captures all such cases, secondary to any source.
These findings support using biomarkers as co-primary outcome measures to select study populations for clinical trials designed to test KAT II-modifying cognitive enhancers, by recruiting "high KYNA" populations. This approach of prospective enrichment of the patient

population through phenotyping is expected to reduce the size of trials as well as increase the likelihood of success by targeting SZ people with a “high KYNA” fingerprint.

Disclosures: **C.A. Tamminga:** 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.; 1/5 - Selective Antipsychotic Response to Clozapine in B-SNIP Biotype-1 (Clozapine). F. Consulting Fees (e.g., advisory boards); Astellas – Ad Hoc Advisor < $10,000, National Institutes of Health— member of a study section and do Ad Hoc, Sunovion Pharmaceutical – Ad Hoc Advisor < $10,000 Kynexis – Chairman of the Clinical Advisory Board, <$10,000, Kynexis – Chairman of the Clinical Advisory Board, <$10,000, Karuna—Scientific Advisory Board Member, <$10,000. Own company stock., Merck – Chairman of a Merck DSMB. **M. Hudgens-Haney:** None. **B. Clementz:** None. **E. Gershon:** None. **S. Keedy:** None. **G. Pearlson:** None. **M. Keshavan:** None. **S. Hill:** None. **J. Lund:** None. **C. Kohler:** None. **R. Schwarcz:** None.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.14

Topic: H.13. Schizophrenia

Support: U54 NS108874
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Stanley Center for Psychiatric Research

Title: Coding variants of CACNA1G derived from patients with schizophrenia, epilepsy, and neurodevelopmental delay display distinct biophysical phenotypes.

Authors: *S. JO[1], L. A. WANG[1], **N. BUDNIK**[1], D. BAEZ-NIETO[1], S. PARTHASARATHY[2], A. LINDY[3], K. RETTERER[3], I. HELBIG[2], J. Q. PAN[1];
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Abstract: *CACNA1G* encodes the low-voltage-activated $Ca_V3.1$ T-type calcium channel that is highly expressed in Purkinje neurons of the cerebellum, as well as throughout the thalamus and cortex. $Ca_V3.1$ T-type channels play important roles in sleep stabilization, cognitive function and motor memory. *CACNA1G* was implicated in schizophrenia, epilepsy and neurodevelopmental delay by exome sequencing studies. However, the molecular phenotypes of *CACNA1G* underlying the pathophysiology of these disorders are unknown. We analyzed coding variants of *CACNA1G* derived from three regions of the $Ca_V3.1$ channel where disease-derived variants were significantly enriched, together with those derived from control subjects in gnomAD. We performed functional evaluation of a set of 80+ *CACNA1G* variants using established recording

protocols (SyncroPatch384) and compared the functional properties among variants derived from SCHEMA (Schizophrenia exome meta-analysis consortium), Epi25 (Collaborative for Large-Scale Whole Genome Sequencing in Epilepsy) and population controls. The biophysical properties of each cell encoding a variant channel were extracted including the whole cell current density, the voltage-dependent activation/inactivation and the kinetics of inactivation and deactivation. We then compared the biophysical properties across the disease and control groups. Cells expressing variants derived from schizophrenia patients display significantly reduced maximum current density, while cells expressing variants derived from epilepsy patients show a significant leftward shift in the voltage-dependent activation. Interestingly, variants derived from neurodevelopmental delayed patients demonstrated significantly slowed inactivation kinetics. We obtained clinical information from a subset of variants and found that variants derived from patients with seizure have a significant leftward shift in the voltage-dependent activation, recapitulating the finding that shift in the voltage-dependent activation of $Ca_V3.1$ may contribute to epilepsy. In addition, leftward shift in the voltage-dependent activation is also found in patients with developmental abnormalities and delays. Taken together, the results of our functional analysis of an allelic series of *CACNA1G* variants across three disease and control cohorts reveal that distinct functional phenotypes of $Ca_V3.1$ channel may be associated with different pathophysiology in humans.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.15

Topic: H.13. Schizophrenia

Support: NIH grant P50 MH103222

Title: Positioned to “talk”: kynurenine aminotransferase II (KAT II) in the human prefrontal cortex is localized in astrocytes proximal to parvalbumin-containing neurons

Authors: G. E. HOFFMAN[1], S. M. CLARK[2], *R. SCHWARCZ[1];
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Abstract: A reduction in parvalbumin (PV)-containing inhibitory neurons in the prefrontal cortex (PFC) is believed to play a significant role in the pathophysiology of schizophrenia (SZ). As abnormal expression of several genes regulating astrocytic function in the PFC is observed in persons with SZ, dysregulation of astrocyte/neuron interactions may participate in functional abnormalities. Disruption of prefrontal GABAergic function by nanomolar concentrations of kynurenic acid (KYNA), an endogenous inhibitor of NMDA and α7 nicotinic receptor function,

has been shown in rats and may be of significance in this context since increased PFC KYNA levels are seen in SZ. In the rat brain, KYNA's major biosynthetic enzyme kynurenine aminotransferase II (KAT II) is almost exclusively found in protoplasmic astrocytes, but its cellular localization in the human brain is unknown. In an initial approach, we now used post-mortem PFC tissue (BA 9; N=4) from control subjects to visualize the cellular localization of KAT II and PV. In parallel, we evaluated coded tissue specimens from 4 people with SZ to gain preliminary insight into possible qualitative group differences (all tissues were provided by the Maryland Brain Collection). After confirming species cross-reactivity, our own anti-rat KAT II antibodies were used at dilutions up to 1:100,000. PV antisera (kindly provided by K. Baimbridge) were diluted up to 1:300,000. To reduce background staining and enhance detection of low antigen levels, a microwave-based methodology was applied together with the classic ABC Elite immunoperoxidase method using Ni-DAB ± tyramine signal amplification. In both control and SZ tissues, KAT II was identified in cells that were morphologically identifiable as protoplasmic astrocytes, as indicated by strong labeling of fine KAT II-containing processes and absence of staining in sites of the glial limitans. KAT II^+ cells were most numerous in PFC layers III-V and in underlying white matter, and staining appeared to be more prominent in SZ specimens. As anticipated, PV^+ neurons in layers II-V were less numerous in SZ, though amplification of the staining increased the number of detectable cells. Interestingly, greater KAT II staining seemed to be accompanied by lower PV immunoreactivity. These data raise the possibility that the reduced expression of PV in the PFC in SZ may be *causally* related to increased KYNA formation in adjacent KAT II-containing astrocytes. Detailed anatomical features of the contact between KAT II- and PV-containing cells in the PFC and their functional significance under normal and pathological conditions clearly require careful further examination.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.16

Topic: H.13. Schizophrenia

Support: CONACYT Grant 320520

Title: Molecular mimicry between Toxoplasma gondii Bcell epitopes and neurodevelopmental proteins: An immunoinformatics approach

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Abstract: *Toxoplasma gondii* is an obligate intracellular parasite that can infect humans and cause severe damage during pregnancy due to its ability to cross the placenta and infect the fetus. Congenital toxoplasmosis can cause long-term neurodevelopmental disabilities, including mental, behavioral and personality disorders. The aim of this study was to identify the occurrence of antigenic mimicry between *T. gondii* epitopes and key neurodevelopmental proteins. First, we obtained the FASTA sequences of *T. gondii* membrane and vesicular proteins using the ToxoDB platform; aminoacid sequences for brain proteins (neural progenitor cell, immature, and mature neuronal markers) were obtained from NCBI database. Then, we used the linear B cell epitope predictor from the Immune Epitope Database (IEDB) to identify B-cell epitopes using five different methods of prediction: Chou-Fassman, Emini, Kolaskar-Tongaonkar, Parker and Karplus-Schulz. All *T. gondii* and brain proteins were divided into consecutive polypeptides of 6 to 7 amino acids and for each polypeptide the probability of being part of an epitope was calculated. Thereafter, multiple alignments were performed to identify shared sequences between brain proteins and *T. gondii* predicted epitopes. For *T. gondii* membrane proteins, 647 (Emini), 181 (Karplus-Schulz), 139 (Kolaskar-Tongaonkar), 197 (Parker) and 97 (Chou) sequences were shared with brain proteins; and, 265 (Emini), 71 (Karplus-Schulz), 47 (Kolaskar-Tongaonkar), 73 (Parker) and 33 (Chou) sequences for *T. gondii* vesicle proteins. These shared sequences include several key neurodevelopmental proteins such as Brg1, Musashi1, Neurod1, Nestin, Cd15, Ascl1, Caspr and Ncam. We also identified shared antigenic epitopes in mature neuron markers specific for cholinergic (Ache, Chat and Schr), glutamatergic (Glutaminase, glutamine syntethase, NMDAR, vGlut), dopaminergic (Darp32, Nurr1, Th1,3,4, Foxa2, Net1, Lmx1b and Dat), and gabaergic lineages (Gabr1,2, Gad65,67 and vGAT). These results indicate that several *T. gondii* epitopes share specific sequences with proteins essential for neural differentiation, neural network formation and neurotransmission thus confirming molecular mimicry between *T. gondii* antigens and brain proteins.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.17

Topic: H.13. Schizophrenia

Support: UC Davis-Hong Kong University Collaboration Grant

Title: Polygenic scores are associated with early psychosis and specific symptom profiles of people with psychotic disorders

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Abstract: Much of the research in psychiatric genetics aims to delineate the links between genetic risk, neurological phenotypes, and clinical symptom presentation. With this goal in mind, we conducted a study aiming to associate polygenic burden with psychosis and with psychotic disorder endophenotypes. Patients presenting with early psychosis were recruited at the UC Davis Medical Center for psychiatric and neurological phenotyping as part of an ongoing research effort. For the present study, we included from this cohort 225 cases (22% female, average age 19.6) who progressed to a diagnosis of a psychotic disorder, as well as 126 matched controls (42% female, average age 19.7) with no neurological or psychiatric diagnoses. Subjects represented the demographics of the area served by the UC Davis Medical Center. DNA was extracted from subject blood draws and genotyped on the Illumina Infinium PsychArray-24 kit, which tests for 271,000 variants and is enriched for psychiatric markers. We also analyzed measures of global functioning, IQ, and task-based fMRI data. For psychosis subjects, we additionally looked at clinical ratings for positive symptoms, negative symptoms, and cognitive functioning. Schizophrenia (SZ) polygenic scores (PGSs) - based on recent GWAS data from the Psychiatric Genomics Consortium - were calculated using the lassosum method. As a secondary, exploratory outcome, we determined PGSs of pathways we defined that contained genes involved in different neurotransmitter systems. SZ PGS was significantly associated with psychosis case versus control status in our cohort. SZ PGS was also associated IQ in all subjects and negative symptoms in bipolar disorder (BD) subjects. fMRI association analyses are ongoing. In the exploratory analyses of neurotransmitter pathways, preliminary data show that GABA PGS was associated with case versus control status and with IQ in psychosis subjects. Glutamate PGS was associated with IQ in psychosis subjects and with negative symptoms in BD subjects. The findings from this study extend upon PGS as a risk factor for early psychosis and build a case for the use of PGS and pathway-specific PGS for the study of psychiatric symptoms.

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Poster

327. Psychosis: Cellular Mechanisms

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Title: Mechanism allowing environmental enrichment to rescue social discrimination memory in a 22q11.2DS mouse model

Authors: M. LOISY[1], M. AHMADI[1], A. FAFOURI[1], M. CHATEAU[1], C. VIOLLET[1], L. THERREAU[1], A. FARAH[1], J. COGNET[1], M. NOGUERES[2], J. CHABRY[2], V. CHEVALEYRE[1,3], ***R. A. PISKOROWSKI**[1,3];
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Abstract: Environmental factors are well-accepted to play a complex and interdependent role with genetic and epigenetic factors in numerous psychiatric diseases. Of particular importance is social cognition, as social impairment is among the earliest features of schizophrenia, correlating highly with poor functional outcome. While it has been clearly demonstrated that environmental factors can either preclude or precipitate pathogenesis in psychiatric diseases, the physiological and cellular mechanisms underlying these phenomena are poorly understood. In human postmortem studies for numerous disorders, hippocampal area CA2 has been shown to be particularly affected. Using a mouse model of the 22q11.2 deletion syndrome (22q11.2DS), we have shown that both parvalbumin-expressing (PV+) interneurons and pyramidal neurons (PNs) in this region undergo alterations that result in reduced activity in this region. We have found that three weeks in an enriched environment (EE) results in a depolarization of the resting membrane potential of CA2 pyramidal neurons is more depolarized for deletion mice and littermate controls. Furthermore, we show that this depolarization is due to changes in TREK-1 potassium channel conductance. Regulation of the TREK-1 conductance is linked to increased levels and activity of sPLA2G5 in EE, a secreted lipase enriched in hippocampal area CA2. The consequence of this environmentally induced change in intrinsic properties is that an endocannabinoid-mediated synaptic plasticity of inhibitory transmission is rescued in these animals. 22q11.2DS mice also have rescued social recognition memory after three weeks in an enriched environment, indicating that hippocampal area CA2 is highly sensitive to intrinsic states of the animal. We are optimistic these results may shed light on the mechanisms underlying effective non-pharmacological treatment for psychiatric disorders.

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Poster

327. Psychosis: Cellular Mechanisms

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Program #/Poster #: 327.19

Topic: H.13. Schizophrenia

Support: NIH Grant R01MH080234

Title: Unveiling the mechanisms of cortical dysfunction in a Setd1a-deficient mouse model of Schizophrenia risk

Authors: *P. APOSTOLOU[1], Y. CHEN[1], E. CANNAVÒ[1], Z. SUN[2], B. XU[2], S. LOMVARDAS[1], J. A. GOGOS[1];
[1]Mortimer B. Zuckerman Mind Behavior Inst., Columbia Univ., New York, NY; [2]Dept. of Psychiatry, Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Schizophrenia (SCZ) is a debilitating neuropsychiatric disorder that affects the mental, cognitive, and social abilities of ~1% of the human population worldwide. Extensive sequencing studies have identified rare *de novo* or inherited copy number variations that confer high risk for the disease. Within that context, our lab has previously linked loss-of-function (LoF) *De Novo* Mutations in *SETD1A* to risk for SCZ. This finding was recently confirmed by large scale exome sequencing studies, which identified SETD1A as the risk gene with the largest statistical support for SCZ. *SETD1A* encodes for a lysine methyltransferase that is best known as part of the Set/COMPASS complex (that mediates mono-, di-, and tri-methylation of the lysine 4 on the histone H3 protein) although it has additional roles in regulating gene expression. In enhancers, SETD1A associates and modulates the activity of cell-type specific auxiliary transcription factors, such as Myocyte Enhancer Factor-2 (MEF2). A number of these transcription factors are known regulators of activity-inducible gene expression raising the possibility that Setd1a function may be required for proper regulation of the activity-dependent transcriptome. To identify if and how *SETD1A* mutations lead to altered cortical pyramidal gene expression we are using primary cultures *in vitro* and a chemogenetic approach, in which neuronal activity is synchronously induced in mouse cortical pyramidal neurons, *in vivo*. We found that SETD1A binds enhancers in a neuronal activity-dependent manner and modulates activity-dependent gene expression. In addition to direct effects on chromatin, we show that Setd1a deficiency significantly enhances depolarization induced calcium signaling, a critical component in the activation of gene transcription. Looking for potential behavioral outcomes of altered activity dependent gene expression we found that spatial novelty preference on Y-maze assay, previously linked to neuronal activity and altered gene expression, is significantly impaired in SETD1A deficient mice.

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Poster

327. Psychosis: Cellular Mechanisms

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Topic: H.13. Schizophrenia

Title: The Effects of Chronic Antipsychotic Exposure on Memory and Memory-Related Trophic Factors in Rats

Authors: J. J. CHANDRA[1], **W. X. HAYNES**[4], A. PETTY[5], A. K. WALKER[2], *C. S. WEICKERT[3];
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Abstract: Chronic antipsychotic exposure is associated with cognitive deficits and midbrain inflammation in humans with schizophrenia. It is not clear whether long-term antipsychotics are causing behavioural and/or molecular brain changes. To explore these relationships, we tested for differences in novel object recognition (NOR) performance and vacuous chewing movements (VCMs), and in brain-derived neurotrophic factor (BDNF) and interleukin 1 receptor, type I (IL1R1) mRNAs in both the hippocampus and midbrain of rats following prolonged typical or atypical antipsychotic exposure. We anticipated that both classes of antipsychotics would negatively impact NOR, increase VCMs, decrease BDNF, and increase IL1R1 gene expression in the two brain regions investigated. Additionally, we expected to find correlations between hippocampal BDNF/IL1R1 and NOR (positive/negative, respectively). We randomly assigned 48 male Sprague-Dawley rats to either risperidone (1mg/kg), haloperidol (1mg/kg), or vehicle (n=16) administration twice-daily in cookie dough for 7 months. Cognition was assessed through a NOR test, performed 6 months after treatment start. NOR performance was indexed by the percentage of exploration time the animal spent investigating the familiar object. VCMs were tallied during 3-minute fortnightly observations. Midbrain and hippocampal gene expression for BDNF (exon III) and IL1R1was measured using qPCR. Correlations between NOR performance, VCMs, and gene expression were assessed with Pearson's. We confirmed previous findings that NOR performance was impaired in rats treated with haloperidol [$F(2,38)=4.71$, $p=0.002$ (LSD $p<0.05$)], but surprisingly found risperidone did not cause NOR impairments. Unexpectedly, midbrain BDNF mRNA was negatively correlated ($n=9$, $r=-0.679$, $p=0.044$) and hippocampal IL1R1 mRNA was positively correlated ($n=10$, $r=0.663$, $p=0.037$) with percentage of exploration time spent investigating the novel object in the risperidone group. VCMs were significantly greater in the haloperidol group than controls [$F(2,40)=8.097$, $p<0.001$ (LSD, $p<0.05$)]. However, we failed to find a significant relationship between VCMs and midbrain transcript levels of BDNF or IL1R1 mRNAs. In conclusion, we find evidence that long-term administration of the typical antipsychotic haloperidol negatively impacts both cognition and oral dyskinesias. Furthermore, the negative correlation between midbrain BDNF expression and NOR, and the positive correlation between hippocampal IL1R1 expression and NOR in the risperidone group are unexpected findings that warrant further investigation.

Disclosures: J.J. Chandra: None. **W.X. Haynes:** None. **A. Petty:** None. **A.K. Walker:** None. **C.S. Weickert:** None.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.21

Topic: H.13. Schizophrenia

Support: NIMH-IRP ZIC MH002903

Title: Single nucleus differential gene expression of schizophrenia implicates neurodevelopmental and synaptic dysfunction

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Abstract: Schizophrenia is a prevalent and debilitating mental disorder with complex psychopathology and unclear etiology. Genetic and epidemiological studies have suggested the role of neurodevelopment, including *in utero*. Still, the neuropathological consequences and cell-specific alterations resulting from altered brain development remain unknown.
We performed single nucleus RNA sequencing on the dorsolateral prefrontal cortex (BA9/BA46) of 23 cases with schizophrenia and 23 neurotypical controls (29 African and 16 European ancestry), resulting in a dataset of 370,000+ nuclei belonging to 16 excitatory neuron, 20 inhibitory neuron, and 10 glia cell populations. Cell-class specific differential gene expression revealed marked dysregulation in the majority of upper and deep layer excitatory neurons and parvalbumin-expressing inhibitory neurons, with only modest alterations in glial cells (astrocytes, OPCs, and endothelial cells). Gene function and spatiotemporal enrichment analysis of differentially expressed genes revealed enrichment for 1) neurodevelopmental genes typically expressed in the prenatal subplate/cortical plate and 2) synaptic genes expressed postnatally during childhood/adolescence. Likewise, many excitatory subpopulations presented with downregulated expression of synaptic plasticity related genes and were associated with transcription factors mediating the homeostatic regulation of neuronal activity.
Taken together, our findings suggest the role of neurodevelopment and post-natal synaptic functioning, primarily observed through excitatory neuronal populations, as salient hallmarks of dysfunction in a diverse sample of individuals with schizophrenia.

Disclosures: Y. Patel: None. **A. Schulmann:** None. **D. Kim:** None. **A. Mandal:** None. **N. Feng:** None. **Q. Xu:** None. **C.L. Dalgard:** None. **M. Kelly:** None. **B. Kolachana:** None. **P.K. Auluck:** None. **S. Marenco:** None.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.22

Topic: H.13. Schizophrenia

Title: Neuroinflammation interferes with trophic support in the midbrain of people with schizophrenia

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Abstract: Neuroinflammation is increasingly implicated as a pathological feature in schizophrenia, yet understanding how this impacts schizophrenia-relevant circuitry remains necessary. We find increased inflammatory markers in the midbrain of about 50% of schizophrenia cases. Midbrain dopamine dysfunction is central to schizophrenia neurobiology. Increased interleukin 1 (IL1) signaling leads to dopamine neuron demise through IL1R1. Here, we asked if midbrain mRNA of IL1R1 is altered in people with schizophrenia and elevated cytokines, as well as the neurotrophin Brain-Derived Neurotrophic Factor (BDNF) and its full-length ($TrkB^{TK+}$) or truncated receptor ($TrkB^{TK-}$), which may also be altered to promote neuronal survival in inflammatory conditions. Antipsychotics may aid in dampening inflammation and increasing trophic support; however, we find cytokines are positively correlated with antipsychotics in the human midbrain and so the neuroprotective role of antipsychotics is unclear. Next, we tested if chronic antipsychotic exposure impacts inflammation and trophic transcript levels in the rat midbrain. We measured IL1R1, BDNF (exon IV-human and III-rat), $TrkB^{TK+}$ and $TrkB^{TK-}$ mRNA by qPCR in a combined cohort of human midbrain tissue from the NSW TRC and SMRI (66 control/66 schizophrenia), and in midbrain tissue from adult male rats treated with 1mg/kg/day haloperidol (HAL), risperidone (RISP) or vehicle for 7 months (n=16/group). Schizophrenia cases were previously defined as high/low inflammation by 2-step clustering of cytokines. We found IL1R1 ($F(1,115)=32.72$, $p<0.001$) and $TrkB^{TK-}$ ($F(1,117)=7.50$, $p=0.007$) mRNAs were increased, while BDNF ($F(1,110)=7.59$, $p=0.007$) and $TrkB^{TK+}$ ($F(1,116)=19.77$,$p<0.001$) mRNAs were reduced in schizophrenia compared to controls, with a greater magnitude of change in high inflammation schizophrenia (all, $p<0.001$). Duration of illness ($r=0.270$, $p=0.039$) correlated with IL1R1 mRNA and did not correlate with BDNF or TrkB mRNAs in the human midbrain. In rats, IL1R1 mRNA was increased with chronic RISP (37%) compared to controls ($p=0.021$) and BDNF mRNA was reduced with RISP (35%) compared to HALO ($p=0.024$). BDNF and $TrkB^{TK+}$ mRNAs negatively correlated with IL1R1 mRNA in the human cohort ($r= -0.291$, $p=0.032$), but not in rodents. In sum, increased IL1R1 mRNA may contribute to inflammation-related neuropathology, which combined with reduced neurotrophic support could lead to midbrain dopamine neuron damage in schizophrenia. Additionally, chronic RISP treatment induces an inflammatory response in healthy rodents, and may be associated with the putative downregulation of BDNF in the schizophrenia midbrain.

Disclosures: J. Chandra: None. **A. Petty:** None. **W. Haynes:** None. **A. Walker:** None. **C.S. Weickert:** None.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.23

Topic: H.13. Schizophrenia

Support: F31MH126565

Title: The Role of the Melanin Concentrating Hormone System in Cognitive Functions in Mice

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Abstract: MCHR1 KO animals and MCH neuronal ablation show schizophrenic like phenotypes such as cognitive, social, and sensorimotor gating deficits. Data shows a difference between the two animal models, where there is social deficits, were seen in the MCHR1 KO animals but not in the ablation of MCH neurons. This may indicate that the disruption of the MCH system during neurodevelopment processes will contribute to cognitive deficits. Also the models used to understand the functions of the MCH systems have limitations as both the deletion of MCHR1 gene may mask or change behavioral phenotypes. While the diphtheria toxin induced ablation of MCH neurons provide insights on the distinct role of the MCH system it is less selective as the induction of death of MCH neurons can effect co expressing other neurotransmitters. The effects we see may not be contributed solely to MCH ablation. Thus, I hypothesize that the timing of MCH/MCHR1 dysregulation contributes to the pathogenesis of the schizophrenic phenotype. I will employ a creative approach that takes advantage of both CRISPR/Cas9-gRNA and Cre/LoxP technologies to enable us to selectively delete MCH precursor from the lateral hypothalamus while keeping normal neuronal function. Pmch-sgRNA is packaged in an AAV serotype 9, and the sgRNA-AAV is infused into the LH using stereotactic surgery. Mice then will be tested in a series of behavioral tests including: memory functions: T-maze, novel object recognition test, novel location recognition, contextual fear conditioning. I predict that selectively removing pMCH in the lateral hypothalamus would result in an array of cognitive deficits as the lateral hypothalamic area mediates motivation-cognition interfaces. MCH is mainly produced in neurons localized to the LH, and projects extensively throughout the brain. The lateral hypothalamus plays an important role in the brain reward system influenced by the dopamine system through GABA neurons. GABA neurons in the lateral hypothalamus project to the ventral tagmental area, which is enriched with dopamine neurons important for learning and reward. In the proposed model taken together we are able to remove pMCH to understand the role in the LH to then draw logical conclusions of the probable results individually and as a whole.

Disclosures: W. Alhassen: None. **A. Alachkar:** None.

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.24

Topic: A.03. Stem Cells and Reprogramming

Support: R01NS114977

Title: Excitatory dysfunction drives network and calcium handling deficits in 16p11.2 duplication schizophrenia iPSC-derived neurons

Authors: *E. L. PARNELL[1], L. CULOTTA[1], M. P. FORREST[1], H. JALLOUL[1], B. ECKMAN[1], D. LOIZZO[1], K. HORAN[1], M. DOS SANTOS[1], N. PIGUEL[1], D. J. TAI[3], H. ZHANG[4], T. S. GERTLER[5], D. SIMKIN[1], A. SANDERS[6], M. TALKOWSKI[7], P. V. GEJMAN[4], E. KISKINIS[2], J. DUAN[4], P. PENZES[1];
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Abstract: Schizophrenia (SCZ) is a debilitating psychiatric disorder with a large genetic contribution; however, its neurodevelopmental substrates remain largely unknown. Modeling pathogenic processes in SCZ using human iPSC-derived neurons (iNs) has emerged as a promising strategy. Copy number variations (CNV) confer high genetic risk for SCZ, with duplication of the 16p11.2 locus increasing risk 14.5 fold. To dissect the contribution of excitatory (iENs) versus GABAergic (iGNs) neurons to SCZ pathophysiology, we induced iNs from CRISPR-Cas9 engineered isogenic and SCZ patient-derived iPSCs, and analyzed SCZ-related phenotypes in iEN monocultures and iEN/iGN cocultures. In iEN/iGN cocultures, we found reduced neuronal network firing rate and synchrony at later, but not earlier, stages of *in vitro* development. These were fully recapitulated in iEN monocultures, indicating a primary role for excitatory neurons. Transcriptomic analysis of isogenic 16p11.2 duplication (DUP) iENs revealed pathway dysregulation related to neuroarchitecture and calcium ion binding. Consistent with this, isogenic DUP iENs showed reduced dendrite length and calcium imaging revealed deficits in calcium handling. Analysis of iENs from 16p11.2 duplication-carrying SCZ patients (SCZ) revealed overlapping deficits in network synchrony, dendrite arborization and calcium handling. These results indicate 16p11.2 duplication-dependent alterations to SCZ neurodevelopment. Calcium ion binding was the only altered transcriptomic gene set shared between isogenic and patient-derived iENs, suggesting a central role of calcium signaling in 16p11.2 duplication-mediated pathogenesis. Our results support excitatory dysfunction during early neurodevelopment as the basis of SCZ pathogenesis in 16p11.2 duplication carriers and identifies network synchrony and calcium handling deficits directly linked to this genetic mutation.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.25

Title: WITHDRAWN

Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.26

Topic: H.13. Schizophrenia

Support: Fogarty/NIMH
R21 MH120692-01A1
Higher Education Commission, Pakistan

Title: Genetics of non-comorbid severe psychosis in multiplex consanguineous pedigrees

Authors: *J. V. PARDO[1], A. KANWAL[2], S. A. SHEIKH[3], F. ASLAM[2], A. IFTIKHAR[4], S. YASIN[2], S. NAZ[2];
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Abstract: Mendelian genes show monogenic inheritance with simple segregation of traits (dominant, recessive, sex-linked) and large effects on phenotype. If related to pathology, these genes are rare and are likely directly involved in disease pathophysiology. Although these genes feature prominently in many medical fields, few are associated with common-appearing psychiatric disorders such psychosis or schizophrenia. The latter is a mental health condition frequently involving psychosis, defined as the presence of hallucinations or delusions in a clear sensorium. Many mental disorders appear polygenic with complex inheritance. However, Mendelian genes for mental illness are rarely sought. If Mendelian genes exist in psychiatry, a highly successful approach derived from other fields is the study of consanguineous (e.g., first cousin) unions. Extreme phenotypes especially without comorbidities have also proven invaluable for ascertainment. Such families enable genome sequencing and homozygosity mapping. A directed, unbiased search for variants of interest (VOI) in single genes follows filtering. Once identified, detailed study of the variant is required to define causal mechanism(s) or rule out its candidacy. Several multiplex consanguineous families in which affected

individuals were suffering with a severe psychosis/schizophrenia phenotype were examined in this way. Whole exome sequencing and homozygosity mapping revealed multiple variants of interest. Different filters were used to isolate likely pathogenic/causal alleles. The filters included rare allele frequencies MAF < 0.01; linkage to phenotype; protein conservation; in silico analysis of damage to protein; exclusion in normative databases; and absence in indigenous ethnic healthy population. Three VOIs were identified; one from each of three independent multiplex families affected with psychosis. These include ubiquitin specific protease 53 (*USP53*), p.Cys228Arg, a non-catalyic deubiquitinase; regulator of G protein signaling 3 (*RGS3*), p.Arg217Cys, a GTP-ase activator inhibiting G-protein mediated signalling; and interleukin 1 receptor accessory protein 1 (*IL1RAPL1*), p.Thr234Ala, associated with mnemonic and learning abilities as well as synaptic formation and stabilization. Thus, these proteins are transcribed in the brain and appear to have important roles in neurotransmission and brain functions. Using this approach, false positive VOIs were rare as each family carried only one VOI (or none could be found in other pedigrees). Functional studies are underway to confirm pathological involvement and causal mechanisms of these variants.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 327.27

Topic: A.09. Adolescent Development

Support: NIMH R01-MH128176-01

Title: Reduced predictive processing mimics schizophrenia-like deficits in a genetic model of adolescent dendrite loss

Authors: *A. M. RADER[1], A. D. FERRELL[1], C. G. GALLIMORE[1], T. J. SUTTON[1], R. A. SWEET[2], M. J. GRUBISHA[2], J. P. HAMM[1];
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Abstract: The neural mechanisms underlying context processing are disrupted in many neuropsychiatric disorders, including schizophrenia (SZ). Such disorders tend to develop during late adolescence and early adulthood, suggesting a vulnerable period in development. Exactly *how* cortical circuits critical for context processing mature during adolescence - and what might go wrong to produce disordered context processing in SZ — remains an open question. We have previously shown the importance of a fronto-visual feedback circuit for processing stimuli in context and producing mismatch negativity like responses to stimuli deviant from context. Here we sought to characterize how this circuit changes across adolescence in wild-type mice and in

mice with a SZ-relevant mutation (KALRN-PT). Importantly, we previously demonstrated that KALRN-PT mice display *adolescent-onset* reductions in auditory cortical dendrite length and complexity, which align with anatomical neuropathology observed in SZ. Using dual local field potential (LFP) recordings from primary visual cortex (V1) and anterior cingulate area (ACa; a prefrontal region projecting to V1), we measured i) interregional phase synchrony between ACa and V1 and ii) deviance detection responses in V1 (i.e., increased responses to contextually rare stimuli) during a visual oddball paradigm. This context processing paradigm is also used to quantify mismatch negativity deficits during electroencephalography (EEG) recordings, which serve as a biomarker for SZ. To compare interregional synchrony and V1 responses to deviant stimuli across development and pathological conditions, we recorded from adult (P84-126; n=7 per genotype) and pre-adolescent (P28-31; n=8 per genotype) male and female mice either i) homozygous for KALRN-PT or ii) wild-type littermate controls (WT). Fronto-visual synchrony in the 6-12 Hz range and V1 "deviance detection" responses increased across adolescence in WT mice, but not KALRN-PT mice, coinciding with the anatomical defects seen in KALRN-PT dendrites. These data suggest a critical period for the maturation of context-processing circuitry in the visual cortex, during which SZ-like deficits arise in KALRN-PT mice. Overall, these results illuminate typical and disrupted adolescent brain development following dendritic loss and beg further investigation of the role of dendritic fields in context processing for future SZ therapies.

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Poster

327. Psychosis: Cellular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 327.28

Topic: G.08. Other Psychiatric Disorders

Support: NIMH R01MH120080
NIMH R01MH123245
Kavli Institute for Neuroscience Postdoctoral Fellowship for Academic Diversity
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NUS Yong Loo Lin School of Medicine NUHSRO/2020/124/TMR/LOA
Singapore National Medical Research Council LCG OFLCG19May-0035
NMRC STaR STaR20nov-0003

Title: White matter connectivity differentially predicts mental health traits in children and adolescent males and females

Authors: *E. DHAMALA[1], L. Q. R. OOI[4], Y. QU[2], J. RICARD[3], E. BERKELEY[5], B. YEO[4], A. J. HOLMES[2];

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Abstract: Behavior and personality emerge from the functional and structural properties of the brain. Individual differences in cognition, personality, and mental health can be predicted by the spatial topography, size, and connectivity of functional networks as well as associated patterns of white matter connectivity and regional anatomy. While prior work suggests the presence of sex differences in predictive accuracies and neurobiological correlates of cognitive abilities, the extent to which sex differences in brain-based predictions may exist across mental health traits remains to be established. Here, we investigate whether white matter connectivity can comparably predict a range of psychiatric symptoms in males and females. We examined diffusion tensor MRI data from 1823 children and adolescents (ages 9-11; 979 females) from the Adolescent Brain Cognitive Development study. We computed the pairwise streamline count between regions from the 400-region Schaefer cortical parcellation to predict mental health traits obtained from the Child Behavior Checklist using k-fold group-matched cross-validated linear ridge regression over 25 distinct train/test splits. Models were trained separately in males and females and evaluated across both sexes. Models trained in males successfully ($p<0.05$) predicted withdrawing depression, attention, and stress in males, and attention, rule-breaking, and conduct disorders in females (Fig 1). Models trained in females successfully predicted attention, rule-breaking, aggressiveness, ADHD, and conduct disorders in males, but no traits in females (Fig 1). These accuracies are generally weaker than those reported for predictions based on functional connectivity, suggesting muted relationships exist between psychiatric symptoms and white matter connectivity compared to functional connectivity. Moreover, the generally unsuccessful predictions in females, regardless of which sex the models were trained on, suggests psychiatric symptoms may be more strongly associated with white matter connectivity changes in males than in females.

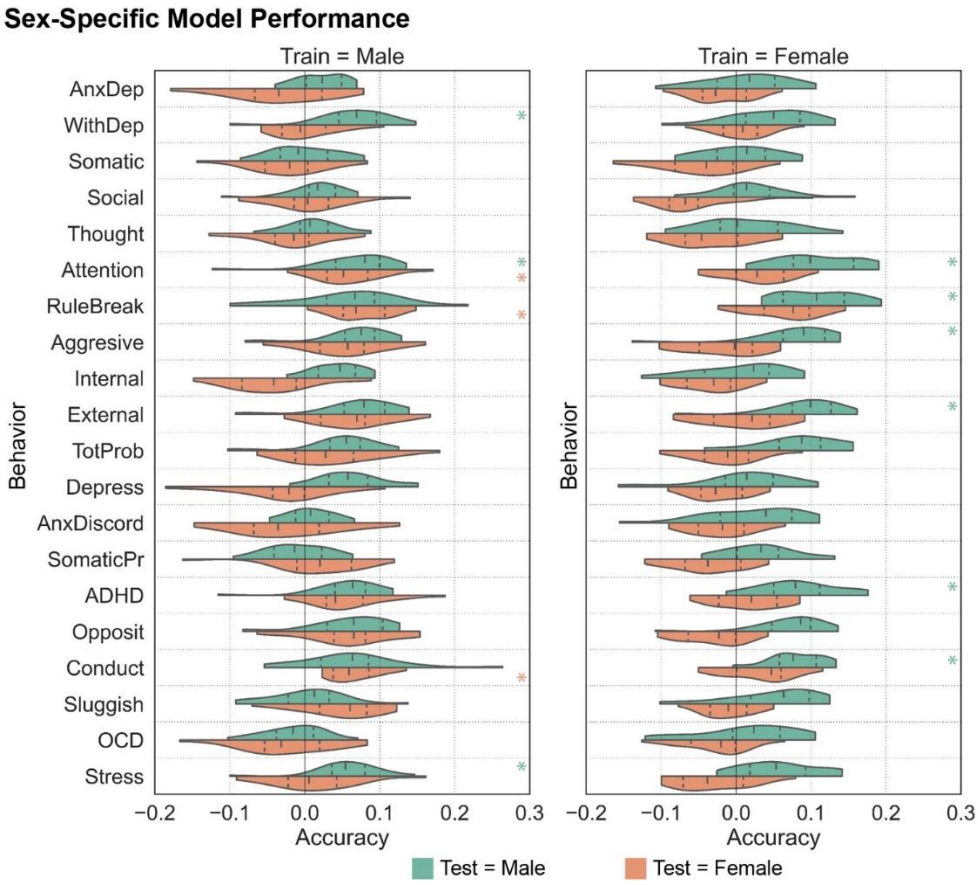

Figure 1: Sex-Specific Model Performance for the Prediction of Mental Health Traits

Prediction accuracy (correlation between true and predicted traits) violin plots for models trained on males (left) and females (right) to predict mental health traits in males (green) and females (orange) using white matter connectivity. Green and orange asterisks (*) denote that the model performed above chance levels based on permutation tests ($p<0.05$).

Prediction accuracy across all behavioral traits for models are $r=0.038\pm0.05$ (mean ± standard deviation) for models trained and tested on males, $r=0.019\pm0.06$ for models trained on males and tested on females, $r=0.050\pm0.06$ for models trained on females and tested on males, and $r=-0.011\pm0.05$ for models trained and tested on females.

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 328.01

Topic: I.03. Anatomical Methods

Support: National Health and Medical Research Council (APP1194206)
National Science Centre (2019/35/D/NZ4/03031)

Title: Distribution of calbindin-immunoreactive neurons throughout the entire cerebral cortex of the marmoset (Callithrix jacchus)

Authors: N. ATAPOUR[1,2], S. BEDNAREK[3], A. KULESZA[3], G. SAWORKSA[3], K. H. WORTHY[1,2], P. MAJKA[3], ***M. G. P. ROSA**[1,2];
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Abstract: Precise data on the distribution and prevalence of different brain cell types is key to the creation of biologically realistic models that can help us understand the emergence of complex patterns of neural activity. One of the most important criteria for the characterization of neuronal classes is the cellular expression of molecules such as neurotransmitters, neuromodulatory peptides and calcium-binding proteins. Here we present the first complete map of the distribution of a neurochemically-defined class of neurons in the primate cerebral cortex: cells expressing the calcium-binding protein calbindin-D28K (CB+ neurons). This was achieved based on the combined use of artificial intelligence-based algorithms for cell detection and computerized reconstructions, and validated through the use of human expert-based stereological techniques. The method allowed the 3-dimensional mapping and full quantification of the density of CB+ neurons across areas and layers of 3 hemispheres of marmoset cortex. The density of CB+ neurons varied within a greater than 3-fold range across most of the cortex, with particularly high densities in auditory (core, belt and parabelt), primary somatosensory (3a, 3b) and visual (extrastriate) areas. The striate cortex was a notable outlier, with a CB+ neuron density nearly twice as high as that in other densely populated areas, such as A1 and V2. In the visual cortex CB+ densities decreased from caudal to rostral, whereas in auditory cortex an opposite gradient was observed (increasing towards rostral). In the prefrontal cortex, ventrolateral areas showed higher CB+ densities than dorsolateral areas. Low CB+ neuronal densities were observed in the anterior cingulate, motor and ventral temporal (parahippocampal and perirhinal) regions. Given that the total neuronal density estimated by NeuN staining is also known to vary across a greater than 3-fold range between cortical areas, a natural question is whether these observations can be explained by a simple model whereby CB+ neurons form a constant fraction of the neuronal population across areas. Our analyses demonstrate that this is not the case: a strong proportional relationship was found, demonstrating the principle that areas containing higher densities of CB+ neurons also tend to have these cells forming a higher percentage of the total neuronal population. These results demonstrate the feasibility of accurately mapping the entire distribution of neurochemically-defined cells across the entire cortex in a primate, and opens the way for a full characterization of other cell types based on the use of deep neural networks and computerized reconstructions.

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 328.02

Topic: I.03. Anatomical Methods

Support: This work was supported by the program for Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) from the Japan Agency for Medical Research and Development AMED (JP15dm0207001)
It was also supported by Scientific Research on Innovative Areas (22123009) from MEXT, Japan and National Science Centre of Poland (2019/35/D/NZ4/03031).

Title: The Brain/MINDS Marmoset Connectivity Atlas

Authors: *H. SKIBBE[1], M. F. RACHMADI[1], K. NAKAE[2], C. E. GUTIERREZ[4], J. HATA[5], H. TSUKADA[6], C. POON[1], K. DOYA[4], P. MAJKA[7], M. G. P. ROSA[8], H. OKANO[5], T. YAMAMORI[1], S. ISHII[3], M. REISERT[9], A. WATAKABE[1];
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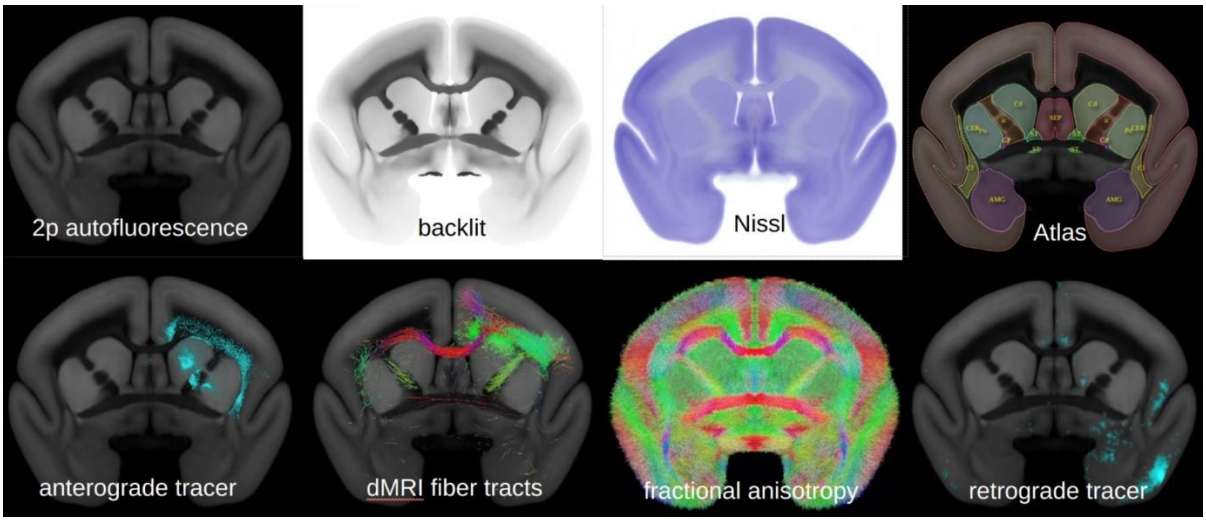
Abstract: We introduce the Brain/MINDS Marmoset Connectivity Atlas (BMCA) standard image preprocessing pipeline and the tools for exploring and reviewing the BMCA. The BMCA is a new resource that provides open access to 164 retrograde (Majka et al.,2020,2016) and 52 anterograde neuronal tracer imaging data (Watakabe et al. 2021) in the cortex of the marmoset brain. The BMCA also integrates measurements derived from structural diffusion MRI (dMRI). dMRI is a widely used technique for studying brain connectivity in primates, including humans, but estimates of true structural connectivity based on this technique are imperfect. In this context, the BMCA provides a rich platform to enable studies aimed at refining and validating dMRI (Gutierrez et al. 2020). We added visualization tools to explore the aligned images at a resolution that is sufficiently high to identify individual axon structures, and added mapping tools to integrate with other available marmoset brain image databases. We also provide a map for interspecies comparisons between the marmoset and the human brain image space.



Disclosures: **H. skibbe:** None. **M.F. Rachmadi:** None. **K. Nakae:** None. **C.E. Gutierrez:** None. **J. Hata:** None. **H. Tsukada:** None. **C. Poon:** None. **K. Doya:** None. **P. Majka:** None. **M.G.P. Rosa:** None. **H. Okano:** None. **T. Yamamori:** None. **S. Ishii:** None. **M. Reisert:** None. **A. Watakabe:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 328.03

Topic: I.03. Anatomical Methods

Support: National Science Centre (2019/35/D/NZ4/03031)
National Health and Medical Research Council (APP1194206)

Title: Deep learning approaches to identification and delineation of cortical areas in the marmoset cortex

Authors: A. KULESZA[1], S. BEDNAREK[1], G. SAWORSKA[1], M. G. P. ROSA[2,3], ***P. MAJKA**[1,3];
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Abstract: The cerebral cortex is subdivided into functionally distinct areas, which have been traditionally distinguished based on the distribution and size of the neuronal bodies (cytoarchitecture) and the direction and density of myelinated axons (myeloarchitecture). The delineation of the cortex into areas and layers is, in most cases, performed manually in a laborious process requiring substantial expertise in neuroanatomy. This manual approach is

typically a bottleneck in large-scale, high-throughput applications where several areas are to be delineated using objective criteria in large numbers of animals.
We report on a deep learning pipeline for automated identification of cortical areas in the entire cortex of the marmoset monkey (Callithrix jacchus). The process relies on microscopic (0.5 µm/px) images of serial sections stained with the Nissl method and consists of three components: segmentation of the cerebral cortex, estimation of the density and size of the neuronal somata, and identification of the cortical areas based on series of cortical profiles (i.e. unambiguously defined lines that connect the pial with the white matter).
To train the network, we took the advantage of the repository of several hundred outlines of selected cortical areas in twenty hemispheres of the marmoset cerebral cortex, in which areas were delineated manually based on both cyto- and myeloarchitecture, as part of the Marmoset Brain Connectivity Atlas project (https://www.marmosetbrain.org/). This allowed us to compare the performance of two networks: one relying on cortical profiles derived from 3D reconstructions of the brain hemisphere, and another utilising individual 2D sections independently. The preliminary results demonstrate that the cytoarchitectonic profiles are a reliable source of training data for deep-learning solutions aimed at identifying cortical areas based on their cytoarchitectonic characteristics.
While the primary purpose of the pipeline is to automate the delineation of cortical areas, the close examination of the resulting neural networks might provide an insight into the specific features of the cytoarchitectonic features that contribute the most to the identification of the cortical areas.

Disclosures: **A. Kulesza:** None. **S. Bednarek:** None. **G. Saworska:** None. **M.G.P. Rosa:** None. **P. Majka:** None.

Poster

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Program #/Poster #: 328.04

Topic: I.03. Anatomical Methods

Support: Canadian Institute of Healt Research Grant FRN 148365
Canada First Research Excellence Fund
Discovery grant by the Natural Sciences and Engineering Research Council of Canada

Title: An action-observation network in the common marmoset identified by ultra-high field fMRI

Authors: *A. ZANINI, A. DUREUX, S. EVERLING;
Western Univ., London, ON, Canada

Abstract: Thirty years have passed since the first description of neurons in Old World macaque monkeys that fire both during the execution and the observation of goal-directed actions. Since then, these so-called “mirror neurons” have been shown to play important roles in understanding the actions of others, learning by imitation, theory of mind, and social cognition in macaques and humans. Moreover, their dysfunction is reported in various neurodegenerative pathologies and in autism spectrum disorders, making the study of the mirror neuron system of fundamental clinical importance. The results from electrophysiological and neuroimaging studies have allowed us to identify an "action-observation network" (AON) that includes premotor-prefrontal, superior temporal and parietal areas in both human and non-human Old World primates. In contrast to these lasts, an AON has not been identified in New World primates. Here we used ultra-high field fMRI at 9.4T in 4 awake common marmosets (*Callithrix jacchus*) while these small primates watched 12 seconds-videos depicting goal-directed (grasping food) vs non-goal-directed actions. Their small size, their lissencephalic (smooth) cortex and the social similarities they share with humans (i.e., prosocial behaviour, imitation) make marmosets an excellent candidate for the study of the neural basis of action observation. We found that the observation of goal-directed actions activated a fronto-temporo-parietal network sharing strong similarities with macaques and humans’ mirror neuron system, including areas 6 and 45 in premotor and prefrontal cortices, and areas PGa-IPa, FST, and the TE complex in temporoparietal regions. Moreover, time-series analysis showed stronger responses for goal-directed vs non-goal-directed actions in parietal area PG, another fundamental hub of humans and macaques’ mirror neurons network. These results demonstrate the existence of an evolutionary conserved AON in primates that likely predates the separation of Old and New World monkeys.

Disclosures: **A. Zanini:** None. **A. Dureux:** None. **S. Everling:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.05

Topic: I.03. Anatomical Methods

Support: Brain/MINDS: JP15dm0207001
KAKENHI 22123009
Brain/MINDS: JP22dm0207088

Title: Characterization of the prefrontal cortex projections of the common marmoset

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Tochigi, Japan; [7]Tokyo Metropolitan Univ., Tokyo, Japan; [8]Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: The prefrontal cortex (PFC) positions at the highest stage of neural integration and exerts top-down influences on various brain regions. To clarify the structural basis of control by PFC neurons, we performed a projectome mapping of PFC using common marmosets as the model primate. Based on high-resolution 3D datasets, we characterized two contrasting types of corticocortical and corticostriatal projections that may exert differential effects on the target neurons. One was the "focal projection", which is characterized by multiple foci of axonal terminations packed into narrow spots. These foci were scattered within the extra-PFC association areas or in the caudate nucleus in a topographic manner. The other was the "spread-type projection", exhibiting wide coverage of cortical and striatal regions with various intensity and sparsity. Quantitative characterization of these features revealed recapitulation of the PFC gradients in the local and global distributions of the axonal projections in the cortical association fields. Finally, we observed area-specific PFC-collicular projection patterns to emerge in a topographically restrained way. These data provide insight into the organization of PFC areas and their influences on their projection targets.

Disclosures: A. Watakabe: None. **H. skibbe:** None. **K. Nakae:** None. **H. Abe:** None. **N. Ichinohe:** None. **M.F. Rachmadi:** None. **J. Wang:** None. **M. Takaji:** None. **H. Mizukami:** None. **A. Woodward:** None. **R. Gong:** None. **J. Hata:** None. **H. Okano:** None. **S. Ishii:** None. **T. Yamamori:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.06

Topic: I.03. Anatomical Methods

Support: Japan AMED JP15dm0207001

Title: Semi-supervised semantic segmentation of in situ hybridization gene expression in the marmoset brain

Authors: *C. POON, M. F. RACHMADI, M. BYRA, T. SHIMOGORI, H. SKIBBE;
Ctr. for Brain Sci., RIKEN, Wako, Japan

Abstract: Gene expression brain atlases are widely used in neuroscience research. The Marmoset Gene Atlas, created by the Brain Mapping by Integrated Neurotechnologies for Disease Studies (Brain/MINDS) project in Japan, is an *in situ* hybridization (ISH) database of gene expression in the marmoset brain. To characterize gene expression, ISH signals must be segmented. Traditional methods of segmentation, such as manual thresholding and engineering handcrafted features, are limited by the confines that define them and prone to human error. Deep learning techniques have been widely used for segmentation tasks. Supervised

architectures have led to impressive segmentation results, but require large labelled training datasets, which are expensive to obtain and also prone to human error. Recently, deep learning models that require less human instruction, known as semi-supervised learning, have shown impressive results. The goal of our work is to use a semi-supervised model to automate the segmentation of ISH gene expression in the marmoset brain. The model is based on a U-Net architecture. In addition to the supervised loss, the proposed model uses a contrastive loss. In contrastive learning, labelled images are not required as the model is trained in the latent space, thereby reducing the human error associated with manual labeling and increasing the training dataset size. We use the contrastive loss introduced in SimCLR, which calculates the loss between positive pairs of examples, i.e., by maximizing agreement between features of different augmented views of the same image patch. Gene expressions segmented by the model are registered to a common 3D template of the marmoset brain, into which other imaging data, such as neuronal tracer data, has also been registered to [Skibbe et al., bioRxiv, 2022]. We hope that our contribution will facilitate more holistic and comparative analyses of connectivity and development in the marmoset brain [Kita et al., PNAS, 2021]. This work was supported by the Japan AMED (JP15dm0207001).

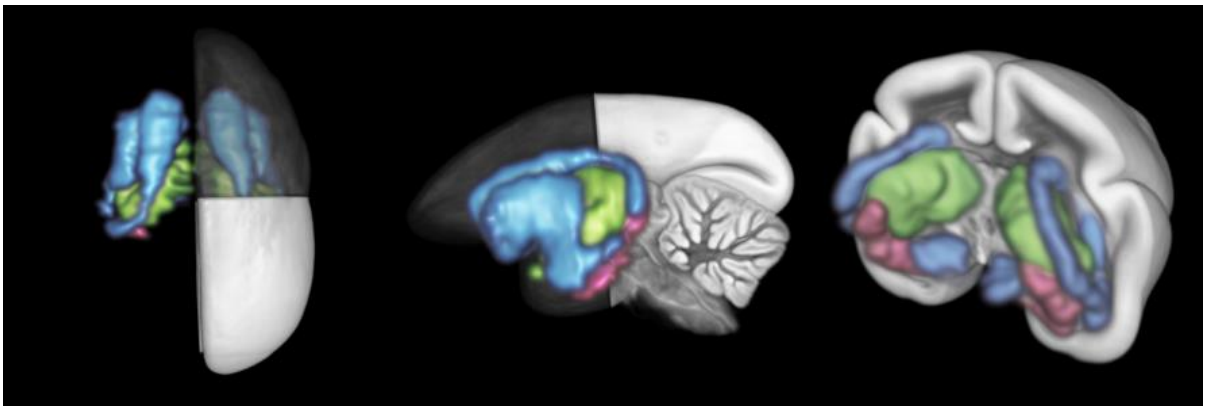

Figure 1. 3D rendering of 3 genes in the marmoset brain.

Disclosures: **C. Poon:** None. **M.F. Rachmadi:** None. **M. Byra:** None. **T. Shimogori:** None. **H. Skibbe:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.07

Topic: I.03. Anatomical Methods

Support: Canadian Institutes of Health Research (FRN 148365)
Canada First Research Excellence Fund to BrainsCAN

a Discovery grant by Natural Sciences and Engineering Research Council of Canada

Title: Functional correspondences between marmoset and human brain areas during the perception and attribution of mental states to animated shapes

Authors: *A. DUREUX, A. ZANINI, J. SELVANAYAGAM, R. S. MENON, S. EVERLING; Robarts Res. Institute, Western Univ., London, ON, Canada

Abstract: Theory of Mind (ToM) refers to the ability to attribute mental states to others. Research in human ToM studies has shown that mental state attribution can be evoked by animations of simple geometric shapes with different movement patterns (i.e., Frith-Happé animations developed by Abell et al., 2000). fMRI studies using Frith-Happé animations have revealed a distinct pattern of brain activations during the observation of "ToM conditions" - showing two animated triangles moving in a way which indicates that one triangle reacts to the other object's mental state - compared to the "Random conditions" - showing the same two triangles moving and bouncing like objects - in medial frontal and temporoparietal regions.ToM is a fundamental aspect of social cognition for species living in complex social groups. However, to the best of our knowledge, no neuroimaging studies have investigated ToM capacities in nonhuman primates with moving animated shapes. Here, we used ultra-high field fMRI to directly compare neural networks involved in mental state attributions to animated shapes between humans and New World common marmoset monkeys (*Callithrix jacchus*). Ten healthy humans and six marmoset monkeys watched modified versions of the Frith-Happé animations in a 7T and 9.4T scanner, respectively. In order to have an indication of the cognitive processing in marmosets, we also investigated their gaze patterns outside the scanner. In humans, our results show that observation of the "ToM conditions" - compared to the "Random conditions" - activates several regions in frontal, parietal and temporal areas including prefrontal areas, ventral premotor cortex, posterior parietal areas, temporal parietal junction as well as superior and inferior temporal areas. In marmosets, the same contrast reveals activations mostly in motor and premotor regions, posterior parietal cortex, as well as in superior and inferior temporal regions. Moreover, marmosets spent also more time fixating the triangles during "ToM conditions" compared to "Random conditions". Therefore, these findings show that the observation of Frith-Happé ToM animations activates a similar network in humans and marmosets. This opens the possibility that marmosets, like humans, may be able to attribute mental states to these animations.

Disclosures: A. Dureux: None. **A. Zanini:** None. **J. Selvanayagam:** None. **R.S. Menon:** None. **S. Everling:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.08

Topic: I.03. Anatomical Methods

Support: NIH Grant R01 NS094499
NIH Grant R01 NS039444
Allen Institute for Brain Science (JLS, SJS)

Title: Collaborative use of conjugate array tomography to characterize synapses in mouse neocortex

Authors: J. L. SCHARDT[1], A. K. SIMHAL[2], K. D. MICHEVA[3], S. J. SMITH[4], *R. J. WEINBERG[5];
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Abstract: Conjugate light - electron microscopy offers a powerful tool to study the structure and composition of synapses. However, the diverse resources and expertise needed for such experiments is available to only a handful of laboratories. Here we present a collaborative approach to perform conjugate array tomography of the same samples of mouse neocortical tissue, carried out in multiple geographically distant locations, each specializing in different aspects of the task. Harnessing the strengths of each lab enabled effective study of a wide array of synapses. Labs at North Carolina, Stanford, Seattle, and New York have united for an in-depth exploration of synapses in mouse cortex. We have generated a catalogue of individual synapses with associated features including molecular composition, ultrastructure, and tissue context. While each of these labs could in principle perform this project, the collaboration allowed each lab to focus on its core competence, with UNC providing the samples, Stanford the light microscopy, Allen Institute the electron microscopy, and Sloan Kettering the computerized synapse detection. Data were uploaded onto the German software platform webKnossos, allowing all parties to work together on analysis. Probes included DAPI (for sample alignment), antibodies to identify excitatory and inhibitory synapses, and multiple antibodies against specific ionotropic glutamate receptors, allowing proteometric characterization of excitatory synapses. We focused on the immunofluorescence ratio of NMDAR signal (defined by probes for GluN1 and GluN2B) to AMPAR signal (defined by probes against GluA1-GluA4). Beyond confirming the expected pattern that small synapses display higher NMDAR/AMPAR ratios, we find intriguing patterns for proteometrically-defined subpopulations. The automated synapse detector can locate specific synapse types. This feature allows us to efficiently target rare synapses, such as those enriched in GluA4, for further analysis. We conclude that the workflow of conjugate AT projects can be efficiently distributed across multiple geographic locations to allow a comprehensive investigation of cortical synapses, including molecular composition and ultrastructure of both common and rare synapse types.

Disclosures: J.L. Schardt: None. **A.K. Simhal:** None. **K.D. Micheva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC. **S.J. Smith:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aratome, LLC., Aratome, LLC. **R.J. Weinberg:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.09

Topic: I.03. Anatomical Methods

Support: R01 NS094499 (KDM)
R01 NS39444 (RJW)
Allen Institute for Brain Science (JLS, SJS),

Title: Conjugate IF-SEM: A tool to provide ground truth for synapse detection and analysis

Authors: *A. K. SIMHAL[1], J. L. SCHARDT[2], S. J. SMITH[3], R. J. WEINBERG[4], K. D. MICHEVA[5];
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Abstract: When working with large anatomical datasets comprising millions of synapses, the advantages of using automated tools for analysis are obvious. However, automated synapse detection is imperfect, and the accuracy of detection can vary depending on type and size of synapses, introducing bias into downstream analyses. Datasets from mouse cerebral cortex imaged with conjugate immunofluorescence - scanning electron microscopy (IF-SEM) allowed us to evaluate automated IF-based synapse detection, using manual IF-SEM as a robust gold standard. Tissue from adult mouse visual cortex was immunolabeled with antibodies against general synaptic markers for excitatory and inhibitory synapses, and for ionotropic glutamate receptor subunits. Synapses were detected with the previously developed probabilistic synapse detector using combinations of at least two markers, one presynaptic and one postsynaptic. The automated synapse detector reliably identified the large majority of excitatory synapses, but approximately 10% of synapses observed in the electron micrographs had insufficient immunofluorescent signal to be detected. These were usually very small synapses, with a postsynaptic density diameter <200 nm; thus, the smallest synapses can be detected only with electron microscopy. On the other hand, the conjugate immunofluorescent signals facilitated detection of synapses that are often missed when using only electron microscopy data. Thus, manual detection of synapses using only the SEM data missed 9% of excitatory synapses that were *en face*, i.e. sectioned parallel to the synaptic cleft. Immunofluorescent signal also improved identification of synapse type, because the morphological distinction between excitatory (asymmetric) and inhibitory (symmetric) synapses is not always clear. Our analysis allowed us to define conditions where automated synapse detection is highly effective, and to specify situations where it becomes unreliable. We conclude that automated IF-based synapse detection effectively measures overall synapse densities and the relative prevalence of different synapse types, and is ideally suited to investigate correlations between synapse size and receptor

composition. However, when the focus is on very small synapses, or multi-synapse arrangements such as dually innervated spines, electron microscopic information becomes indispensable.

Disclosures: **A.K. Simhal:** None. **J.L. Schardt:** None. **S.J. Smith:** None. **R.J. Weinberg:** None. **K.D. Micheva:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); K.D.M. has founder's equity interests in Aratome, LLC (Menlo Park, CA), an enterprise that produces array tomography materials and services.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.10

Topic: I.03. Anatomical Methods

Support: NIH Grant EY011488
Max Planck Florida Institute for Neuroscience

Title: Two novel approaches for source identification of functional synaptic input architecture

Authors: *J. JAEPEL[1], R. SATTERFIELD[1], N. SHULTZ[1], C. I. THOMAS[3], A. FERGUSON[2], N. T. URBAN[2], N. KAMASAWA[3], D. FITZPATRICK[1];
[1]Functional Architecture and Develop. of Cerebral Cortex, [2]Light Microscopy Core Facility, Max Planck Florida Inst. For Neurosci., Jupiter, FL; [3]Electron Microscopy Core Facility, Max Planck Florida Inst., Jupiter, FL

Abstract: Cortical neurons integrate information from a large number of inputs originating from various input sources, including local connections and long-range projection to generate well-tuned somatic responses. Functional imaging of dendritic spines has revealed functional diversity rather than specificity and organization of inputs on multiple spatial scales. However, it remains unclear how input sources contribute to the observed functional synaptic architecture. Answering these questions requires both physiological characterization of inputs in vivo and identification of synaptic connections between neurons. Conventional optical microscopy generally lacks the required resolution for synapse identification and large-scale electron microscopy is limited to local networks. To address this issue, we developed two new approaches: Source Identification in Correlative Light Microscopy with Stimulated Emission Depletion Microscopy (SI-CLM-STED) and Source Identification in Correlative Light and Electron Microscopy (SI-CLEM). Both approaches required three steps: 1) Labeling axon terminals from distinct input sources with structural markers; 2) Functional characterization of individual spines in vivo using two-photon imaging of calcium responses and 3) High resolution imaging of labeled axon terminals and previously in vivo characterized dendritic spines. For SI-CLM-STED, we labeled axon terminals with fluorophores and performed multi-colored 3D STED imaging of axon terminals, spines and synaptic markers for synapse identification. This high-throughput tool allowed us to

visualize all inputs from one source onto an entire cell, linking functional properties and anatomical characteristics such as input frequency within the dendritic tree, allowing for a more targeted SI-CLEM approach. For SI-CLEM, we combined CLEM with viral reporter constructs with peroxidase targeted to subcellular compartments to differentiate two input sources onto previously described target regions. This tool not only allowed us to identify synapses from multiple inputs onto single neurons without the need to fully reconstruct the presynaptic partners, but also enabled us to quantitatively characterize the synaptic ultrastructure of these connections such as bouton and spine size. In summary, these methods are reliable tools enabling us to successfully link functional properties of spines and their location within the dendritic field to defined input sources. In the future, these approaches will permit us to disentangle how specific input sources are contributing to the observed functional diversity, spatial organization and somatic drive of spines.

Disclosures: **J. Jaepel:** None. **R. Satterfield:** None. **N. Shultz:** None. **C.I. Thomas:** None. **A. Ferguson:** None. **N.T. Urban:** None. **N. Kamasawa:** None. **D. Fitzpatrick:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.11

Topic: I.03. Anatomical Methods

Support: NIH Grant DP2-NS116768
Simons Foundation Grant SCGB #543003
NSF CPBF Grant PHY-1734030
Boehringer Ingelheim Fonds
Swartz Fellowship for Theoretical Neuroscience

Title: Mapping the Functional Connectome in C.elegans

Authors: *S. DVALI, F. RANDI, A. K. SHARMA, A. M. LEIFER;
Physics, Princeton Univ., Princeton, NJ

Abstract: Measuring how signals propagate through the nervous system is important for understanding how neural dynamics are generated and uncovering how neuroanatomy relates to neural function. Here we sequentially perturb individual neurons in *C .elegans* optogenetically and simultaneously measure the response of all neurons in the brain via calcium imaging in order to measure functional connectivity at whole-brain scale and cellular resolution. We measure the sign, strength, causal relations, and temporal properties of effective connections between neurons. We present a preliminary functional connectivity map covering 90 of 118 neuron classes recorded from 43 animals. The functional map recovers properties of previously well-studied circuits and connections, such as AFD to AIY, and provides clarity to other connections that had remained ambiguous. Our measured functional description of the network better predicts

correlations in spontaneous neural activity than an anatomical one. To investigate differences between the anatomical and functional descriptions, we constructed detailed simulations of whole-brain neural dynamics that are constrained either by anatomy or by our measured functional connections and found that the two make different predictions of neural dynamics. Neural connections that are extra-synaptic and therefore not visible to anatomy may explain some of the differences we observe between the anatomical and functional descriptions of the network. We investigated responses to activation of neuron RID, which has very few outgoing anatomical connections, but which is thought to communicate via neuropeptides released extra-synaptically from dense-core vesicles. We measured functional connections between RID and other neurons that express receptors for peptides released by RID. We found that the responses of these neurons to stimulation of RID were reduced in *unc-31* mutants, that are deficient for dense-core vesicle mediated extra-synaptic signaling when compared to the wildtype. This provides direct evidence that RID communicates extrasynaptically and, importantly, these connections are captured by our functional connectivity map.

Disclosures: S. Dvali: None. **F. Randi:** None. **A.K. Sharma:** None. **A.M. Leifer:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 328.12

Topic: I.03. Anatomical Methods

Support: Digital Research Alliance of Canada

Title: Recapitulating the circuit of Papez using diffusion MRI in rhesus macaque

Authors: *B. G. KARAT[1], J. KAI[2], A. R. KHAN[2], J. C. LAU[3];
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Abstract: The limbic circuit, known eponymously as the circuit of Papez, encapsulates the major white matter bundles that connect hubs playing essential functions including memory related processes. While this circuit has been described previously through dissection, it is relatively unknown if it can be feasibly recapitulated with non-invasive techniques such as diffusion MRI (dMRI). Accurate recapitulation is a crucial step for identifying key connections non-invasively to improve upon current understanding of limbic function. In this work we show that the limbic circuitry can be reconstructed using dMRI in a single post-mortem Rhesus macaque brain. Preprocessed diffusion-weighted images scanned on a 4.7 Tesla MRI were obtained with 0.5mm3 isotropic voxels with b-values of 1610, 4100, and 7700 s/mm2 and 420 directions per shell, with 108 b-values of 0 s/mm2 acquired (Ambrosen et al., 2020). Tractography was performed by seeding in the whole-brain white matter and selecting 20 million streamlines where connections between nodes were weighted by their underlying diffusion

signal. The NMTv2 cortical and subcortical atlas was transformed to the space of the diffusion images, where regions related to the limbic circuitry were identified. Streamlines connecting the nodes of the circuit of Papez were extracted, where streamline endpoints terminated within a 1.5mm radius of the node of interest. Trajectories were visually examined and compared to tract-tracing and dissection literature. Major connections were identified with tractography between the following nodes of the circuit: entorhinal cortex, hippocampus, mamillary bodies of the hypothalamus, anterior nucleus of the thalamus, and cingulate cortex. These connections corresponded to the following white matter bundles: perforant path, fornix, mammillothalamic tract, genu of the internal capsule, and the cingulum. Our findings demonstrate the feasibility of applying dMRI for identifying the limbic circuitry. Previous studies have demonstrated preservation of connections throughout the brain across different families of primates. These results suggest that the circuit of Papez, which has been previously studied in humans, is structurally preserved in rhesus macaques and likely other primates. Future work will look to parse any differences between humans and non-human primates within this circuit. Ambrosen, K. S., Eskildsen, S. F., Hinne, M., Krug, K., Lundell, H., Schmidt, M. N., van Gerven, M. A. J., Mørup, M., & Dyrby, T. B. (2020). Validation of structural brain connectivity networks: The impact of scanning parameters. NeuroImage, 204, 116207. https://doi.org/10.1016/j.neuroimage.2019.116207

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 328.13

Topic: I.03. Anatomical Methods

Support: NIH Medical Research Scholars Program

Title: Tractography Based White Matter Atlas of the Rhesus Macaque

Authors: *P. M. NADAR, K. S. SALEEM, A. V. AVRAM, P. J. BASSER;
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Abstract: The rhesus macaque monkey is an important model for many human neurological and psychiatric conditions. Digitized atlases that enable parcellation of multimodal neuroimaging data are critical for understanding brain structure and function and for designing translational studies for human health. Using previously acquired high-resolution imaging data from a macaque monkey brain, our group has previously delineated 219 subcortical structures, including the cerebellar cortex and hippocampal formation, and the subcortical white matter fiber bundles (Saleem et al., 2021). These segmented subcortical regions were then registered to a standard D99 cortical atlas (Saleem et al., 2021). Currently available atlases for mapping structural

connectivity use lower resolution data and extrapolate the location of certain tracts from human data for ease of automated segmentation. In this study, we integrate 1) a high-resolution DWI data (200 um), 2) a fine-grain cortical and subcortical atlas from our recent studies (see above), and 3) matched histology comparison from the same specimen to the construct of a draft of the most comprehensive white matter pathways (atlas) of the rhesus macaque to date. A perfusion-fixed adult male rhesus monkey (*Macaca mulatta*) brain was scanned using a Bruker 7T scanner with a 72 mm quadrature RF coil to acquire high-resolution MTR and DW images. Using the MTR image as a template, we preprocessed DWIs using the TORTOISE package to correct for Gibbs ringing artifacts, motion (drift), and imaging distortion due to magnetic field inhomogeneities and diffusion gradient eddy current. We then used Mrtrix3's probabilistic tractography algorithm IFOD2 to perform ROI-based parcellation of whole-brain tractography and delineated white matter tracts of interest (e.g., fornix, optic radiation, MLF, ILF, anterior commissure, cingulum, callosal tracts). We confirmed the spatial location of our fiber tracts with publicly available neuronal tracer studies and matched histological sections with five different stains derived from the same animal. We then compared our initial results to the automated white matter segmentation available through FSL's XTRACT. With the addition of major white matter tracts, the D99 atlas will provide a reference for standardized analysis of functional and structural macaque neuroimaging data.

Disclosures: **P.M. Nadar:** None. **K.S. Saleem:** None. **A.V. Avram:** None. **P.J. Basser:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 328.14

Topic: I.03. Anatomical Methods

Support: Brain Initiative 1RF1MH123233
NSF CAREER Award 1942963

Title: Heritability of Human Structural Connectome

Authors: *J. CHUNG, E. W. BRIDGEFORD, M. POWELL, J. T. VOGELSTEIN;
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Abstract: A connectome is a map of the connections in the brain, and the degree to which the connectivity patterns may be constrained by genetics and environment is only beginning to be understood. Here, we investigate the heritability of human connectomes by framing heritability as a causal question, that is, do changes in genetics cause changes in connectomes? We first define three statistical models for comparing connectomes, denoted exact, global scale, and vertex scale from simplest to most complex, based on statistical modeling of connectomes, which considers the inherent structure and dependencies within networks. We then leverage distance correlation (Dcorr) and conditional distance correlation (CDcorr), which can detect

linear and non-linear dependency between two properties by comparing the distances between all pairs of subjects of each property, to measure unconditional and conditional heritability, respectively (Figure 1). Unconditional heritability measures dependency of connectomes on genomes without accounting for potential confounders (e.g. neuroanatomy), while conditional heritability accounts for such confounders. We used the HCP 1200 dataset (n=1023) to estimate connectomes from diffusion MRI. We validate the connectomes by comparing the distances between same-sex MZ and DZ twins, which controls the shared environment. Using a one-sided Kolmogorov-Smirnov test, we find that the connectomes are different for almost all parcellations at $\alpha = 0.05$ after correcting for multiple comparisons (Figure 2a). This suggests the connectomes encode genomic differences. We then test for unconditional and conditional heritability of connectomes by comparing MZ, DZ, Sib, and unrelated individuals (Figure 2b&c). The key result is that for most parcellations, Dcorr and CDcorr tests show that for all three heritability models, the genome and the connectome were significantly dependent. This is strong evidence that connectomes are heritable. Crucially, the heritability of the connectome is not entirely explained by the heritability of the neuroanatomy.

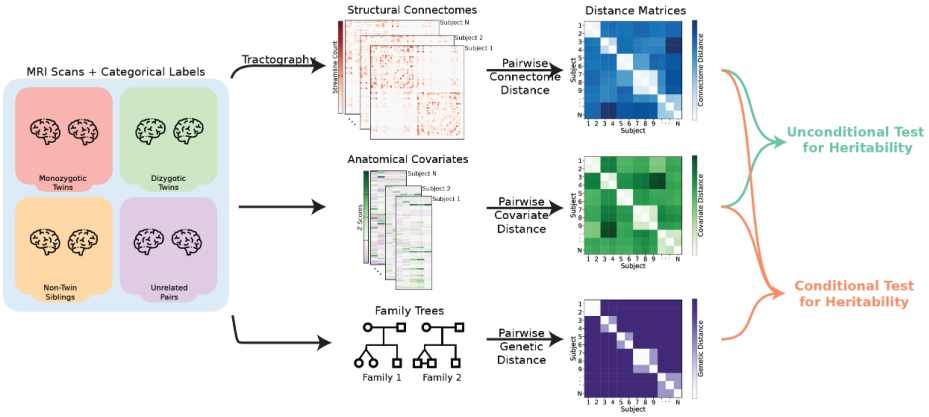

Figure 1: Overview of the framework for measuring heritability of connectomes. (Left) Diffusion and structural MRI (dMRI and sMRI) images are pre-processed and have associated categorical labels. Labels correspond to whether a pair of individuals are monozygotic twin, dizygotic twin, non-twin siblings, or unrelated pairs. Parcellations (e.g. Schaeffer, Desikan, AAL) are used to define a common set of vertices of the brain. (Center left) For each subject, connectomes and anatomical covariates are estimated from dMRI scans. Covariates (brain volume, fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity) are computed using both dMRI and sMRI scans. (Center right) For each modality, the distance between all pairs of individuals are computed. We consider three connectome distance funactions, each corresponding to a statistical model of heritability: 1) absolute distance between connectomes (exact), 2) distance after scaling so both connectomes have the same norm (global scale), and 3) distance but each ROI is scaled to have the same norm (vertex-wise scale). The genetic distance function is a categorical function where monozygotic (MZ) twins are encoded as zero, siblings (Sib) that are not MZ twins, including dizygotic (DZ) twins as one, and unrelated pairs as two. The covariate distance function is the Euclidean distance function. (Right) Distance correlation (Dcorr) is used to test for dependence between the connectomes and familial relationships, represented by the genetic distances, and conditional distance correlation (CDcorr) is used to test for dependence while controlling for potential effect confounders, such as the anatomical covariates.

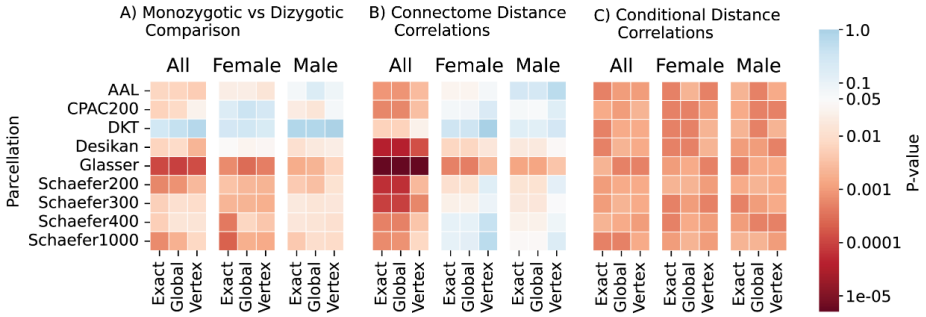

Figure 2: Testing for unconditional and conditional heritability of connectomes. Red squares indicate significant tests while blue indicate non-significant tests using Holm-Bonfferoni corrected α=0.05. The shared and non-shared environmental effects are controlled by comparing only the females or males. Each test is repeated for the three statistical heritability models. (A) Validating connectomes by comparing monozygotic and dizygotic twins using a one-sided, two-sample Kolmogrov-Smirnov test. We find significance on most parcellations and heritability models, suggesting that the differences in connectomes are most likely to be due to differences in genome. (B) Testing for unconditional heritability of connectomes using Dcorr. When considering all subjects, unconditional test is significant for all parcellations under all three heritability models, suggesting the connectomes are heritable. However, when separating the samples to same sex, the tests are significant for few parcellations, such as Glasser, Schaefer200 and Schaefer300. (C) Testing for conditional heritability of connectomes while controlling for neuroanatomy (e.g. brain volume, fractional anisotropy, mean diffusivity) using CDcorr. The tests are significant for all parcellations, meaning the connectivity of the brain are still heritable after conditioning on the anatomical covariates.

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

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Program #/Poster #: 328.15

Topic: I.03. Anatomical Methods

Support: NIH UG3MH126864
NIH RF1MH117820

Title: High resolution mapping of individual axonal projections in human white matter tracts

Authors: *E. TURSCHAK[1], B. MACLENNAN[1], A. HELLEVIK[1], E. PETERSON[1], P. BALARAM[1], K. TAKASAKI[1], M. J. TAORMINA[1], R. TORRES[1], O. GLIKO[1], S. SESHAMANI[1], S. BHASKARAN[1], E. PERLMAN[1], D. VUMBACO[1], J. ZHUANG[1], C. D. KEENE[2], R. C. REID[1];
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Abstract: Studies of white matter organization in human brains are crucial to advancing our understanding of both normal and neurodegenerative disease states. Current methods for studying white matter organization are typically limited in resolution, and the high levels of fixation used in archived human tissue make it difficult to label and visualize structures at high resolution in dense white matter regions. These issues have posed barriers for the ability to trace individual axons from source to termination. Recently, the Allen Institute for Brain Science has developed an axonal connectomics pipeline that combines hydrogel-based tissue processing, lightsheet microscopy, and computational segmentation to visualize individual axons at <1um resolution. Here, we employ this pipeline to visualize the structural organization of dense white matter regions in human brains.
Tissue slabs from human brains are fixed in paraformaldehyde, serially sectioned, and labeled with fluorescent markers to visualize cell bodies (DAPI and/or Nissl), vasculature (lectin), myelin (myelin basic protein), and axonal scaffolds (heavy, medium and light chain neurofilaments). The tissue is transformed into a swellable tissue-hydrogel matrix, which is then subjected to a detergent-based delipidation process, rendering it optically clear and >2x expanded when placed in water. Lightsheet data collection is performed using a modified commercially available (ASI Imaging) microscope.
The data from individual tissue samples are then digitally stitched to reconstruct axonal trajectories, and stitched volumes from sequential tissue samples are digitally aligned and transformed into 3D volumes. Individual axon segments are automatically detected with machine learning algorithms, then manually proofread to generate accurate skeletons of individual axons in white matter tracts below cortex. Combining detection of cell bodies with axonal skeletons then enables the tracing of single neuronal projections across large distances. Further analyses will quantify differences in axon caliber, direction, and cabling patterns within individual white matter tracts, thereby advancing our understanding of structural organization of dense white matter regions in human brains. Future studies combining high resolution axonal connectomics with diffusion magnetic resonance imaging and hierarchical phase contrast tomography will offer an unprecedented multi-scale interpretation of neuronal connectivity in large brain volumes.

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

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Topic: I.03. Anatomical Methods

Support: Simons Foundation

Title: A connectome project of the brain of the micro wasp Megaphragma amalphitanum

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Abstract: With the dramatic improvements in 3D electron microscopy (EM) and large-scale image data processing techniques, EM-based connectomics research has made significant achievements in recent years. Attempts have been made to identify neurons and synaptic connections between them comprehensively and quantitatively, focusing on the central nervous systems of various model organisms, including the fruit fly, *C. elegans*, mouse, fish, and primates. Our study aims to obtain the complete connectome of the brain of *Megaphragma amalphitanum*, a parasitoid wasp approximately 0.3mm in length. *Megaphragma*, one of the smallest flying insects, exhibits extreme miniaturization both in its body plan and the nervous system, with only about 5,000 neurons in the brain. Many of these neurons lose their cell nuclei during pupal formation, making adult neurons mostly anucleate. Despite this unusual nervous system, the brain of *Megaphragma* is highly homologous to the brain of other insects. We are identifying all neurons and synapses in a wasp whole-brain dataset scanned with focused ion beam-aided scanning EM (FIB-SEM), utilizing automated neuronal segmentation, synapse prediction, as well as manual proofreading. Traced neurons are systematically annotated based on the innervating brain regions and homology to neurons identified in other insects. As a partial result, we have reconstructed and annotated approximately 600 neurons in the optic lobe, the visual information processing center of the brain. In addition to the photoreceptor cells and their direct synaptic partners, we have identified neurons highly homologous to the T4 and T5 cells, neurons classified as primary components of the elementary motion detector in the fly's optic lobe. Many neurons and brain regions identified in other insects, including honeybee and fruit fly, have also been found in the *Megaphragma* brain, which is merely 0.02% of the honeybee brain and 1% of the fly brain by volume. These comparisons suggest that these neurons and neuronal circuits, although comprise much fewer number of neurons than other species, are essential for an insect's survival.

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Poster

328. Holistic Imaging to Imagine Brain Function

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Program #/Poster #: 328.17

Topic: I.03. Anatomical Methods

Support: ANR (Agence Nationale pour la recherche)
NIMH, Baszucki Brain Research Fund, Pittsburgh Foundation
Australian NHMRC
DFG
German Research Foundation DFG; Grants DA 1151/5-1, DA 1151/5-2
Health Research Board (HRA-POR-324)
R01MH116147

Title: Large-scale investigation of structural brain connectivity in bipolar disorder: a graph theory analysis of 959 individuals from the enigma bipolar disorder working group

Authors: *L. NABULSI[1], N. JAHANSHAD[1], E. VIETA[2], F. HOWELLS[3], M. WESSA[4], M. L. PHILLIPS[5], P. BRAMBILLA[6], G. DELVECCHIO[7], M. BELLANI[8], P. FAVRE[9], M. BERK[10], T. T. KIRCHER[11], U. DANNLOWSKI[12], P. MITCHELL[13], B. HAARMAN[14], C. MCDONALD[15], G. ROBERTS[13], D. C. GLAHN[16], M. BREAKSPEAR[17], J. HOUENOU[18], P. M. THOMPSON[1], O. A. ANDREASSEN[19], C. R. K. CHING[1], D. M. CANNON[15], &. THE ENIGMA BIPOLAR DISORDER WORKING GROUP[20];
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[18]APHP, Mondor Univ. Hospitals, UPEC, INSERM U955, Créteil, France & NeuroSpin neuroimaging platform, PsyBrain team, UNIACT Lab, CEA Saclay, Creteil, France; [19]Oslo Univ. Hosp. - Ulleval, Oslo, Norway; [20]Imaging Genet. Center, Mark and Mary Stevens Neuroimaging and Informatics Institute, Univ. of Southern California, Marina del Rey, CA 90292, USA, Los Angeles, CA

Abstract: Neuroanatomical abnormalities in fronto-limbic systems have been associated with disorders of emotion regulation, such as bipolar disorder (BD). Findings have not always been replicated due to small samples and differences in analysis methods used. Understanding disruptions in the rearrangement and connections of fronto-limbic regions with other functionally specialized cortico-subcortical subnetworks is key to understanding how the human brain's architecture underpins abnormalities of mood and emotion, particularly in BD. In the largest graph theory analysis of structural brain connectivity in BD to date, we investigated topological patterns with the anatomically improved precision conferred by combining ENIGMA-standardized subject-specific nodal parcellation/segmentation (Desikan-Killiany atlas) with non-tensor-based tractograms derived using a high angular resolution diffusion-weighted approach (Explore DTI v4.8.6). Individual structural connectivity matrices - binary, and weighted by streamline count and fractional anisotropy - were obtained for sixteen independent sites, contributing 449 BD (55% female) and 510 healthy (62% female) datasets (age range 18-65 years). Whole-brain metrics of segregation and integration, and permutation-based statistics (NBS v1.2) were used to investigate topological differences in BD relative to controls. Adjusting for age and site, we examined a main effect of diagnosis, sex, and the interaction of diagnosis-by-sex on global measures of integration, segregation, and regional dysconnectivity. Relative to controls, the BD group displayed differences across metrics of integration (range of p=.003-.03); these differences appeared to be driven by dysconnectivity between limbic regions and the basal ganglia (p=.03), and between fronto-temporo-parietal nodes (p=.04). While there was no detectable diagnosis-by-sex interaction in this sample (p>.05); male brains displayed differences across several whole-brain metrics ($p=3.6E^{-07}$-.04), driven by dysconnectivity between limbic regions and basal ganglia (p<.001), and fronto-temporo-parietal nodes (p=.01), compared to females. These large-scale, multisite findings lend stronger evidence for BD topological dysconnectivity, at the whole-brain level and regionally involving limbic and basal ganglia networks. The implications of these structural findings for functional network topology and structure-function coupling in BD are a focus of future investigation by the ENIGMA-BD Working Group.

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 328.18

Topic: I.03. Anatomical Methods

Support: PA Department of Health SAP #4100083102 to ACS

Title: Direct interhemispheric cortical communication via thalamic commissures: a new white-matter pathway in the primate brain

Authors: *D. SZCZUPAK[1], D. J. SCHAEFFER[1], X. TIAN[1], S. CHOI[1], F. YE[1], P. MENESES[2], F. TOVAR-MOLL[3], R. LENT[2,3], A. C. SILVA[1];
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Abstract: The Thalamic commissures (TCs) are a recently found fiber pathway that connects the cortex to the contralateral thalamus. Thus far, only characterized in rodents. In this work, we leverage high-resolution diffusion-weighted imaging, viral axonal fiber tracking, and functional MRI methods to accurately characterize the TCs in the primate brain. Furthermore, we show how the TCs development is similar to the rodent TCs and that they contribute to a clear functional connection of the thalamus with the contralateral cortex. Finally, we show that the TCs are present in humans with corpus callosum dysgenesis but were not identified yet in the healthy human brain. This set of results poses the TCs as an important fiber pathway in the primate brain

that allows for more robust interhemispheric connectivity and synchrony. Additionally, present the TCs as a fiber pathway that is common among eutherian mammals.

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Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 328.19

Topic: I.03. Anatomical Methods

Support: NIH Grant R01MH122957

Title: Mammalian brain network distances and their relationship to taxonomy and phylogeny

Authors: ***J. FASKOWITZ**[1], M. PUXEDDU[1], M. P. VAN DEN HEUVEL[4], B. MISIC[5], Y. YOVEL[6], Y. ASSAF[7], R. BETZEL[2], O. SPORNS[3];
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Abstract: Modeling neuroanatomy as a mathematical network allows for the extraction of quantitative features describing brain organization (Sporns 2012, Betzel and Bassett 2017). We can compare features derived from brains of varying shapes, sizes, and even species, without knowledge of precise homological neuroanatomy. Through such comparisons, we can assess how organization differs between brains, and further, observe how this relates to our understanding of between-species differences via taxonomies and phylogenies. In the present study, we use the MaMI database, a collection of brain networks reconstructed from post-mortem anatomical and diffusion MRI (Assaf, Bouznach et al. 2020, Suárez, Yovel et al. 2022) spanning 125 species and 12 taxonomic orders/super-orders. For each mammal, white matter tracts were reconstructed using diffusion tractography, and the volume-normalized streamline count was taken between grey matter regions (n=200) to form a network. To measure between-network distances we use the network portrait divergence (Bagrow and Bollt 2019), which compares networks based on shortest-path-length profiles. We found that within-taxonomy order distances are significantly closer than between-taxonomy distances (non-parametric Mann-Whitney U-test; p=0.006; Fig 1 a,c,d). Furthermore, 10k phylogenetic trees were downloaded and distances were obtained (Fig 1b) to estimate the evolutionary divergences between species (Upham, Esselstyn et al. 2019). The anatomical network distances were rank correlated with phylogenetic distances 10k times, creating a distribution of coefficients within the range 0.05 to 0.21 (Spearman's rho; Fig 1g). Collectively, these analyses demonstrate species-level organization across scales and informational sources: we relate brain networks distances, derived

from MRI, with evolutionary distances, derived from genetic and fossil information. Future work will evaluate the robustness of these relationships (i.e., with alternative measures; Fig 1 e,f,g) and explore fine-grained differences between mammalian species.

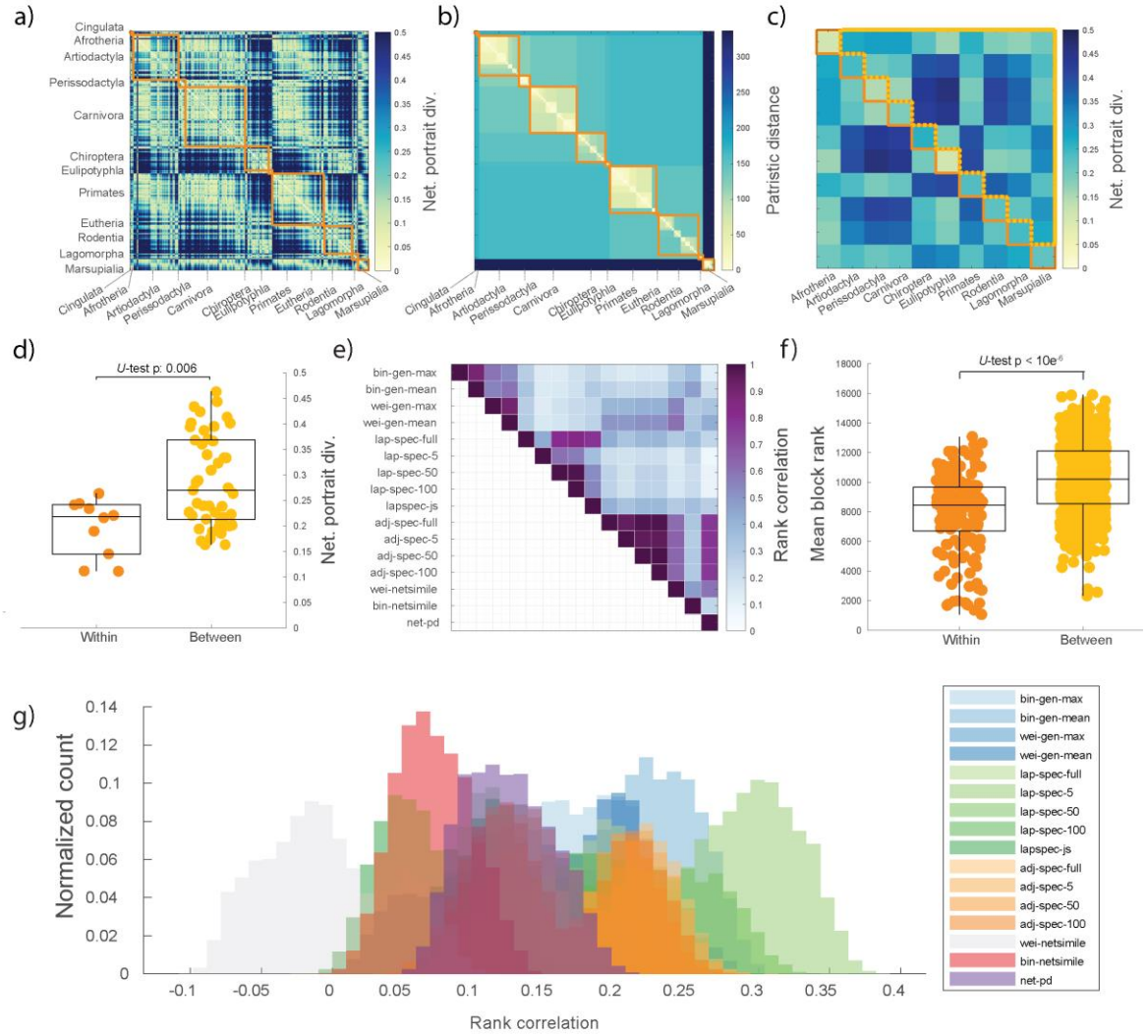


Figure 1. a) Network portrait divergence between all mammals, with orders outlined by orange blocks. b) Consensus phylogenetic distance between all mammals, using data from Upham et al. 2019. c) Network portrait divergence values averaged within- (orange) and between- (yellow) orders; block values are plotted as bar plot in d. e) Alternative measures can be used to obtain additional between-network distances (like in panel a) whose patterns we rank correlate. f) Alternative distance measures also show less distance within-order than between-order. g) Histograms of correlation coefficients derived from the comparison between network distance and phylogenetic distance between mammals; each histogram (color) contains data from comparisons to 10k estimated trees; histograms are colored if the bottom 1% correlation coefficient exceeds zero.

Disclosures: J. Faskowitz: None. **M. Puxeddu:** None. **M.P. Van den Heuvel:** None. **B. Misic:** None. **Y. Yovel:** None. **Y. Assaf:** None. **R. Betzel:** None. **O. Sporns:** None.

Poster

328. Holistic Imaging to Imagine Brain Function

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

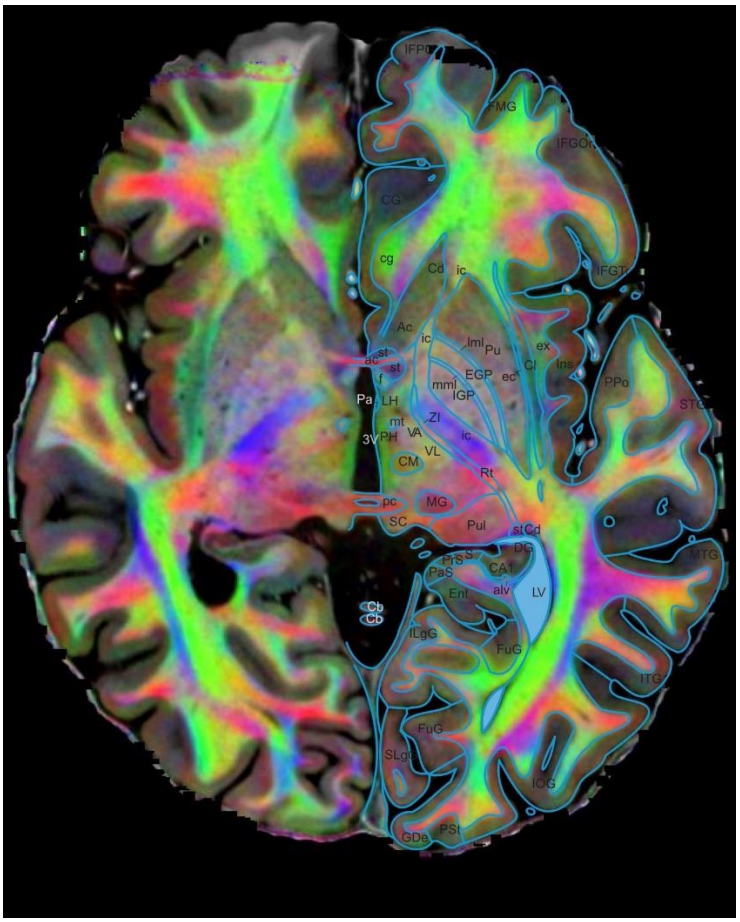
Program #/Poster #: 328.20

Support: NHMRC APP1140295

Title: Human Brain Atlas: an in vivo MRI dataset of sufficient resolution for detailed segmentations

Authors: *M. S. KASSEM[1], M. M. SCHIRA[2], Z. J. ISHERWOOD[3], M. BARTH[4], T. SHAW[5], G. PAXINOS[1];
[1]Neurosci. Res. Australia, Randwick, Australia; [2]Sch. of Psychology, Univ. of Wollongong, Wollongong, Australia; [3]Sch. of Psychology, Univ. of New South Wales, Sydney, Australia; [4]Ctr. for advanced imaging, Univ. of Queensland, Brisbane, Australia; [5]Ctr. for Res. on Ageing Hlth. and Wellbeing, Australian Natl. Univ., Canberra, Australia

Abstract: Virtually all major discoveries in neuroscience for the last 100 years have been underpinned by an increasingly detailed understanding of the architecture and connectivity of the central nervous system. We introduce herein *HumanBrainAtlas,* an initiative to construct a highly detailed, comprehensive, open-access atlas of the living human brain. The project combines high-resolution *in vivo* MR imaging and in-depth delineations, previously only available on histological preparations, within one segmented brain. We present the first step of this initiative: a comprehensive dataset of two healthy male volunteers reconstructed to a 0.25^3 mm^3 isotropic resolution for T1w, T2w and DWI contrasts. These contrasts act as windows to the anatomist, allowing the consideration of white matter directionality while delineating the grey matter and subcortex of the brain. Multiple high-resolution acquisitions were collected for each contrast and each participant, followed by averaging using symmetric group-wise normalisation (Advanced Normalisation Tools). The resulting image quality permits structural parcellation rivalling histology-based atlases, while maintaining the advantages of *in vivo* MRI. For example, components of the thalamus, hypothalamus, and hippocampus are typically difficult to identify within MR images while easy to do so within histology, however, now, they can be identified within the present MRI. This dataset is virtually distortion free, fully 3D and compatible with existing *in vivo* Neuroimaging analysis tools, such as AFNI, FSL, FreeSurfer and MRTrix. The dataset is suitable for research and teaching, and is approaching a fidelity of segmentation needed for clinical use. The dataset is currently publicly available via our website (www.hba.neura.edu.au), where we also provide the data processing scripts used. We invite the researcher, clinician, and perhaps the SfN enthusiast, to enjoy the detail of an ultra-high-field MRI and join us in ‘caressing the detail’ of the human brain.



Disclosures: **M.S. Kassem:** None. **M.M. Schira:** None. **Z.J. Isherwood:** None. **M. Barth:** None. **T. Shaw:** None. **G. Paxinos:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.01

Topic: I.04. Physiological Methods

Title: Development of vigilance-sleep platform to reveal drugs' EEG specific pattern and phenotype relevant rodent models

Authors: *C. HABERMACHER, J. VOLLE, C. DUMONT, B. CARABALLO, E. GRONLIER, Y. ROCHE;
SynapCell, Saint Ismier, France

Abstract: Chronic sleep insufficiency affects one third of the population and impacts physical and mental health. Alteration of sleep quality occurs also in a wide range of neurological and neurodegenerative disorders and could appear during the prodromal phase before the onset of typical clinical symptoms. In this context, characterization of drugs' effect on sleep and restoration of altered sleep architecture by pharmacological compounds represents a challenge for pharmaceutical industry.
Despite a highly fragmented sleep pattern compared to humans, rodents represent a relevant translational model with four identified vigilance stages: active and quiet wake, rapid-eye movement (REM) and non-REM (NREM) sleep. The combination of electroencephalogram (EEG) and electromyogram (EMG) recordings provides objective markers for monitoring vigilance states. However, manual scoring is prone to subjectivity. We took advantage of recent advances in artificial intelligence to develop an in-house platform to automatically detect sleep stages using artificial neural network-based algorithms.
Mice were implanted with parietal and nuchal electrodes, to allow EEG and EMG recordings over 8 to 24 hours. Selected reference compounds were tested to assess their effect on sleep architecture. Compounds were administered in a cross-over design and recordings were scored in 4 second epochs to identify sleep stages. Hypnogram, sleep stage occurency, sleep fragmentation and spectral analysis of the different stages were explored. We first validated our machine learning based scoring and then identified specific effects of tested compounds. The psychoactive caffeine increased wakefulness during 90 minutes after administration whereas medication used commonly for sleep disturbances such as zolpidem or suvorexant shortened the time to achieve sleep. Suvorexant, an orexin receptor antagonist, affected REM more prominently. In addition to the modification of hypnogram, diazepam induced a shift towards lower frequencies of REM peak frequency and a reduction of delta activity (1-4Hz) during NREM stages. These results highlighted the need to provide spectral analysis in relevant frequency bands to detect potential power changes specific of one or more vigilance states.
The use of state-dependent frequency bands analysis opens up a new dimension in the identification of sleep-wake stages EEG biomarkers. This project paves the way for the development of a drug screening process aiming at identifying potential side effects of drugs in development on circadian rhythms or aberrant sleep-vigilance states patterns in pathological models of neurological disorders.

Disclosures: **C. Habermacher:** A. Employment/Salary (full or part-time):; Synapcell. **J. Volle:** A. Employment/Salary (full or part-time):; Synapcell. **C. Dumont:** A. Employment/Salary (full or part-time):; SynapCell. **B. Caraballo:** A. Employment/Salary (full or part-time):; SynapCell. **E. Gronlier:** A. Employment/Salary (full or part-time):; SynapCell. **Y. Roche:** A. Employment/Salary (full or part-time):; SynapCell.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.02

Topic: I.04. Physiological Methods

Support: Simons Foundation

Title: Non-stationarity in neuronal dynamics and behavior produce predictable errors in spike sorting

Authors: D. F. ENGLISH[1], L. M. KLAVER[1], E. AKBAR[1], K. C. ARNDT[3], E. GILBERT[3], C. BUHLER[2], ***J. BASSO**[1];
[2]English Lab., [1]Virginia Tech. Neurosci. PhD Program, Blacksburg, VA; [3]Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Mammalian brains encode information in the spiking activity of populations of neurons. To study population coding in behaving animals, spikes from ensembles of hundreds of neurons can be recorded with extracellular electrodes (i.e. tetrodes, silicon probes, Neuropixels) and assigned to their source neurons using spike sorting. For decades, one problem has reduced the rigor and reproducibility of spike sorted data: it has so far been impossible to meaningfully quantify sorting accuracy because of insufficient empirically validated data, termed 'ground truth.' Ground truth data for spike sorting consist of extracellular recordings in which a subset of neurons is also recorded with a second, unambiguous technique, such as a glass electrode: a significant technical challenge. Ground truth data are essential for quantifying spike sorting performance and developing automatic spike sorters to replace manual methods which are time-consuming and error prone. Such automatic sorters are urgently needed, as novel technologies will soon enable the recording of so many neurons that manual curation will no longer be possible.Existing ground truth data are insufficient because: (1) short recordings and low sample size do not meet statistical power requirements; (2) almost all data are from anesthetized animals, while user data are from awake, behaving animals exhibiting different neuronal activity (e.g., spike train statistics), and signal artifacts (e.g., muscle contractions) which respectively obviate quantification of sorting performance and investigation of error sources; and (3) available data are from a limited number of brain areas, and the unique physiology of different areas and their cell types presents specific challenges to spike sorting. Here we address these challenges using silicon-juxtacellular hybrid microelectrode probes to obtain ground truth data in awake head-fixed mice. We recorded >40 single neurons with a juxtacellular electrode whose action potential waveform detected on the silicon probe was of sufficient amplitude to apply existing spike sorting algorithms. To investigate how physiological and behavioral factors affected the sorting of such spikes among the ~10-30 other neurons recorded by the silicon probe, we used three spike sorting algorithms and examined errors in spike sorting during periods of varying neuronal activity and behavior. We found that all three sorters were similarly susceptible to errors driven mainly by variability in spike waveform, burst spiking, increased population spike rate, electromyographic activity and less so by high frequency oscillatory activity and ambulation.

Disclosures: D.F. English: None. **L.M. Klaver:** None. **E. Akbar:** None. **K.C. Arndt:** None. **E. Gilbert:** None. **C. Buhler:** None. **J. Basso:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.03

Topic: I.04. Physiological Methods

Support: McKnight Foundation
Brain Initiative RF1NS113287
Brain Initiative RF1NS126044

Title: Cranial exoskeleton assisted multisite neural recordings in mice navigating 2D physical environments

Authors: J. HOPE[1], T. BECKERLE[2], P.-H. CHENG[4], Z. VIAVATTINE[5], M. FELDKAMP[6], S. FAUSNER[7], K. SAXENA[2], E. TROESTER[2], R. CARTER[3], T. J. EBNER[3], *S. B. KODANDARAMAIAH[1];
[1]Univ. Of Minnesota, Twin Cities, Minneapolis, MN; [2]Mechanical Engin., [3]Neurosci., Univ. of Minnesota, Minneapolis, MN; [4]Mechanical Engin., UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MN; [5]Univ. of Minnesota - Twin Cities, Minneapolis, MN; [6]Mechanical Engin., UNIVERSITY OF MINNESOTA - TWIN CITIES, Minneapolis, MN; [7]Mechanical Engin., Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Over the last few years, there has been considerable progress made on engineering neural recording devices, such as multiplexed high-density silicon probes that allow neuroscientists to record from hundreds of neurons simultaneously. Multi-site insertion of these probes allows simultaneous recordings from several brain regions, to provide a relatively comprehensive view of how computations in multiple disparate brain regions mediate behavior. Current technologies for brain-wide recording require either physical restraint of mice, which limits behavioral complexity; or extreme miniaturization of recording hardware which results in sharp tradeoff in device performance and capabilities. We have engineered a robotic cranial exoskeleton that allows a mouse to move in 4 degrees of freedom at physiologically realistic speeds in 2D environments while docked with a 1.5 kg ‘pixel-drive’ headstage. The exoskeleton uses the milliNewton scale forces applied by the mouse as input to an admittance controller to determine which direction it should maneuver and allows mice to traverse an arena of up to 60 x 60 x 20 cm (x, y, z) with unlimited rotation about their yaw axis. Mice are able to maneuver the recording headstage using the exoskeleton while navigating a linear track with velocity and acceleration profiles comparable to untethered mice moving in an open field, with walking velocities in the range of 3 to 10 cm/s on the exoskeleton as compared to 3 to 16 cm/s observed when freely behaving. Markerless tracking of gait dynamics indicates conservation of natural gait dynamics in mice tethered to the exoskeleton. Further, mice can perform a variant of the T-maze alternating choice task, with full control over their forwards/backwards, sideways, and yaw directions at the junction of the two goal arms while maneuvering the pixel-drive headstage. To demonstrate the utility of the exoskeleton, we acquired multi-site neural recordings from two silicon probes and simultaneously monitored pupil direction with a behavioral camera while mice performed the T-maze alternating choice task. In the future, the pixel-drives can be designed to accommodate and manipulate 6-8 high density silicon recording probes (see Poster

2022-S-9105-SfN for inverse kinematic strategies for probe insertion), allowing recordings from 1000s of neurons distributed across several brain regions simultaneously in mice moving and navigating physical environments, enabling completely novel experimental paradigms to understand nervous system function.

Disclosures: J. Hope: None. **T. Beckerle:** None. **P. Cheng:** None. **Z. Viavattine:** None. **M. Feldkamp:** None. **S. Fausner:** None. **K. Saxena:** None. **E. Troester:** None. **R. Carter:** None. **T.J. Ebner:** None. **S.B. Kodandaramaiah:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.04

Topic: I.04. Physiological Methods

Support: Branfman Family Foundation

Title: Mid-range frequency amplification of neural signals to improve spike detection

Authors: *E. ASHBOLT, G. MCCONNELL;
Stevens Inst. of Technol., Hoboken, NJ

Abstract: Microelectrode recordings (MERs) are commonly used for target confirmation during deep brain stimulation (DBS) electrode placement surgery. The neurosurgeon uses MERs to listen to characteristic neural activity patterns and identify brain regions. These recordings are challenging to interpret due partly to noise from environmental and biological sources. Here, we outline a strategy to amplify frequencies in the mid-range (0.5kHz - 1.5kHz) using a modified parametric equalizing (EQ) circuit. As human hearing is the most sensitive to mid-range frequencies, even subtle amplification in this zone may facilitate spike detection. The active analog amplification circuitry uses potentiometers to set the center frequency and gain, allowing for tailorable amplification. The circuit was designed *in silico* and tested using intraoperative neural recordings in anesthetized rats. Fourteen neural recordings (duration=10s) with over 1300 spikes were played back through the circuit. Spike detection ability was quantified in both the temporal and frequency domains by comparing the changes in signal-to-noise ratio (SNR) and power spectral density of neural recordings with and without amplification. In the temporal space, the SNR increased by 56% pre- vs. post-amplification (p &lt 0.05). In the frequency space, the power increased by 97% pre- vs. post-amplification (p &lt 0.01). We describe a parametric EQ circuit that increased the maximum amplitude of spikes and the power of the signals at a target frequency. The real-time and tunable features of the circuit design may enhance sonified intraoperative MERs by accelerating electrode implantation surgeries and minimizing the risk of electrode misplacement.

Disclosures: E. Ashbolt: None. **G. McConnell:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.05

Topic: I.04. Physiological Methods

Support: Israeli Ministry of Science and Technology #3-16486
Bar-Ilan Institute of Nanotechnology and Advanced Materials

Title: Magnetic actuators for remote neuronal guidance and extracellular recording

Authors: *D. LEVENBERG, E. VARON, G. INDECH, T. BEN ULIEL, Z. SHAPIRA, A. SHARONI, O. SHEFI;
Bar-Ilan Univ., Ramat Gan, Israel

Abstract: The ability to record individual neuron activity within a neural network is of great importance in understanding the brain in health and disease, with many potential implications in therapeutics and in the development of nano-chip devices. Recording neural activity by extracellular recording using multi electrode array (MEA) allows long term monitoring in vitro, and with potential for monitoring in vivo. However, currently MEA recordings are not specific due to cell migration and have a low efficiency in the number of electrodes that interact with cells. In this work we suggest a novel approach to locate cells at electrode sites by magnetic forces, achieving stable and specific cell-electrode correspondence. We transform neurons and neuron-like cells into magnetic units by an uptake of magnetic nanoparticles as well as by coating the cells with magnetic microparticles. Using live cell imaging, we kinetically analyzed the cell migration toward an external magnet. We develop and fabricate magnetic patterned substrates and a magnetic-embedded MEA device by photolithography process and sputter deposition. Our design is based on simulation of the magnetic fields which would be generated by the magnetic electrodes. We combine the magnetized cells and the magnetic substrates and analyze neuronal growth and activity. Viability and neurite length of the magnetized cells are similar to those of the control. The presence of electrical activity in the neural network is demonstrated by MEA recordings. The efficiency of the magnetic actuators for remote neuronal guidance is assessed and found to be high since the percentage of cells that reached the magnetic sample is significantly higher than that we would expect in a uniform distribution. In all, our study opens new possibilities for organizing and analyzing neural networks for long time periods and for the design of novel neural-chip interfaces.

Disclosures: D. Levenberg: None. **E. Varon:** None. **G. Indech:** None. **T. Ben Uliel:** None. **Z. Shapira:** None. **A. Sharoni:** None. **O. Shefi:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.06

Topic: I.04. Physiological Methods

Support: EU Project NeuraViPeR Grant 899287

Title: Novel alignment and insertion method for ultra flexible probe arrays

Authors: C. BOEHLER[1,2], A. SAEED[1], K. SHARMA[1], R. VAN DAAL[4], T. HOLZHAMMER[4], A. AARTS[4], T. L. TAY[5,6,3,2], *P. RUTHER[1,2], M. ASPLUND[1,2,3]; [1]Dept. of Microsystems Engin. (IMTEK), [2]BrainLinks-BrainTools Ctr., [3]Freiburg Inst. for Advanced Studies (FRIAS), Univ. of Freiburg, Freiburg, Germany; [4]ATLAS Neuroenginieerng, Leuven, Belgium; [5]Dept. of Biol., [6]Dept. of Anat. and Neurobio., Boston Univ., Boston, MA

Abstract: Chronic glial scaring limits the functionality of implantable intra-cortical neural probes. There is a great deal of evidence for that ultra-flexible and ultra-thin neural implants substantially minimize the chronic inflammation of tissue, enabling longer and more reliable stimulation or recording of neural activity. However, handling and implantation of such ultra-thin probes presents a challenge, especially for probes providing multiple shanks which are required to meet the demand for high channel count and large tissue volume coverage. In order to address this challenge, we introduce a simple yet efficient, precise, and easy to use implantation solution for ultra-thin and ultra-flexible implants. A set of polyimide neural probes with multiple shanks of different widths (35, 70 and 105 µm) at thicknesses of 2, 5, 15 and 25 µm and shaft length of 1.6 mm were fabricated to evaluate our implantation concept in a large parameter space. Micromachined silicon shuttles, adapted to the contour of the implants, were realized as mechanical insertion support. These shuttles were locally thinned to a thickness of 15µm in a wafer-level process. This is to reduce tissue damage during probe implantation yet providing precise insertion of the flexible implants, which were temporarily attached to the shuttles using a biocompatible dissolvable adhesive, i.e. polyethylene glycol. Since placement and alignment of micro-scale polyimide shanks onto the thinned Si shuttles is rather challenging, a 3D printed tool was engineered to easily and efficiently facilitate such alignment even for untrained individuals. Moreover, we introduce a 3D printed inserting mechanism that is capable of inserting several multi-shank probes simultaneously (parallel or sequentially). This inserter is laid out as a cartridge, which can be mounted on a stereotaxic arm so the overall probe positioning and accuracy can still be provided by the stereotaxic system. Lastly, our polyimide probes embed an insertion depth control feature that allows a precise insertion depth for each probe. The Innovative implantation concept has been tested by multiple neuroscience labs, where flexible probes were successfully implanted into the mouse cortex for chronic evaluation. In conclusion, a complete, reliable and efficient solution for ultra-thin probes was introduced and tested successfully. These outcomes encourage the long-term use and investigation of ultra-thin neural probes in the future.

Disclosures: C. Boehler: None. **A. Saeed:** None. **K. Sharma:** None. **R. Van Daal:** None. **T. Holzhammer:** None. **A. Aarts:** None. **T.L. Tay:** None. **P. Ruther:** None. **M. Asplund:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.07

Topic: I.04. Physiological Methods

Title: Exploiting CMOS-based probes' resolution for on-board and real-time data reduction techniques

Authors: *M. VINCENZI[1], A. M. FOSSATI[1,2], A. PERNA[1,2], G. ORBAN[1], J. F. RIBEIRO[1], F. BOI[1], G. ANGOTZI[1], L. BERDONDINI[1];
[1]Microtechnology for Neuroelectronics Lab., Fondazione Inst. Italiano di Tecnologia, Genova, Italy; [2]The Open Univ. Affiliated Res. Ctr. at Inst. Italiano di Tecnologia (ARC@IIT), Genova, Italy

Abstract: Brain Computer Interfaces (BCIs) are communication links capable of connecting the brain to external artificial machines, both for neurophysiological studies, as well as for breakthrough consumer and biomedical applications. The recent advances in active CMOS-based implantable probes led to the integration of dense electrode arrays of thousands of closely spaced microelectrodes, together with circuitry for signal conditioning and sub-millisecond recordings at ultra-low power consumption. On the acquisition side, although electrically wired approaches remain the easiest solution for first laboratory experiments, wireless-BCIs (W-BCIs) are better candidates for chronic experiments and applications.
However, because of the very high data rates that would be necessary for the transmission of full-resolution neural data acquired from high density CMOS-probes (e.g., hundreds of Mbps) and the bandwidth limitations typical of on-body wireless transmission technologies, W-BCIs need to be designed as a trade-off between resolution, form factor/lifetime, and range of transmission. It is therefore fundamental to study and implement data reduction strategies enabling to extract, transmit and store only the significant information contained in neural recordings.
Typically, sorting methodologies proposed in the literature are based on feature extraction, clustering and labelling, however, no definitive and unsupervised solution exists yet, especially for embedded applications. Indeed, the high-complexity typically associated with these strategies makes extremely hard their integration in embedded systems with low form factor and reduced power and computational budgets. Besides, existing sorters have a limited agreement on the extracted units and, typically, a manual curation step is required. In this study, we exploit the high-density and space-time redundancy provided by the SiNAPS CMOS-probes developed in our laboratory for exploring novel data reduction techniques, as an alternative to traditional sorting techniques based on the recognition of action potentials' waveforms. In particular, our approach focuses on measuring the intrinsic correlation between nearby electrodes' signals for implementing adaptive clustering of the recoding sites and for enhancing recordings' quality. This work demonstrates a high fidelity of the proposed data reduction approach with respect to

well-known sorters and ground truth dataset. It opens the path to the exploration of novel architectures for on-board and real-time processing of neural data.

Disclosures: **M. Vincenzi:** None. **A.M. Fossati:** None. **A. Perna:** None. **G. Orban:** None. **J.F. Ribeiro:** None. **F. Boi:** None. **G. Angotzi:** None. **L. Berdondini:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.08

Topic: I.04. Physiological Methods

Title: Using camera-guided electrode microdrive navigation for precise 3-dimensional positioning of electrodes in brain target volumes

Authors: *M. A. CRAYEN[1,2], S. SCHAFFELHOFER[3], D. HOEHL[4], U. THOMAS[4], M. ESGHAEI[1], S. TREUE[1];
[1]Cognitive Neurosci. Lab., German Primate Ctr., Goettingen, Germany; [2]Intl. Max Planck Res. Sch. for Neurosciences, Goettingen, Germany; [3]cortEXplore GmbH, Linz, Austria; [4]Thomas RECORDING GmbH, Giessen, Germany

Abstract: When investigating the functional role of neuronal activity in macaque cortex, an exact localization of recording sites is of critical importance. Traditional approaches for electrode navigation feature mostly a detailed record keeping of the electrode microdrives' position set by micromanipulators, or the use of grids that are inserted into recording chambers. Both approaches suffer from the accumulation of positioning errors, which we address with a novel navigation system, cortEXplore. Here, we mount the electrode microdrive on a sturdy frame, rather than a recording chamber, allowing 3D navigation with increased degrees of freedom. A 3D-representation of the target volume is created, based on co-registered MRI and CT scans. Thereby, electrode trajectories are defined in advance to a recording session by choosing the desired target- and entry-points. The Microdrive (Thomas Recording, customized for cortEXplore compatibility) and the target location are continuously tracked by cortEXplore's infrared camera system that allows precise evaluation of electrode positions relative to the target volume when advancing recording electrodes. To verify the actual electrode position after the end of experiments, we created local metal deposits at the recording site by applying a current to iron electrodes. These are detected in MRI scans and their relative offset to the planned target position evaluated, showing electrode navigation in agar filled containers is possible within a mean offset of 600 µm to the planned target. Here we considered three sources of positioning errors. 1. error due to optical co-registration of the virtual model of objects to their physical counterpart. This error depends on precision of technical drawings of involved tools as well as the quality of the 3D models of the target. This combined RMS error has been reported to be below 150 µm. 2. error due to the quality of the co-registration of MRI and CT images used for planning the trajectories. CT is used for skin and skull models, required for the co-registration

with the physical target. MRI is used to identify the target location in the soft tissue. Any offset between these two scans results in an equivalent physical error of the electrode to the target position. 3. the displacement between the planned and the final navigated microdrive location, due to aggregation of errors in micro-manipulation of the microdrive's position. We evaluate this offset online and correct it during the navigation. In summary, this system allows a more accurate 3D-positioning of recording electrodes, improving flexibility and accuracy of traditional methods.

Disclosures: M.A. Crayen: None. **S. Schaffelhofer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CEO of cortEXplore GmbH that developed the camera system and neuronavigation software used in this study. **D. Hoehl:** A. Employment/Salary (full or part-time):; Technical Director of Thomas RECORDING GmbH that developed the electrodes and microdrives used in this study. **U. Thomas:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Owner of Thomas RECORDING GmbH that developed the electrodes and microdrives used in this study. **M. Esghaei:** None. **S. Treue:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.09

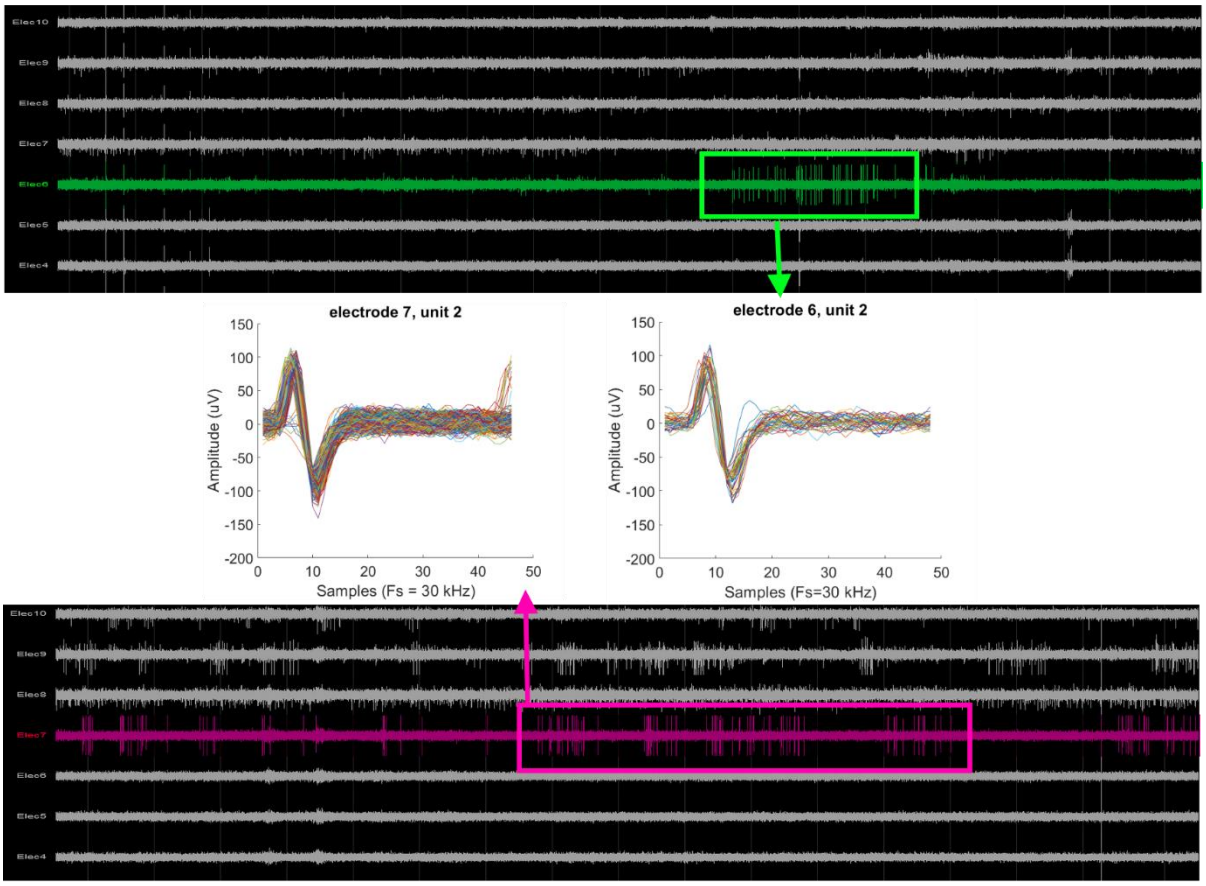
Topic: I.04. Physiological Methods

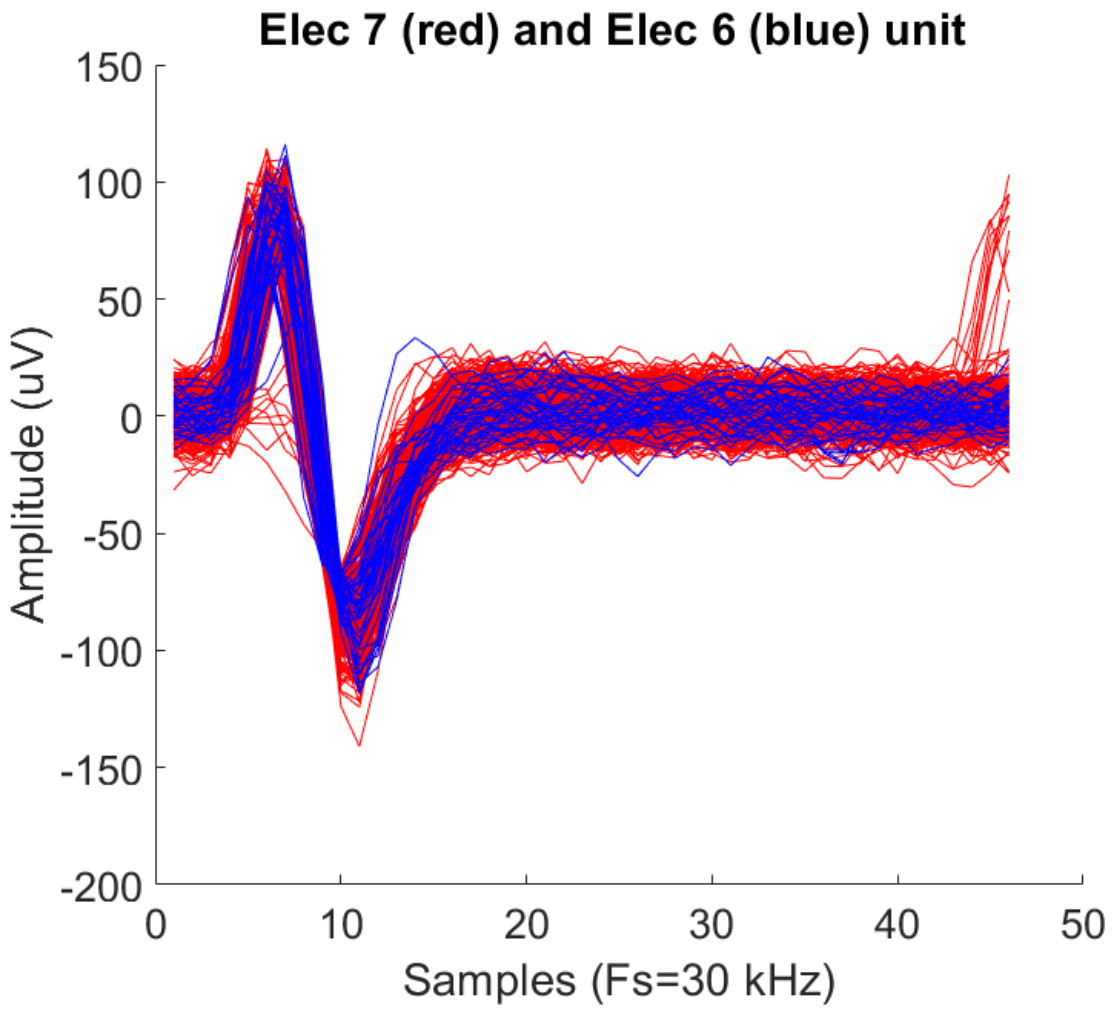
Title: Multi-contact laminar depth electrode for microelectrode recording during deep brain stimulation lead placement

Authors: *T. MAXFIELD[1], M. SORENSON[1], R. SURESH[1], M. BAILEY[1], S. HOU[1], S. WIEBE[1], B. NOUDOOST[2], R. BHANDARI[1];
[1]Blackrock Neurotech, Salt Lake City, UT; [2]Ophthalmology and Visual Sci., Univ. of Utah, Salt Lake City, UT

Abstract: Multi-contact laminar micro-electrodes are novel for human use. We have fabricated a penetrating depth electrode intended for use in deep brain stimulation (DBS) lead placements. The design consists of a laminar array of 12 sputtered iridium oxide film electrode contacts. The contacts are embedded in a polyimide substrate and attached to a flexible circuit board and rigid carrier. This feature is important since it enables our electrode to access deeper brain structures than wafer processed electrodes would normally reach. The current standard of care uses single contact probe, advanced incrementally, for physiological recordings to confirm deep brain target structures. We present the design and preliminary data collected in a rhesus macaque visual cortex. We were able to record single unit activity with a median signal to noise ratio (SNR) of 3.2, with a maximum SNR observed of 9.2. We were able to trace the same single unit activity on multiple electrodes as the laminar electrode was advanced through the cortical tissue. When

the electrode was fully inserted into the cortex, we were able to record several active single units simultaneously. This novel probe has the potential to decrease the time to confirm deep brain targets, as it bypasses the need for incremental advancement of a single electrode. Globally, only 2% of patients eligible for DBS therapy today move forward with the procedure. Technology that improves the efficiency and speed of this surgery has the potential to increase patient acceptance.





Disclosures: **T. Maxfield:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. 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.; ClearPoint Neuro. **M. Sorenson:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **R. Suresh:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **M. Bailey:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **S. Hou:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **S. Wiebe:** A. Employment/Salary (full or part-time):; Blackrock Neurotech. **B. Noudoost:** None. **R. Bhandari:** A. Employment/Salary (full or part-time):; Blackrock Neurotech.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.10

Topic: I.04. Physiological Methods

Support: U01NS115588

Title: High-density, large-scale recording and stimulating neural probes with integrated ASIC

Authors: *Y. MA[1,2], Y. FAN[1,3], L. SUN[3], P. ZOLOTAVIN[1,3], W. WANG[1,4], E. ALTUN[1,5], H. ZHU[1,3], T. CHI[1,3], C. XIE[1,3,6];
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Abstract: Implanted electrodes provide an electrical neural interface that resolves individual neurons and millisecond dynamics. They are critically useful for both fundamental neuroscience and clinical applications. Traditional rigid probes can hardly maintain a constant and stable recording because of the drastic mechanical mismatch at the tissue-probe interface. In contrast, the ultraflexible probes enabled a glial scar-free neural interface without blood-brain barrier (BBB) leakage or neuronal degeneration after the implantation (Luan *et al*., 2017). However, flexible probes, including nanoelectronic thread (NET), have limitations on scaling to an equivalent channel count compared to most recent rigid probes (Steinmetz *et al*., 2021). The micrometer-resolution photolithography (PL) techniques currently used to fabricate flexible probes are not capable of yielding such high an electrode density. On the other hand, there are also challenges in connecting large-scale electrodes with external or integrated acquisition electronics. Although multiple emerging solutions can scale up to thousand-channel per animal using high-density connectors and stackable electronics, this strategy is space and effort demanding (Chung *et al*., 2019; Zhao *et al*., in print). Here, we present the development of an approach to integrate application-specific integrated circuit (ASIC) with ebeam-lithography (EBL) based ultraflexible neural probes, that embody 5376 electrodes simultaneous recording sites. The first design will have 12 penetrating shanks, each of which has 448 recording sits at a density of 100 sites per millimeter depth. This high-density, ultraflexible probe will provide neuroscience studies in animal models with chronic, stable electrophysiology data at large scale.

Disclosures: Y. Ma: None. **Y. Fan:** None. **L. Sun:** None. **P. Zolotavin:** None. **W. Wang:** None. **E. Altun:** None. **H. Zhu:** None. **T. Chi:** None. **C. Xie:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.11

Topic: I.04. Physiological Methods

Support: R44AA020676

Title: Multichannel multi-sensor electrical, optical, electrochemical and fluidic tethered workstation system for long duration studies of behaving rodents

Authors: *D. WOODWARD*;
Neurosci. Res. Inst. of North Carolina, Winston Salem, NC

Abstract: A key feature of a new system design is a rotating tethered overhead platform system permitting recording and stimulation during behavior over months. The cable system links a rodent overhead system that tracks movements. Connections to the animal include head stage plugs, interface devices, cables, imagers, light sources, and multiple fluid connections to parallel computational devices above in the chamber. The rotating design allows for multiple connections to the animals by eliminating need for a complex swivel system. Multiple stepper motors control injection of drugs or micro dialysis probes. **A second component is an array of simple field programmable gate arrays (FPGAs) for interface with ADs, DACs, DIGIO, Neuropixel, and imager sensors.** FPGA/CPUs capable of computation then conduct complex waveform, image analysis, and stream data to parallel cpus on a network. CPUs both inside and outside the chamber manage connections directly to the animal and outside the chamber to control logic and timing of behavioral devices. Cpus include a real time software state machines to manage logic, timing, and data flow between electrical, chemical, and optical sensors, and stimulation devices during behavioral protocols. We recently developed arrays of carbon fibers as chemical sensors with creation of new data streams. Programmable fast scan waveforms require use of real time state machines for optima use of fibers in arrays. The new fiber array design demonstrated capability of alternate recording of spike trains, field potentials and electrochemical signals from the same fiber. **A third critical feature is implementation of Precision Time Protocol (PTP) that allows all acquisition processors and data to synch to a common high 100 nsec resolution 64-bit digital clock running continuously on a single CPU.** The use of PTP allows time synchronization to be reestablished for all data. This feature greatly simplifies controlled access to separate data segments in files accumulated across days. **A fourth feature in progress toward a structure for data organization with naming of files for real time extraction and analysis of behavioral events of interest.** Multiple files, settings, and notes are auto stored in directories created for each epoch and phase of behavioral protocols. Structure data design facilitates remote extraction of complex data around time points of interest for statistical analysis of transitions and trends. Query software modules permit remotely shared access to terabytes of local stored data. **Development of common remotely shared data local servers is essential for a future community to share effort toward large research efforts.**

Disclosures: D. Woodward*: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); BIOGRAPHICS INC OWNER.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.12

Topic: I.04. Physiological Methods

Support: T32 MH 119168-3
U01 NS113279
R21 DA049592
R01 NS102725
R01 NS089688
R21 DA043817

Title: Polysaccharide-based Bioactive Coating for Recording Microelectrodes

Authors: *V. DHAWAN[1,2], X. T. CUI[1,2,3];
[1]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for Neural Basis of Cognition, Pittsburgh, PA; [3]McGowan Inst. for Regenerative Med., Pittsburgh, PA

Abstract: Implantable neural electrodes can record neural activity, stimulate neurons, and sense neuroactive species with high spatial selectivity. However, upon their invasive implantation, neural devices are exposed to a foreign body response (FBR) which can contribute to deterioration in device function for chronic implantation. Bioactive coatings have shown promise in camouflaging the electrode surface to promote seamless integration with the surrounding tissue while maintaining device performance. Existing protein-based coatings are difficult to source and are vulnerable to denaturation during processing. Chondroitin sulfate (CS) is a naturally occurring polysaccharide in the brain, is commercially available and can offer a more stable alternative with specific bioactivity for improving device-tissue interface. Negatively charged sulfate groups of CS are involved in neurite-growth promoting properties, while surface bound CS can be utilized to sequester MCP-1 and dampen the downstream inflammatory cascade. Due to the abundance of hydroxyl and sulfate groups in the CS, the hydrophilicity imparted to the surface can help prevent non-specific protein adsorption, the first step in the FBR. Silicon electrodes were functionalized with CS via divinyl sulfone linker chemistry and immobilization was confirmed via Fourier-transform infrared spectroscopy, ellipsometry and water-contact angle measurements. An *in vitro* protein-adsorption test demonstrated a significantly lower protein coverage on CS-coated probes as compared to the uncoated control ($p<0.01$). Primary cortical neurons were grown on CS and control SiO_2 glass coverslips ($n=4$ cultures) and total neurite length was quantified. The normalized neurite length was significantly higher in CS groups (433+/-142%, $p<0.0001$) as compared to the control (100+/-39%). Preliminary experiments with primary microglia grown on CS-coated coverslips had smaller morphology with reduced cellular extensions and a lower cell count, as compared to uncoated controls which seem to promote normal growth. Future work will investigate interactions between microglia and CS using *in vitro* inflammation assays. *In vivo* recording and histological assessments will be performed to investigate the effect of the CS coating on neural recording quality and immune response to implantation. This study utilizes the untapped potential of polysaccharide-based coatings which can improve device performance by aiding the seamless integration of neural electrodes with the native tissue.

Disclosures: V. Dhawan: None. **X.T. Cui:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.13

Topic: I.04. Physiological Methods

Support: NIH 1 R01 DK120307-01

Title: Pelvic nerve injuries in aging and multiparous rabbits result in stress urinary incontinence-like behavioral, anatomical and functional deficits

Authors: *F. S. RAHMAN[1], Z. YOUSUF[1], M. I. ROMERO-ORTEGA[1], P. ZIMMERN[2]; [1]Biomed. Engin., Univ. of Houston, Houston, TX; [2]Dept. of Urology, 2The Univ. of Texas Southwest Med. Ctr., Dallas, TX

Abstract: Introduction: Pelvic floor neuromuscular damage has been linked to stress urinary incontinence (SUI), affecting approximately 26% of adult women in the United States. A well-established animal model is required for further SUI treatment-oriented research. While previous research has shown that multiparous female rabbit models (retired breeders or RB) are viable SUI models due to their similarities in pelvic floor muscle, specific information on micturition behavior and physiology, and correlation to nerve morphometry is missing. This study seeks to establish standardized methods to quantitatively evaluate SUI in the rabbit animal model in RB animals at baseline and after targeted neuromodulation treatments.**Methods:** New Zealand female rabbits were used for the study. Absorbent pads are placed underneath animal cages to collect their urinary voids during daytime and overnight hours to provide a full 24-hour window of monitoring. Pads are weighed to record total micturition masses and wet pads are imaged and analyzed to identify “spotting” (urinary leakage) behavior. Micturition pad logging is done during baseline (2 weeks) and during treatment period (4 weeks) where the animals are treated by electrical stimulation for 28 days using a miniaturized wireless neural stimulator “NeruoClip” implanted unilaterally on the bulbospongiosus nerve (BsN). The pad data is analyzed for pre- and post-treatment results and compared between groups: active implant vs sham/no implant. Additional analysis included BsN and bulbospongiosus muscle (BsM) electrical stimulation thresholds, cystometry data, and nerve and muscle histology for a comprehensive effort to determine what aspect of this animal model relevant to the SUI condition and can be used as metrics for evaluating treatment efficacy.**Results and Discussion:** Daily micturition pad weight was approximately 20-50 g in most RB rabbits, and 100-150g in most young nulliparous (YN) control animals. Intermittent leakage was observed as small areas outside their normal voiding corner. Animals with active implants show a significant increase in micturition pad weight to levels comparable to that in YN animals. This behavioral SUI-like deficits were confirmed using BsN and BsM activation thresholds, cyctomery and nerve-muscle histology.Together, the data defines quantitative metrics that can be used to diagnose SUI-like symptoms in the RB rabbits and that can be used as a valid metric to inform the treatment efficacy, particularly of neuromodulation-based treatments.**Funding:** NIH 1 R01 DK120307-01**Disclosure:** MRO is a

shareholder of RBI Medical, a company that has commercial interest in neuromodulation of the PFM.

Disclosures: **F.S. Rahman:** None. **Z. Yousuf:** None. **M.I. Romero-Ortega:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regenerative Bioelectronics Inc.. **P. Zimmern:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.14

Topic: I.04. Physiological Methods

Support: R01DA045664
R01MH116904
R01HL150566

Title: Neurostring: a tissue-like neurotransmitter sensor for the brain and gut

Authors: *J. LI[1], X. CHEN[2], S. P. PASCA[3], B. CUI[3], Z. BAO[3];
[1]Michigan State Univ., Michigan State Univ., Okemos, MI; [2]Biol., [3]Stanford Univ., Stanford, CA

Abstract: Neurotransmitters play essential roles in regulating neural circuit dynamics both in the central nervous system as well as at the peripheral, including the gastrointestinal tract. Their real-time monitoring will offer critical information for understanding neural function and diagnosing disease. However, bioelectronic tools to monitor the dynamics of neurotransmitters in vivo, especially in the enteric nervous systems, are underdeveloped. This is mainly owing to the limited availability of biosensing tools that are capable of examining soft, complex and actively moving organs. Here we introduce a tissue-mimicking, stretchable, neurochemical biological interface termed NeuroString, which is prepared by laser patterning of a metal-complexed polyimide into an interconnected graphene/nanoparticle network embedded in an elastomer. NeuroString sensors allow chronic in vivo real-time, multichannel and multiplexed monoamine sensing in the brain of behaving mouse, as well as measuring serotonin dynamics in the gut without undesired stimulations and perturbing peristaltic movements. The described elastic and conformable biosensing interface has broad potential for studying the impact of neurotransmitters on gut microbes, brain-gut communication and may ultimately be extended to biomolecular sensing in other soft organs across the body.
Reference: Nature 606, 94-101 (2022).

Disclosures: **J. Li:** None. **X. Chen:** None. **S.P. Pasca:** None. **B. Cui:** None. **Z. Bao:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.15

Topic: I.04. Physiological Methods

Title: Patterned hippocampal culture with single cell resolution electrode interfacing for stimulation and recording

Authors: *K. MRITUNJAY, J. C. STURM;
Electrical and Computer Engin., Princeton Univ., Princeton, NJ

Abstract: In this paper we describe experimentally the ability to spatially pattern hippocampal neurons on a single cell resolution to interface them with individually patterned electrodes for one-cell to one-electrode stimulation and recording of neuronal signals. The process starts with 8-micrometer wide gold electrodes on a fused silica substrate, underneath and perpendicular to the subsequent axons. To guide the neuron growth at specific desired locations, the electrode-patterned surface is covered with a pattern of cytophilic (N1-(3-Trimethoxysilylpropyl)diethylenetriamine) and cytophobic (Trimethoxyphenylsilane) coatings, with 5-micrometer-wide cytophilic regions to guide single axons. A self-aligned method is used to precisely align cytophilic and cytophobic layers to create sharp boundaries between them. Crucially, we observe single axons growing on the cytophilic regions, crossing and in contact with the underlying perpendicular gold electrodes. For the cell culture, 17-day pregnant Sprague Dawley rats are euthanized to extract embryos. The hippocampus is dissected from the E17 embryonic brain and is cultured on a 12-well cell plate with the patterned electrode substrate at the bottom of the well. A seeding density of ~1500 cells/mm^2 is used and the cells are cultured in Neurobasal media with PSG (1%), B27 (2%), and NGF (0.1%) as supplements. Half of the media is changed after 2 days to remove unattached neurons and subsequently half of the media is changed after every 3 days. The patterned electrode and axons geometries we demonstrated are designed using a full electrical numerical model of the neuron-electrode interface that includes the cytoplasm of the neuron, the membrane and ion pumps (using the Hodgkin Huxley model for Hippocampal cells), the extracellular media and the electrode interface. The model shows that with our demonstrated single cell-single electrode structure, a single neuron can be stimulated using a microampere-scale current, and action potential signals from an excited neuron would create a millivolt-scale signal in the electrode. The study of single cell resolution stimulation and recording will aid in the understanding of hippocampal plasticity and creating artificial Brain on Chip. Such a system will also help in *in vivo* electrode implants' ability to stimulate and record from single cells in human brain.

Disclosures: K. Mritunjay: None. **J.C. Sturm:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.16

Topic: I.04. Physiological Methods

Support: NSF-NCS Grant 1926804

Title: Fully flexible implantable neural probe for electrophysiology recording and chemical stimulation

Authors: *M. MALEKOSHOARAIE[1], B. WU[2], D. KRAHE[2], Z. AHMED[1], X. CUI[2], M. CHAMANZAR[1];
[1]Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA; [2]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Targeted delivery of neurochemicals and biomolecules for chemical stimulation of neural activity in the brain enables the investigation of neural circuit dynamics by adding another dimension of control to the neuromodulation toolset. Existing conventional electrical neural probes can record or stimulate different types of neurons nonspecifically. Localized drug delivery to the nervous system has been recently demonstrated as a more specific modality for neurostimulation. We have designed novel flexible neural implants capable of controlled localized chemical stimulation and high-density electrophysiology recording. To minimize tissue damage and response, the neural probe was implemented with a small cross-sectional dimension (20μm x 330μm) using planar micromachining processes on Parylene C (PC), a mechanically flexible biocompatible substrate. A stack of Platinum/Gold/Platinum metals was sandwiched between two PC layers to form the isolated metal traces and electrodes. Two types of electrodes were microfabricated on the probe shank, including two large chemical sites (40μm x 400μm) for loading sufficient drugs and sixteen small electrodes (30μm x 30μm) for electrophysiology recording to monitor neuronal response to drug release. After multiple CV cleaning cycles, Poly(3,4-ethylenedioxythiophene) (PEDOT) was electrochemically coated on recording electrodes to reduce the impedance while keeping the size of the electrodes small to achieve higher fidelity neural recording. In addition, PEDOT doped with sulfonated silica nanoparticles (SNP) was used on chemical sites to achieve controlled drug loading and releasing. Electrochemical impedance spectroscopy measurement showed an average impedance of 16.5 kΩ for the recording electrodes and 4.5 kΩ for the larger chemical release sites at 1kHz. Different neurotransmitters, including glutamate (GLU), and gamma-Aminobutyric acid (GABA), were incorporated into the SNP and electrically triggered to release repeatedly. The in-vitro experiment was conducted to demonstrate the on-demand chemical release by applying a sinusoidal voltage (0.5 V, 2 Hz). The flexible neural probe was implanted in the barrel cortex of the wild-type Sprague Dawley rats using a Tungsten wire shuttle. GLU and GABA release caused a significant increase and decrease in neural activity, respectively, recorded by the recording electrodes. This novel flexible neural probe technology combining on-demand chemical release and high-resolution electrophysiology recording is an important addition to the neuroscience toolset to dissect neural circuitry and investigate neural network connectivity.

Disclosures: M. Malekoshoaraie: None. **B. Wu:** None. **D. Krahe:** None. **Z. Ahmed:** None. **X. Cui:** None. **M. Chamanzar:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.17

Topic: I.04. Physiological Methods

Support: NIH RF1NS113303-01

Title: High density and mechanically robust microfabricated stainless steel neural interface for large and small animals

Authors: *Z. AHMED[1], V. JAIN[1], V. HASSANZADE[1], I. KIMUKIN[1], T. TEICHERT[2], M. CHAMANZAR[1];
[1]Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Development of accurate models of neural circuits and improving our understanding of brain function and disorders require highly robust tools capable of recording from neurons at the cellular resolution with minimum tissue damage. High resolution microfabrication techniques developed in silicon material platform have led to ultra-high density neural probes for small animals, leading to significant improvement of our ability for recording high spatio-temporal resolution signals from large ensembles of neuronal populations. However, these high-density silicon probes are not mechanically robust. For probing large animal brains with minimal tissue damage, neural interfaces with long shanks and small cross sections are required. The brittleness and fragility of silicon makes such high aspect ratio probes vulnerable to fracturing and breaking in large animal brains during and after implantation. Therefore, for large animal neural interfaces, a more mechanically robust material platform like stainless steel is suitable due to its fracture resistance, corrosion resistance and biocompatibility. However, to realize high density channels on neural probes, high resolution microfabrication processes are necessary. Unlike silicon, such processes are not as mature and well-characterized for stainless steel substrates. We have developed a novel microfabrication process to realize mechanically robust stainless steel probes with high density recording channels based on a hybrid polymer-stainless steel architecture. Using this novel microfabrication platform, neural probes with 8 cm long shanks and 16- 100 channels have been realized. The functionality of the probes have been validated in macaque motor cortex through spontaneous single and multi-unit recording. In addition, for smaller animals, we have fabricated shorter (~1 cm) steel probes with densely distributed channels. We have also demonstrated that these probes can penetrate through intact rat dura without any notable damage to the recording electrodes or the insulations. This obviates the need for surgical removal of dura and leads to less accidental tissue and vascular damage and brain swelling. We have noted stable neural signal quality in rat hippocampus after multiple dura penetrations with the same probe. We have conducted histological evaluations with NeuN immunostaining and observed significantly reduced tissue damage in superficial layers of cortex when steel probe was used for dura penetration as opposed to surgical removal of dura before

implantation. In this presentation, we will discuss the design, characterization and in vivo measurements from both macaque and rat models.

Disclosures: Z. Ahmed: None. **V. Jain:** None. **V. Hassanzade:** None. **I. Kimukin:** None. **T. Teichert:** None. **M. Chamanzar:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

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Program #/Poster #: 329.18

Topic: I.04. Physiological Methods

Support: NIH Grants R01NS110564
NIH Grant R21 DA049592
NIH Grant R01NS089688
NIH Grant R21NS123937

Title: Integration of electrochemical and electrophysiological sensing on flexible multielectrode arrays to achieve multimodality and implant stability

Authors: *E. CASTAGNOLA[1], E. M. ROBBINS[1], M. PWINT[1], B. WU[1,2], X. CUI[1,3,2]; [1]Bioengineering, Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for Neural Basis of Cognition, Univ. of Pittsburgh, Pittsburgh, PA; [3]McGowan Inst. for Regenerative Medicine, Univ. of Pittsburgh,, Pittsburgh, PA

Abstract: The real-time measurement of neurochemical and electrophysiological activities in living brain is of utmost importance for understanding brain functions in normal and pathological conditions to improve diagnosis and treatment of neurological and neuropsychiatric diseases. Devices capable of multimodal readings of neural signals *in vivo* for extended periods of time are a key technological bottleneck. Microfabricated multielectrode arrays (MEAs) are routinely used for measuring neurophysiological activity from multiple sites across different brain depths. Flexible MEAs on thin-film polymeric substrates can seamlessly integrate with the neural tissue and record stable neural signals for months. However, MEAs with metal microelectrodes present poor sensitivity towards electroactive neurotransmitters, such as dopamine (DA) and serotonin (5-HT), when using direct electrochemical detection. To respond to the double need of multimodality and implant stability, we optimized a high-resolution mask-less photolithography process to transfer glassy carbon (GC) MEAs on thin flexible SU-8 substrate with miniaturized features to promote tissue integration. First, GC MEAs demonstrated simultaneous multi-site detection of stimulated phasic DA release in the mouse dorsal striatum (DS) using fast scan cyclic voltammetry (FSCV). Secondly, by optimizing PEDOT/CNT coating and square wave voltammetry (SWV) waveforms, PEDOT/CNT-coated GC MEAs presented high sensitivity towards 5-HT (17.56 nA/µM) and DA (55.63 nA/µM). Furthermore, they showed an excellent selectivity against the most common neurochemical interferences. Our flexible PEDOT/CNT-

coated MEAs were able to clearly discriminate: 1) 5-HT peak at 0.29V at multiple-locations of the mouse hippocampus; and 2) DA peak at 0.12V at multiple-locations of the mouse DS. Finally, our flexible PEDOT/CNT-coated MEAs achieved multi-channel detection of tonic 5-HT and DA concentrations for up to 3 weeks and showed seamless tissue integration. Our results highlight the potential of flexible GC MEAs as a platform for integrated multi-channel neurochemical and electrophysiological recording, a major enabling technological advancement for understanding brain functions and neurochemistry.

Disclosures: E. Castagnola: None. **E.M. Robbins:** None. **M. Pwint:** None. **B. Wu:** None. **X. Cui:** None.

Poster

329. Electrophysiology: Electrode Arrays

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Program #/Poster #: 329.19

Topic: I.04. Physiological Methods

Support: ETH Zürich
Swiss National Science Foundation
Human Frontiers for Science Program
Swiss Data Science Center
OPO foundation
FreeNovation

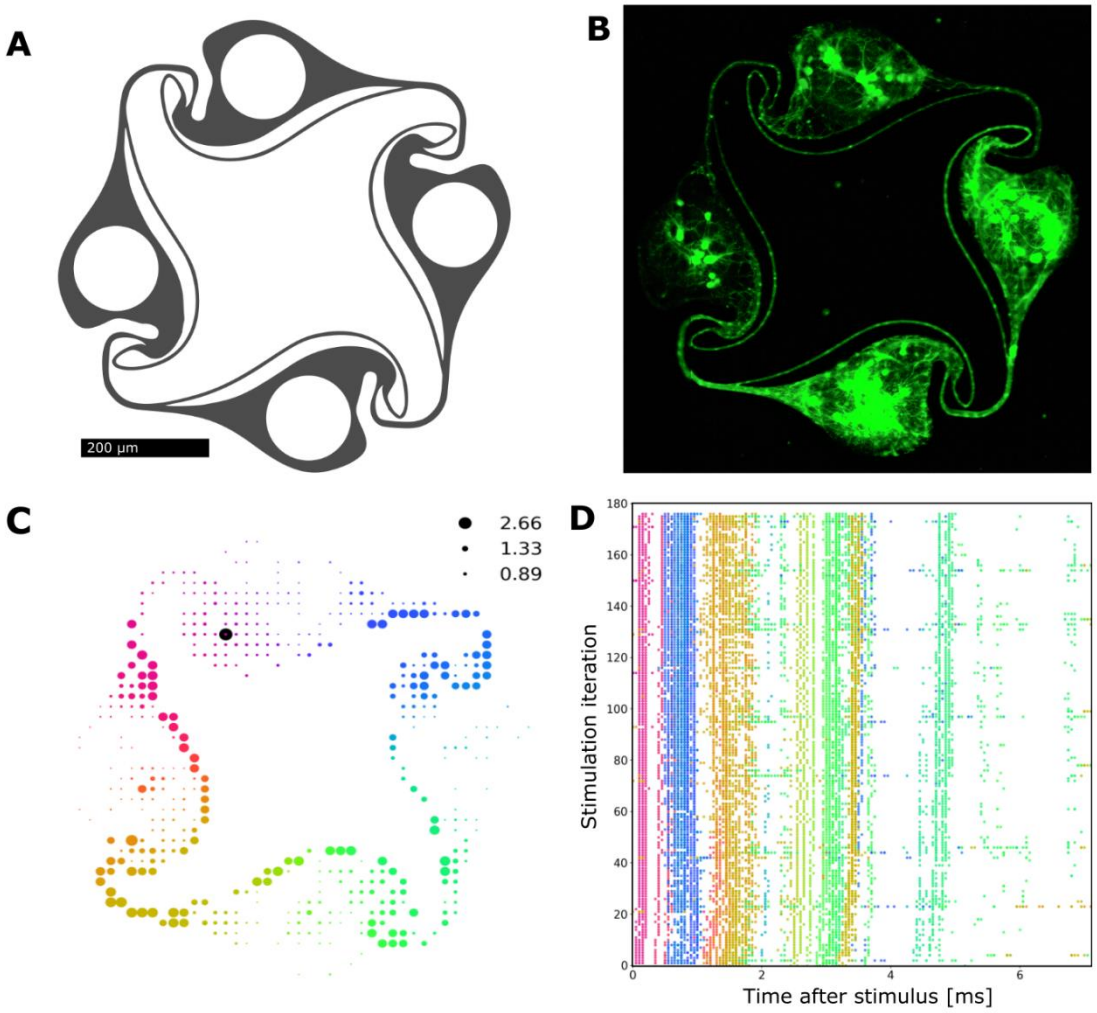
Title: Investigation of the input-output relationship of small engineered neural networks on CMOS microelectrode arrays

Authors: *J. DURU, C. GILES DORAN, B. MAURER, S. J. IHLE, J. KÜCHLER, J. VÖRÖS; Inst. for Biomed. Engin., Zürich, Switzerland

Abstract: Bottom-up neuroscience aims to reduce the complexity of neural networks *in vitro*. Using polydimethylsiloxane (PDMS) microstructures, neural circuits of only a few neurons with controlled connectivity are established on microelectrode arrays (MEA). In contrast to studying neural circuits *in vivo* or *ex vivo*, this bottom-up approach allows the isolation of neural circuits with no interference of adjacent circuitry. Such engineered networks were previously established on low-density MEAs with an electrode pitch of 500 μm (Ihle et al., 2021).
Recently, we have transferred this technology to complementary metal-oxide-semiconductor (CMOS) MEAs, increasing the electrodes covering a four-node PDMS microstructure by a factor of 20, covering both axonal and somatic locations of the network at an electrode pitch of 17.5 μm (Duru et al., 2022).
In this study, we have investigated the input-output relationship of such engineered neural networks composed of primary cells from the cortex of E18 Sprague-Dawley rat embryos. Inputs were given to the network in the form of 4 Hz biphasic voltage stimuli ranging from 100 - 1000

mV amplitude at every single electrode covering the network. The immediate response within the first 15 ms post stimulus for given locations was analyzed as it is highly reproducible over the duration of the stimulation period. We observe that this immediate network response is highly dependent on stimulation location and amplitude. Applying a stimulation voltage of 200 mV within the 10 µm thin axon-guiding microchannels of the microstructures is sufficient to induce a stable network answer, while stimulation voltages of 800 mV are necessary to induce an answer at somatic locations at the openings of the microstructure. Our approach allows to determine the flow of information across the network and reveals the details of the network architecture.

Figure: A Used microstructure. B Network (DIV 8) on the MEA. C Stimulation (black dot) inducing a response on colored electrodes. (dot size = spike count within 15 ms post-stimulus) D Post-stimulus scatter plot following stimulus shown in C.



Disclosures: **J. Duru:** None. **C. Giles Doran:** None. **B. Maurer:** None. **S.J. Ihle:** None. **J. Küchler:** None. **J. Vörös:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.20

Topic: I.04. Physiological Methods

Support: NIH UF1NS107659
NSF 1707316

Title: Carbon Fiber Electrode Arrays for Central and Peripheral Nervous Systems in Various Animal Models

Authors: *P. R. PATEL, J. G. LETNER, J. M. RICHIE, C. A. CHESTEK;
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Abstract: Our lab has developed multiple platforms and processes that have enabled minimally invasive carbon fiber electrodes to detect electrical or chemical activity in different animal models and target regions. Our most popular device consists of a 16-channel array that uses thin, individuated silicon shanks as permanent mechanical supports and shuttles for the fibers. These high-density carbon fiber arrays come in 3mm, 6mm, and 9mm lengths and are designed to target both central and peripheral nervous systems. We can functionalize the protruding carbon fiber tips in three primary ways: laser ablation ideal for dopamine sensing, laser cutting with a poly(3,4-ethylene-dioxythiophene) (PEDOT) coating ideal for electrophysiology, or blowtorch sharpening with either a PEDOT coating for electrophysiology or platinum iridium (PtIr) for the added benefit of stimulation. Recently, we have improved upon our blowtorching methods and reduced the overall exposed length from 100-150um to 50-100um. All three treatments have been used acutely or chronically in the rat brain, to detect dopamine, record electrophysiology, or stimulate neuronal populations. For chronic rodent implants, we have been able to determine the location of the minimally invasive fiber tips using immunohistochemical staining.
While originally created for the rat brain, these arrays have been extensively used in new and novel applications. One group has used the sharpened arrays with PtIr to stimulate and record from *Aplysia.* Another group has used the same configuration to stimulate rodent retina cells and record the elicited activity using calcium imaging. Sharpened arrays functionalized with PEDOT have been used by collaborators to acutely record from rat dorsal root ganglion as well as from the axial nerve cord of an *Octopus bimaculoides* arm. Laser cut arrays functionalized with PEDOT have been used to record chronic neural activity from songbirds and acutely from rat organoids.
To date, we have distributed 1,191 arrays to 47 different labs and work closely with them to meet their unique experimental needs. While we continue to distribute these arrays, we are also developing electrode platforms with smaller form factors that will be better suited for chronic recordings in soft tissue targets such as rat nerves, *Aplysia*, and *Octopus bimaculoides* axial nerve cords.

Disclosures: P.R. Patel: None. **J.G. Letner:** None. **J.M. Richie:** None. **C.A. Chestek:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.21

Topic: I.04. Physiological Methods

Support: 5K08MH107662

Title: Electrophysiological effect of antidepressant-dose ketamine in rat neocortex

Authors: *M. S. RUCKSTUHL[1], D. S. KIM[2], B. O. WATSON[2];
[1]Neurosci. Grad. Program, [2]Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI

Abstract: Depression is a leading cause of disability. Modern antidepressants are reasonably effective but take weeks to elicit improvement. Ketamine, which was first approved for human use in 2019, is effective within hours of delivery. Furthermore, it can sometimes combat depression that is otherwise treatment-resistant, which makes up a third of all cases. Ketamine's time course and proposed mechanism of action have upturned decades of thinking about the root cause of depression. To better understand mechanisms of mood changes, we have decided to investigate the electrophysiologic changes in the hours following delivery of ketamine.
To do this, we decided to take advantage of the powerful neuroscientific tools available to rodent neuroscientists. We implanted silicon probes (multi-electrode arrays) in rat prefrontal cortex and hippocampus to record neurons from those regions before, during, and after ketamine treatment. We administered 10 mg/kg intraperitoneally as this dose has been shown to induce behavioral changes in rats. We recorded for a baseline period of 6 hours and then 24 hours following injection of ketamine versus control. These long recordings allow us to adjust for circadian fluctuations that would otherwise influence our analyses. In this poster, we present investigations of changes in cortical oscillations, firing rate, spike-field coherence, E/I ratio, and functional connectivity. These data constitute novel information about the nature of neural circuit changes in mood-related regions after ketamine administration.

Disclosures: M.S. Ruckstuhl: None. **D.S. Kim:** None. **B.O. Watson:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.22

Topic: I.04. Physiological Methods

Support: MEDISENSE

Title: Boron nitride encapsulated graphene for biosensing

Authors: *C.-A. LERNOUD[1], J. BOUSQUET[2], L. ROUSSEAU[3], V. BOUCHIAT[4], R. OTHMEN[4], B. YVERT[1], C. HÉBERT[1];
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Abstract: The development of liquid-gated graphene field-effect transistors (g-FETs) are subjects of interests in the field of sensors for biomedical applications such as neural interfacing. Thanks to its high-carrier mobility, graphene sensors report the highest sensitivity, along with PEDOT:PSS. Also, the flexibility of this material coupled with an appropriate substrate offers an interesting benefit for implantable technologies. Their biocompatibility allows less rejection from the tissues too. Though, the full potential of g-FETs has not been reached yet. The carrier mobility stays below its theoretical value due to the direct contact with the liquid medium. Also, electrical 1/f noise limits the performances of the device and its miniaturization. In this study, we suggest that encapsulating graphene in between boron nitride layers could be a solution to these issues. Firstly, the electrical transport properties of graphene-boron nitride heterostructures are studied using test devices fabricated on silicon wafers. These heterostructures exhibit a high robustness toward microfabrication residues as well as noise and sensitivity improvements compared to bare graphene. The graphene-boron nitride transistors technology is then transferred on flexible implants for in vivo experiments on the rat model. Consequently, epicortical probes comprising 16 transistors are placed on the rat cortex to perform tonotopy. The preliminary data showed promising results in term of sensitivity and noise, opening the way for high density encapsulated g-FET implants for neural signals sensing.

Disclosures: C. Lernoud: None. **J. Bousquet:** A. Employment/Salary (full or part-time):; DIAMFAB SAS. **L. Rousseau:** None. **V. Bouchiat:** A. Employment/Salary (full or part-time):; Grapheal SAS. **R. Othmen:** A. Employment/Salary (full or part-time):; Grapheal SAS. **B. Yvert:** None. **C. Hébert:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.23

Topic: I.04. Physiological Methods

Support: BBSRC (BB/T016639/1)
EMBO (ALTF 740-2019)

Wellcome Trust (205093)
Wellcome Trust (204915)

Title: Lightweight, reusable chronic implants for Neuropixels probes

Authors: C. BIMBARD, F. TAKACS, M. ROBACHA, T. P. H. SIT, K. D. HARRIS, M. CARANDINI, ***P. COEN**;
Univ. Col. London, London, United Kingdom

Abstract: [Aims] Neuropixels probes have dramatically increased the number of neurons that can be acquired in a single experiment. With chronic recordings, these probes can track neurons across days and behaviors. There is thus substantial interest in developing chronic implants that are recoverable while being light enough to be used in small animals such as mice.
[Methods] Here, we present the "Apollo Implant", a device for reversible chronic implantation of Neuropixels probes. The implant comprises two modules that are combined before implantation. The payload module accommodates up to two Neuropixels 2.0 probes, for a maximum of 8 shanks in the brain, and is recoverable (~€8). The docking module is cemented to the skull during implantation and is not recoverable (~€2). The implant is made of Formlabs Rigid Resin and weighs ~2.5 g with two 1.0 probes and ~1.7 g with two 2.0 probes. Its design is open source and can be readily adjusted to change the angle of insertion or the distance between the probes.
[Results] We used the Apollo Implant to insert the same Neuropixels 2.0 probes across at least 4 head-fixed mice with no noticeable reduction in recording quality. Recordings were stable across weeks and sometimes months, allowing us to successfully track the same neurons over days and record from the entire probe (8 banks) while minimizing set-up time. With these implants, we explored neural responses across various parts of the brain (including superior colliculus, visual cortex, frontal cortex, and striatum) during an audiovisual behavioral task. Doing so, we could obtain many behavioral trials for each brain region, thus allowing finer analysis of the relationship between neural activity and choices of the animal.
[Conclusions] Thus, the Apollo Implant provides a cheap, lightweight, and flexible solution for chronic recordings in head-fixed mice. We are currently testing the versions of the implant optimized for Neuropixels 1.0 probes and for freely moving animals.

Disclosures: C. Bimbard: None. **F. Takacs:** None. **M. Robacha:** None. **T.P.H. Sit:** None. **K.D. Harris:** None. **M. Carandini:** None. **P. Coen:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.24

Topic: I.04. Physiological Methods

Support: ERC Consolidator Award 818179
SNSF CRSII5_198739/1

Title: Cortico-hippocampal coupling during ripples investigated at single-unit resolution with months-long-stable ultra-flexible low-impedance electrode arrays

Authors: T. B. YASAR, *P. GOMBKOTO, A. L. VYSSOTSKI, W. VON DER BEHRENS, M. F. YANIK;
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Abstract: While the number of channels in the state-of-the-art in vivo electrophysiology systems are rapidly increasing, these technologies generally use stiff materials. The mechanical mismatch between these probes and the brain causes damage to the brain tissue, limiting the longevity and quality of recordings. To address this challenge, we developed massively-parallel flexible intracortical microelectrode arrays with minimal footprint. In this study, we fabricated ultra-flexible intracortical microelectrode arrays (MultiBundle arrays) which can be delivered into multiple brain areas at arbitrary locations with practically no depth limitations at a speed of 12.5 µm/s. In these arrays, each channel is mechanically independent to provide maximal compliance with the brain tissue—while also allowing to pack a high density of channels within a minimal footprint at each brain area, with 256 channels in total. During the fabrication of our devices, we robustly achieve 54±16 kOhm impedances for electrode pads with 13x13 μm^2 surface area and 2.4 µm thickness.
We performed simultaneous recordings from the medial prefrontal cortex (mPFC), retrosplenial cortex (RSC), dorsal hippocampus (dHPC) and the intermediate hippocampus (iHPC) in freely moving rats (n=5) using our MultiBundle arrays. We get stable recordings of single-unit activity while tracking putatively the same units for at least up to 3.5 months in some cases. Immunostaining of the brain slices showed no significant long-term damage to the brain tissue surrounding the electrodes. Furthermore, we investigate cortico-hippocampal coupling at single-unit level during the hippocampal ripples. We identified different subclasses of hippocampal ripples according to their location, frequency decomposition and associated ensemble activity patterns in mPFC and RSC. These results imply different ripple-associated information routes between hippocampus and mPFC versus RSC. We are currently scaling up our electrode arrays and recording electronics to cover more brain areas with larger numbers of channels wirelessly for untethered recordings.

Disclosures: T.B. Yasar: None. **P. Gombkoto:** None. **A.L. Vyssotski:** None. **W. von der Behrens:** None. **M.F. Yanik:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

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Program #/Poster #: 329.25

Topic: I.04. Physiological Methods

Support: R01NS123655
U19NS123717

R01MH111359
R01NS123655
UG3NS123723
R01NS108472
R01DA050159

Title: Calcium imaging though semitransparent PEDOT:PSS electrode arrays reveals only a small fraction of layer 2 neurons are synchronous with multi-unit activity recorded at the cortical surface

Authors: *M. THUNEMANN[1], L. HOSSAIN[2], T. V. NESS[4], N. ROGERS[3], K. LEE[3], S. LEE[3], K. KILIÇ[1], H. OH[3], Y. YANAGAWA[5], V. GILJA[3], G. T. EINEVOLL[4], S. DAYEH[3], A. DEVOR[1,6];
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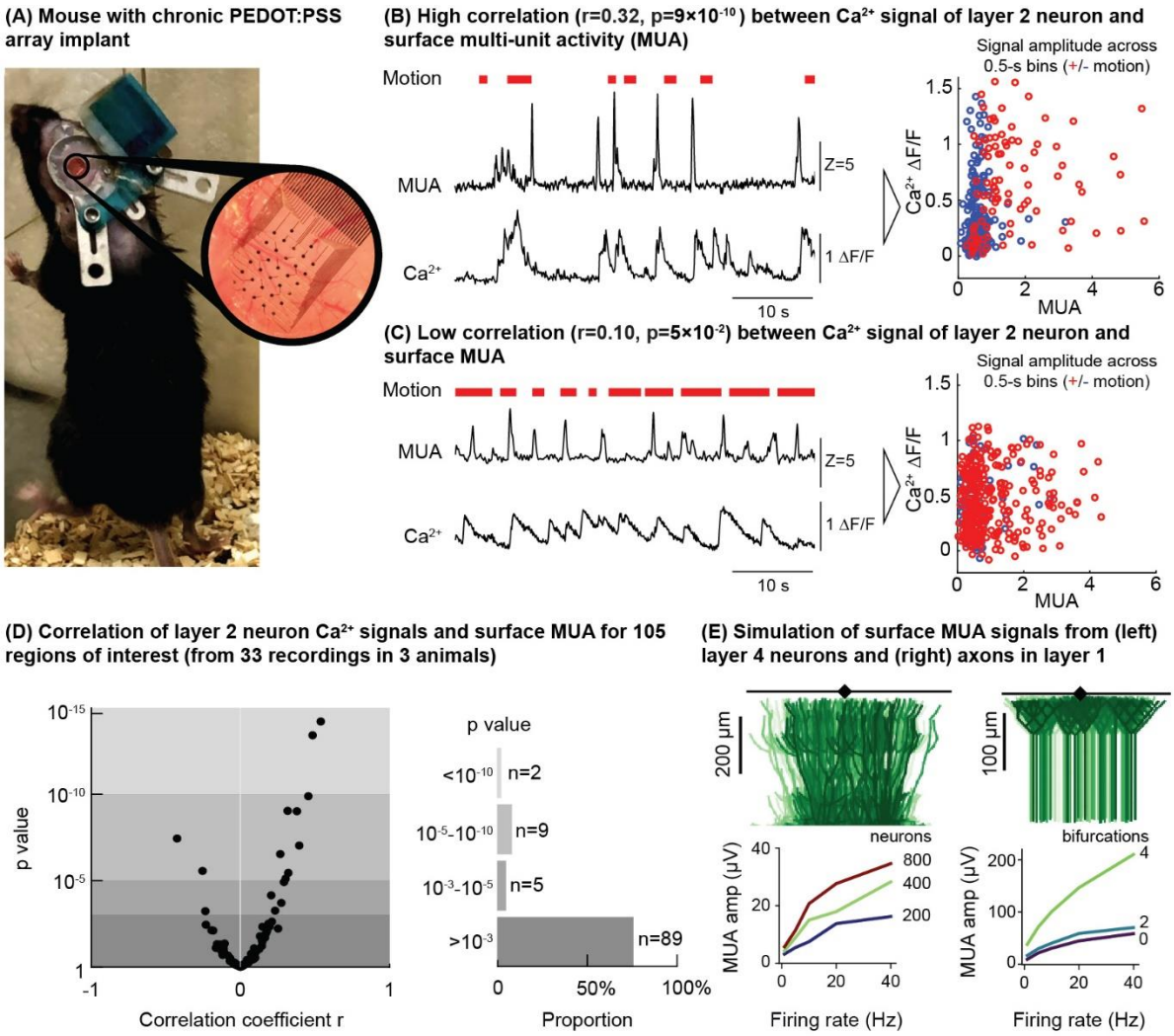
Abstract: Prior studies have shown that spikes and multi-unit activity (MUA) can be recorded with electrocorticography (ECoG) from the cortical surface. However, the etiology of these signals remains unclear. As cortical layer 1 contains very few neuronal cell bodies, it has been proposed that surface spikes or MUA originate from neurons in layer 2. We addressed this question by combining two-photon calcium imaging with jRGECO1a and ECoG recordings in awake mice. We used chronically implanted semitransparent PEDOT:PSS microelectrode arrays integrated with cortical windows offering optical access for high-resolution two-photon imaging. We developed mechanical solutions for installation, connectorization, and protection of recording devices aiming for an unhindered access for high numerical aperture objectives and a lifetime of several months (Figure 1A). We performed simultaneous two-photon Ca2+ imaging with jRGECO1a and recordings of local field potentials (LFP) and MUA from the cortical surface. Contrary to the common notion, our measurements revealed that only few (ca 15%) layer-2 neurons faithfully followed MUA signals recorded at the surface above the imaging field-of-view (Figure 1B-D). We then used computational modeling to explore alternative explanations for the MUA signal and computed extracellular action potentials at the surface due to firing of local cortical neurons and compared the result to that due to axonal inputs to layer 1. Our modeling results show that surface MUA due to axonal inputs was an order of magnitude larger than that due to firing of pyramidal neurons (Figure 1E). In summary, a combination of surface MUA recordings with two-photon calcium imaging can provide complementary information about the input to a cortical column and the local circuit response and will facilitate studies of the input/output relationship in cortical circuits, inform computational circuit models, and improve the accuracy of the next generation brain-machine interfaces.



Disclosures: **M. Thunemann:** None. **L. Hossain:** None. **T.V. Ness:** None. **N. Rogers:** None. **K. Lee:** None. **S. Lee:** None. **K. Kiliç:** None. **H. Oh:** None. **Y. Yanagawa:** None. **V. Gilja:** None. **G.T. Einevoll:** None. **S. Dayeh:** None. **A. Devor:** None.

Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.26

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS030853
NIH Grant R01NS118918
Landon Center on Aging

Title: Stimulation-evoked effective connectivity (SEEC) corresponds with patterns of mesoscale corticocortical effective connectivity

Authors: *D. T. BUNDY[1], S. BARBAY[1], H. M. HUDSON[1], S. B. FROST[1], R. J. NUDO[1,2], D. J. GUGGENMOS[1];
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Abstract: *Background:* Changes in the strength of corticocortical connectivity have been associated with multiple neurological diseases and recovery processes. Unfortunately, traditional methods for assessing the strength of anatomical connectivity are limited to assessing connectivity between a few regions at a single point in time. An alternative approach is to assess effective connectivity by examining the evoked neural activity following focal intracortical micro-stimulation (ICMS). Here we demonstrate a novel method for longitudinal assessments of mesoscale corticocortical connectivity in a technique we refer to as Stimulation-Evoked Effective Connectivity (SEEC).
Methods: We sought to use ICMS in conjunction with recordings of multi-unit action potentials to assess the correspondence between stimulation-evoked effectivity connectivity and known patterns of anatomical connectivity. In 3 male squirrel monkeys (*Saimiri sciureus*) intraoperative neural recordings were made from multielectrode arrays placed into somatosensory, primary motor, and premotor regions. During each recording, single-pulse ICMS was repeatably delivered to a single electrode. After removing stimulus artifact, mesoscale effective connectivity was estimated by determining the ICMS-evoked changes in multi-unit firing. A similar procedure was used in 6 male Long-Evans rats (*Rattus norvegicus*) to compare the specificity of stimulation-evoked effective connectivity across species.
Results: Using our algorithm for stimulus-artifact smoothing, we were able to detect multi-unit action potentials ~1 ms after each ICMS pulse. Across sensorimotor regions, short-latency (< 2.5 ms) ICMS-evoked neural activity strongly correlated with known anatomical connections. Additionally, stimulation-evoked neural responses remained stable across the experimental period, despite small changes in electrode location and anesthetic state.
Conclusions: This study shows that SEEC is a viable method to longitudinally assess effective connectivity *in vivo*. While previous studies have used fMRI and optical imaging in conjunction with ICMS, these methods rely on the relatively slow hemodynamic response, making it difficult to separate action potentials from synaptic potentials. Additionally, this method will allow us to easily move to chronic electrode preparations, enabling studies evaluating the time course of connectivity changes as potential biomarkers for disease progression and recovery potential.

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Poster

329. Electrophysiology: Electrode Arrays

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 329.27

Topic: I.04. Physiological Methods

Support: CIHR
NSERC

Title: A method for chronic and semi-chronic microelectrode array implantation of deep brain structures in macaque using image guided neuronavigation

Authors: *B. MAHMOUDIAN[1], H. DALAL[2], J. LAU[3], B. CORRIGAN[2], K. BARKER[1], A. RANKIN[4], E. C. S. CHEN[5], T. PETERS[6], J. C. MARTINEZ-TRUJILLO[7];
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Abstract: Precise targeting of deep brain structures in humans and large animal models has been a challenge for neuroscientists. Conventional protocols used in animal models typically require large access chambers which are prone to infection and involve assembly and implantation of complex microdrives for semi-chronic applications. Here we present a methodology for improving targeting of subcortical structures in large animals such as macaque monkeys, using image guided neuronavigation. Design of custom cranial caps allowed for incorporation of stable fiducial markers, required for increased targeting accuracy in neuronavigation procedures, resulting in an average targeting error of 1.67 mm over three implantations. Incorporation of anchor bolt chambers, commonly used in human neurosurgery, provided a minimally invasive entrance to the brain parenchyma, allowing for chronic recordings. By leveraging existing 3D printing technology, we fabricated an anchor bolt-mounted microdrive for semi-chronic applications. Our protocol leverages commercially available tools for implantation, decreases the risk of infection and complications of open craniotomies, and improves the accuracy and precision of chronic electrode implantations targeting deep brain structures in large animal models.

Disclosures: B. Mahmoudian: None. **H. Dalal:** None. **J. Lau:** None. **B. Corrigan:** None. **K. Barker:** None. **A. Rankin:** None. **E.C.S. Chen:** None. **T. Peters:** None. **J.C. Martinez-Trujillo:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.01

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF CRCNS #1822517
NSF CRCNS #2112862

NIMH #MH117488
California NanoSystems Institute

Title: High-resolution spatiotemporal dynamics of serotonergic axons in primary brainstem cultures

Authors: *M. HINGORANI, S. JANUSONIS;
Dept. of Psychological and Brain Sci., Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Recent experimental and theoretical work by our group has shown that the self-organization of the brain serotonergic matrix is strongly driven by the spatiotemporal dynamics of single serotonergic axons (fibers). The trajectories of these axons are often stochastic in character and can be described by step-wise random walks or time-continuous processes (e.g., fractional Brownian motion). The success of these modeling efforts depends on experimental data that can validate the proposed mathematical frameworks and constrain their parameters. In particular, further progress requires reliable experimental tracking of individual serotonergic axons in time and space. Visualizing this dynamic behavior in vivo is currently extremely difficult, because of the high axon densities and other resolution limitations. In this study, we used in vitro systems of mouse primary brainstem neurons to examine serotonergic axons with unprecedented spatiotemporal precision. The high-resolution methods included confocal microscopy, STED super-resolution microscopy, and live imaging with holotomography. We demonstrate that the extension of developing serotonergic axons strongly relies on discrete attachments points on other, non-serotonergic neurons. These membrane anchors are remarkably stable but can be stretched into nano-scale tethers that accommodate the axon's transitions from neuron to neuron, as it advances through neural tissue. We also show that serotonergic axons can be flat (ribbon-like) and produce screw-like rotations along their trajectory, perhaps to accommodate mechanical constraints. We conclude that the stochastic dynamics of serotonergic axons may be conditioned by the stochastic geometry of neural tissue and, consequently, may reflect it. Our current research includes hydrogels to better understand these processes in controlled artificial environments. Since serotonergic axons are nearly unique in their ability to regenerate in the adult mammalian brain and they support neural plasticity, this research not only advances fundamental neuroscience but can also inform efforts to restore injured neural tissue.

Disclosures: M. Hingorani: None. **S. Janusonis:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.02

Topic: I.06. Computation, Modeling, and Simulation

Support: Wu Tsai Neurosciences Institute Seed Grant
NSF GRFP
Knight-Hennessy Scholarship

Title: Computational advantages of magnetic sensor arrays for recording neural activity

Authors: *Z. ALI, A. POON;
Stanford Univ., Stanford Univ., Palo Alto, CA

Abstract: Brain implants that use microelectrode arrays to record neural activity suffer from longevity and signal limitations that impede their ability to provide clinical and experimental neuroscience insights. However, magnetic sensors are hypothesized to address longevity issues because they do not require a direct conductive path with tissue. We investigated how the nature of the information obtained by a neural magnetic recording system fundamentally differs from a voltage recording system and have shown how this information can be utilized to improve spike sorting performance as well as perform approximate morphology reconstruction. We developed a simulation platform in which neocortical pyramidal cells are modeled in the NEURON software and their magnetic fields are calculated by using compartmental axonal currents in a differential Biot-Savart Law computation. Using this platform, we first demonstrated the degree to which out-of-plane magnetic fields, electric currents, and in-plane magnetic fields all scale at different rates (slowest to fastest, respectively) with respect to distance from the cell source. We also observed how the polarity of magnetic field measurements is highly dependent on the position of a sensor relative to the cell, often making magnetic field measurements more distinguishable for closely located cells. To quantify this distinction, we combined our platform with MEArec and Kilosort2 to generate simulated recordings of dozens of spiking neurons on a 10x10 array of sensors and compared spiking performance for co-located voltage measurements, in-plane magnetic field measurements, and out-of-plane magnetic field measurements. We have shown that spike sorting on out-of-plane magnetic field measurements outperforms voltage measurement spike sorting for equivalent noise levels due to out-of-plane measurements having opposite polarity on opposite sides of long cellular processes. We have also shown that the difference in scaling properties results in noise from distant neurons being lower for magnetic measurements than voltage measurements. Lastly, we have demonstrated how the polarity properties of out-of-plane magnetic field measurements can be utilized to achieve morphology reconstruction using a support vector machine model with minimal learning. These simulation results suggest that there is utility to conducting clinical and experimental measurements of neural activity using magnetic sensors beyond chronic implant viability, and that out-of-plane sensors are likely to provide the most rich source of information for activity detection, spike sorting, and morphology reconstruction.

Disclosures: Z. Ali: None. **A. Poon:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.03

Topic: I.06. Computation, Modeling, and Simulation

Support: LBNL Contract No. DE-AC02-05CH11231 NERSC award M2043
Action Potential grant from The FamiliesSCN2A Foundation

Title: Compare: method for convexifying the parameter space of highly detailed biophysical neuronal models

Authors: *A. LADD[1], K. G. KIM[1], P. GHOSH[2], K. J. BENDER[3], K. E. BOUCHARD[4], R. BEN-SHALOM[5];
[1]Univ. of California Berkeley, Berkeley, CA; [2]Cupertino High Sch., Cupertino, CA; [3]Ctr. for Integrative Neuroscience, Dept. of Neurol., Univ. of California San Francisco, San Francisco, CA; [4]Scientific Data Div. and Biol. Systems & Engin. Div., Lawrence Berkeley Natl. Lab., Berkeley, CA; [5]Neurol., Univ. of California Davis, Sacramento, CA

Abstract: The neuron is the fundamental unit of computation in the brain and different neuronal classes evolve morphological and electrical properties to perform various computations. A major focus of cellular neuroscience has been determining how ionic conductances are distributed within neuron morphology to support such computations. The difficulty of measuring ionic conductances empirically and numerically modeling them hinders the in-silico study the neuronal computation. Currently, the main method for fitting conductance-based compartmental models to experimental data aims to optimize an objective function that evaluates simulated voltage traces against electrophysiological recordings. Extensive research efforts in neuron modeling are challenged by the nonlinear relationships between conductances that make optimization intractable. To overcome this challenge, we present CoMParE, Conductance-based Model Parameter Evaluation. CoMParE is a method for enabling the tractable fitting of neuronal models to experimental data. This is accomplished by (1) sampling the model's parameter space to find the correlation between the optimization's objectives and the model's conductances, (2) using these correlations to construct a linear combination of electrophysiological score functions and thus a nearly convex representation of the optimization objective function, and (3) applying the evolutionary algorithm to optimize this customized objective function and construct a realistic biophysical neuron model. We demonstrate this treatment of the objective function yields a parameter space that enables the optimization algorithm to find solutions very close to the global minimum in both simple and highly detailed multi-compartmental models. We show the application of CoMParE on biophysical models and experimental data recorded through the Allen Institute Cell Types Database. These results show the robustness of CoMParE in constructing neuronal models which accurately replicate the biophysical dynamics observed in experimentally recorded electrophysiological data. The capacity to construct faithful neuron models has many important implications; for example, applications include modeling pharmacological treatment for ion channel dysfunction and utilizing these models as basic building blocks for more complex cortical models.

Disclosures: A. Ladd: None. **K.G. Kim:** None. **P. Ghosh:** None. **K.J. Bender:** None. **K.E. Bouchard:** None. **R. Ben-Shalom:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.04

Topic: I.06. Computation, Modeling, and Simulation

Title: A thermodynamic model of non-deterministic computation in cortical neural networks

Authors: *E. A. STOLL;
Western Inst. for Advanced Study, Denver, CO

Abstract: Hodgkin and Huxley provided a standard approach for modeling neuron behavior, with partial differential equations denoting the interrelated dynamics between membrane potential, ion conductance, and channel activation. However, cortical neurons have unpredictable firing patterns, with stochastic charge flux and spontaneous subthreshold fluctuations in membrane potential substantially contributing to signaling outcomes (Softky & Koch, 1993; Stern et al., 1997; Dorval & White, 2005). This sensitivity to stochastic charge flux is particularly apparent in cortical up-states, when neurons remain suspended near action potential threshold, allowing random electrical noise to affect signaling outcomes (Steriade et al., 2001). For this reason, the firing patterns of cortical neurons are intrinsically probabilistic and must be modeled accordingly [Beck et al., 2008; Maoz et al., 2020]. In a new approach, cortical neurons are modeled as the mixed sum of all component pure states, or the amount of mathematical information they contain. Here, free energy is distributed to create a physical quantity of information, then free energy is partially recovered as predictive value is extracted from this quantity of information. This physical quantity of information, known as von Neumann entropy, is modeled as a density matrix, accounting for the probabilistic state of every component electron in the system. The encoding thermodynamic system (System A) interacts with its surrounding environment (System B), and the combined density matrix undergoes a unitary change of basis, leading to dimensionality reduction. This process of Schumacher compression results in thermal free energy release, as a single optimal system state is selected in the present context. As predictive value is extracted, information is compressed and free energy is released locally to any reduction in uncertainty. These thermal fluctuations locally reduce membrane resistance, driving inward sodium currents. This effort demonstrates how cortical neurons engaging in probabilistic coding can trap thermal energy to drive a non-deterministic computation.

Disclosures: E.A. Stoll: None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.05

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH/NINDS Grant NS097185

Title: Optimal phase resetting curve for input-output prediction in external globus pallidus neurons

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Abstract: The basal ganglia is populated by oscillatory neurons, whose activity can be predicted using the phase resetting curve (PRC). The PRC describes how a neuron's sensitivity to external inputs varies across the inter-spike interval (ISI) and is traditionally estimated by analyzing the spike time change produced by a brief pulse of current during the ISI. This method can be improved by delivering a barrage of random-amplitude pulses and using multiple regression to determine the PRC. In principle, any broad-bandwidth stimulation can carry the information necessary to retrieve a PRC, but, as far as we know, there is no general method. The PRC can be used in a phase model to simulate the phase-evolution of an oscillating neuron and thereby predict its spike times. While the PRC estimated using regression has proved to have predictive accuracy, it's not clear if it optimizes the predictions of the phase model. In this work, we used an assumption-free evolutionary algorithm (EA) method to extract the PRC that optimized the phase model prediction of experimental data obtained from external globus pallidus (GPe) neurons subjected to broadband stimuli. For the EA we used a parameter space that allowed the algorithm to explore a wide range of PRC shapes and sizes. The algorithm evolves the solutions to optimize an objective function that consists in minimizing the difference between the predicted and experimental inter-spike intervals. The EA approach was validated for stimuli made up of a barrage of random-amplitude pulses, which also allowed PRC estimation by multiple regression. For all 16 GPe neurons studied, the PRCs obtained with the EA were similar to those obtained using multiple regression. The EA method was then applied for different stimuli consisting of pure sine waves with frequencies of 1 to 100 Hz. The multiple regression method was numerically unstable with these stimuli, but the EA method was found to be robust. PRCs obtained using sine wave stimuli closely resembled those calculated using noise pulses in the same cells. Moreover, the PRC calculated from one stimulation protocol could be used to predict spike times observed in response to other stimulus waveforms. These results suggest that a single PRC can provide accurate predictions of spike timing in response to a wide range of stimuli, and the EA can be used to determine the PRC from the spike responses to stimulus waveforms that defy other PRC estimation methods.

Disclosures: E. Olivares: None. **C.J. Wilson:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant #1822517
NSF Grant #2111862
NIMH Grant #MH117488
California NanoSystems Institute

Title: Serotonergic neurons in 3D-hydrogels: Tunable environments to study axon dynamics

Authors: *J. H. HAIMAN[1], M. HINGORANI[1], G. A. DUNN[2], S. JANUSONIS[1];
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Abstract: Serotonergic axons (fibers) have a ubiquitous distribution in vertebrate brains, where they form meshworks with well-defined, regionally-specific densities. In humans, perturbations of these densities have been associated with abnormal neural processes, including neuropsychiatric conditions. The self-organization of serotonergic meshworks depends on the cumulative behavior of many serotonergic axons, each one of which has a virtually unpredictable trajectory. In order to bridge the high stochasticity at the microscopic level and the regional stability at the mesoscopic level, we are developing tunable hydrogel systems that can support causal modeling of these processes. These same systems can support future restorative efforts in neural tissue because serotonergic axons are nearly unique in their ability to robustly regenerate in the adult brain. In the study, we extended our research in 2D-primary brainstem cultures (Hingorani et al., 2022) to 3D-hydrogels. Tunable hydrogel scaffolds can closely mimic the mechanical and biochemical properties of actual neural tissue in all three dimensions and are therefore qualitatively different from 2D-environments. However, the integration of these scaffolds with highly sensitive neurons poses unique challenges. As the first step in building a hydrogel-based platform for the bioengineering of serotonergic axons, we studied primary brainstem neurons in several commercially available hydrogel platforms. The viability and dynamics of serotonergic somata and neurites were analyzed at different days in vitro with immunocytochemistry and high-resolution confocal microscopy. In addition, live imaging of neuron growth cones was performed, and the observed dynamics was compared to our extensive database of holotomographic (refractive index-based) recordings in 2D-cultures. The progress and key problems will be discussed.

Disclosures: J.H. Haiman: None. **M. Hingorani:** None. **G.A. Dunn:** None. **S. Janusonis:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.07

Topic: I.06. Computation, Modeling, and Simulation

Title: In vitro priming and hyper-activation of brain microglia; an-assessment of phenotypes and targeting peptides

Authors: *K. M. KOSS[1,2], T. SON[2], C. LI[3], Y. HAO[3], J. CAO[3], M. A. CHURCHWARD[3], Z. J. ZHANG[2], J. A. WERTHEIM[1,2], R. DERDA[3], K. G. TODD[3];
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Abstract: Microglia are an immune-derived cell critical to development and healthy function of the brain and spinal cord, yet are implicated in the active pathology of many neuropsychiatric disorders. A range of functional phenotypes associated with the healthy brain or disease states has been suggested from in vivo work, which were modeled in vitro as surveying, reactive, and primed sub-types of primary rat microglia. These functional phenotypes and were explored for potential novel peptide binders using a custom M13 phage library to identify unique peptides that bind differentially to these respective cell types. Surveying glia were untreated, reactive were induced with a lipopolysaccharide (LPS) treatment, recovery was modelled with a potent anti-inflammatory treatments dexamethasone (Dex), minocycline (Mino), or a conditioned medium (CM), and priming was determined by subsequently challenging the cells with interferon gamma (IFNɣ). Microglial function was profiled by determining secretion of cytokines, nitric oxide (NO), and neurotrophic factors, expression of inducible nitric oxide synthase (iNOS), major histocompatibility complex class II (MHCII), proliferation, bead and myelin cell-uptake, and the morphology of thinly branched smaller microglia. After incubation with the phage library, populations of phage-positive microglia and astrocytes were isolated and phage DNA was sequenced. Unique peptides were discovered, and their potential functions were assessed using a Basic Local Alignment Search Tool (BLAST), which include a variety of glial roles: synapse support and pruning, inflammatory incitement including cytokine and interleukin activation, and potential regulation in neurodegenerative and neuropsychiatric disorders.

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Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.08

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH MH122023
NSF OAC-1730655

Title: Reduced order realistic neuron models for network simulations

Authors: *D. R. FAGUE[1], J. VON RAUTENFELD[2], T. WILLIAMS[3], S. S. NAIR[1];
[1]Dept. of Electrical & Computer Engin., [2]Dept. of Mathematics, [3]Dept. of Physics & Astronomy, Univ. of Missouri, Columbia, MO

Abstract: Computational models of single neurons are incorporating biological realism at several levels for improved prediction of network activity. Different types of single cell model formulations have, together with realistic synaptic models, helped generate network models that have replicated biological data well and provided useful predictions for the neuroscientist. Findings over the past decade related to various dendritic spikes (e.g., Na+, NMDA+ and Ca2+ spikes) have dramatically changed our perception of the role of dendrites in neuronal function, a topic that continues to remain poorly understood. These findings have, in parallel, resulted in the development of morphologically realistic neuronal models with over 2,000 compartments and over 20,000 synapses. A drawback with morphologically detailed model is the computational overhead when used in network simulations. Here we consider reduced order model structure for cortical pyramidal neurons of L5 and L2/3 layers and a stellate neuron of the amygdala. In each case, we start with morphologically detailed single neuron model from the literature or from a database (e.g., Allen Institute, Neuromorpho, etc.). The reduced order model retains the spatial dimensions of the dendrites but reduces the overall numbers of basal, oblique and apical dendrites. The difference in surface area is made up adding extra passive dendrites for the three types. The in vitro passive and current injection properties are then tuned by adjusting the various conductances in the somatic and periosomatic regions. Information about known dendritic spikes in different dendritic locations as well as about in vivo properties is used to constrain the active conductances along all dendrites. The first example case is of an L5 pyramidal model developed using the NEURON simulation environment that provided good match with biological data. These include in vitro (resting membrane potential, membrane time constant, input resistance, and F-I curve) and several in vivo features. These included match with spatial location of Na, NMDA and Ca2+ spikes, and comparison of output with excitation of the basal and tuft dendrites and mediation of somatic bursts through differing distributions of excitatory inputs and the activation of tuft calcium spikes as reported in a recent full scale L5 single cell model. A similar approach will be used for the L2/3 and stellate cell types. The objective is to develop a principled approach for such model order reduction including quantification of performance tradeoffs, with automation of the overall process being a long term goal.

Disclosures: D.R. Fague: None. **J. von Rautenfeld:** None. **T. Williams:** None. **S.S. Nair:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

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Program #/Poster #: 330.09

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH Grant U19NS104590
NSF LRAC allocation on TACC Frontera (FTA-Soltesz)

Title: Data-driven efficient surrogate-assisted evolutionary method for multi-objective optimization of high-dimensional neural dynamical systems

Authors: P. MOOLCHAND, ***I. RAIKOV**, I. SOLTESZ;
Stanford Univ., Stanford, CA

Abstract: Biophysical models of neurons and neuronal networks are described by dynamic equations with numerous parameters that often cannot be directly determined experimentally and must be constrained with optimization techniques that iterate through the parameter space in order to determine which combinations of parameters recapitulate experimentally measured features of neural activity. These optimization techniques are time consuming and incur a large computational workload.
We present a novel multi-objective optimization framework that combines several approaches, namely surrogate modeling, constrained sampling, sensitivity-guided evolutionary optimization, and distributed parallel computing, in order to estimate a surrogate model with a minimal number of computationally expensive model evaluations through iterative cycles of exploration and optimization.
We demonstrate that our optimization approach can used with neuron models based on the Pinsky-Rinzel formalism to quantitatively reproduce key experimentally-obtained electrophysiological features of several major neuron classes in the dentate gyrus, specifically input resistance, frequency-current relationship, interspike interval adaptation, and spike amplitude. Although ion channel kinetics for the target neuron classes are not known, our approach can generate several suitable models for each neuron type, exhibiting feature variability that is consistent with the experimental data. We further demonstrate that our approach can be applied to constraining the synaptic conductances in a large network model of the dentate gyrus in order to recapitulate mean firing rates for each neuron type that have been observed experimentally in rats during awake behavior.
While the results we present are focused on hippocampal neuron types and networks, our strategy is broadly applicable to nonlinear chaotic, tightly-coupled dynamical and complex systems. Our optimization method is flexible, scales well to high-dimensional parameter spaces, can efficiently utilize distributed computational resources, and its ability to efficiently search large parameter spaces opens up possibilities for numerically identifying regions of criticality in complex systems when no analytical methods can be applied.

Disclosures: P. Moolchand: None. **I. Raikov:** None. **I. Soltesz:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.10

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH U19NS112953

Title: A generative approach to modeling neuron structure, maturation, and diversity

Authors: *E. A. SMITH, R. C. GERKIN;
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Abstract: A neuron's morphology determines its functional processing capabilities and role in neural circuits. Some computational models can classify neurons from morphology using morphometrics; however, that approach does not provide a way to generate novel morphologies. Constructing biologically realistic models of neural circuits requires accounting for a diversity of morphologies, including from the same nominal neuron type, then generating statistically equivalent neuron types in silico.
To classify and generate diverse morphologies, we implemented a variational autoencoder (VAE) model using a Graph Neural Network (GNN) approach to learn a latent feature space that represents this diversity, capturing neuritic branching patterns and local details of neuritic segments. Because the latent representation fully encodes these features, the classification operation can be reversed, creating novel morphologies from provided types, while respecting observed variability within and across types.
This model successfully classified major neurons of mammalian olfactory bulb (OB) with high accuracy, and generated novel morphologies that match key features of their biological counterparts. Importantly, individual neurons with similar morphologies are nearby points in the resulting latent space; the latent space thus represents a smooth manifold on which neuron morphologies exist.
We applied this architecture to mouse OB granule cells, which undergo increasing dendritic arborization over the course of the lifetime of a single cell, and which vary morphologically by laminar position. By sampling from the learned latent space, we can construct new morphologies that recapitulate these stages of arborization, providing a model for generating diverse neuron morphologies that span heterogeneity observed across space and time, even in the same neuron type.

Disclosures: E.A. Smith: None. **R.C. Gerkin:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.11

Topic: I.06. Computation, Modeling, and Simulation

Title: Unraveling the key ion channels shaping the excitability of projection neurons in pain circuits

Authors: A. DE WORM[1], *G. DRION[2], P. SACRÉ[3];
[1]Univ. de Liège, Seraing, Belgium; [2]Univ. of Liège, Liege, Belgium; [3]Dept. of Electrical Engin. and Computer Sci., Univ. of Liège, Liège, Belgium

Abstract: Chronic pain is a major unmet clinical problem and socioeconomic burden worldwide. A prominent contributor to many persistent clinical pain states is the phenomenon of central sensitization—a state of hyperexcitability of the pain system resulting from changes in the properties of neurons and circuits within the dorsal horns of the spinal cord. These circuits of neurons and their plasticity are very challenging to study experimentally. Indeed, a complex network of excitatory and inhibitory interneurons acts on projection neurons to change the functional state of nociception by switching their firing pat- terns. Therefore the lack of understanding of these mechanisms limits the discovery of novel treatments that targets these mechanisms despite their critical need. We propose to use computational models to characterize the key ion channels shaping the excitability of projection neurons in the dorsal horn of the spinal cord. We hypothesize that the neuromodulation of excitability at the cellular level is a critical component to understand the neurophysiology of central sensitisation. This idea is grounded in electrophysiological evidence that neurons of the spinal cord exhibit a rich dynamical repertoire of firing patterns. In addition, it was proposed that these firing patterns (resp. tonic firing, plateau potential, and endogenous bursting) correlate with distinct functional states (resp. healthy processing of innocuous stimuli and nociception, enhanced excitability during central sensitization, distorted processing during persistent pathological pain) [Derjean et al, 2003]. We compare three published conductance-based models of projection neurons in the dorsal horn [Le Franc et al, 2010; Aguiar et al, 2010; Radwani et al, 2016]. We highlight the role of each ion channel in providing positive and negative conductances in the different timescales involved in these firing patterns. We show that a slow negative conductance is key to reproduce these functional switches in the firing patterns of dorsal horn neurons that are robust to cellular heterogeneity. These switches are achieved through the modulation of ultraslow positive and negative conductances. Potassium inward rectifier channels, voltage-gated calcium channels and calcium-gated channels are key for these mechanisms. These results suggest that modulation of the intrinsic properties of the projection neurons in the dorsal horn is likely to be involved in some of the complex alterations in pain processing and that the excitatory and inhibitory interneurons could control the excitability of the projection neurons through metabotropic receptors.

Disclosures: **A. De Worm:** None. **G. Drion:** None. **P. Sacré:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.12

Topic: I.06. Computation, Modeling, and Simulation

Support: R44 MH119842

Title: Using Real-Time Dynamic Clamp to Probe In-silico Dorsal Root Ganglion Neuron

Authors: *M. W. NOWAK[1], L. KORBEL[1], A. KANE[1], B. PANAMA[1,2], R. L. RASMUSSON[2,1], G. C. L. BETT[2,1];
[1]Cytocybernetics, Pendleton, NY; [2]Jacobs Sch. of Med., Univ. of Buffalo, State Univ. of New York, Buffalo, NY

Abstract: Variability in ion channel isoform expression and disease-causing mutations often result in subtle changes in ion channel kinetics making it difficult to predict the effects on cellular responses. Using a high-speed dynamic clamp system, we generated a real-time, *in silico* dorsal root ganglion (DRG) neuron (based on Alsaloum et al., 2021 and Alsaloum et al., 2022) using ion channel models of: NaV1.7 (WT, S241T, I828T) (from Alsaloum, 2022), NaV 1.8 (from Han et al., 2015), Kv (from Berecki et al., 2018). Conductances for NaV1.7/NaV1.8 and Kv were set at 0.002 S/cm^2 and 0.0013 S/cm^2, respectively. Reversal potentials were: NaV1.7 WT: 62.8 mV, NaV1.7 S241T: 63.8 mV, NaV1.7 I828T: 64.9 mV and Kv: -90 mV. The input resistance was set to 333 mΩ using a linear current of 3 nS (E_{rev} = -70 mV). The *in silico* DRG neuron with NaV1.7 WT had a resting membrane potential of 64.9±0.01 mV. Evoked action potentials (AP) (1 nA, 1 ms), had an amplitude of 100±0.02 mV, an afterhyperpolarization magnitude of 12.8±0.01 mV and a duration at 50% of 6±0 ms (n=10). A minimum stimulus of 82 pA (1 s) was required to observe AP spiking. The NaV1.7 S241T and I828T mutations have been shown to underly inherited erythromelalgia (Alsaloum et al., 2022). The NaV1.7 S241T and I828T mutations are gain-of-function resulting in a hyperpolarizing shift in $V_{1/2}$ activation of 11 and 7 mV, respectively. However, only the NaV1.7 I828T mutation affected $V_{1/2}$ inactivation with a depolarizing shift of 9 mV. The minimum current required to observe AP spiking (1 s) in the DRG neuron with NaV1.7 S241T and I828T was 30 and 60 pA, respectively. Using a stimulus of 82 pA (for 1 s), the firing frequencies for NaV1.7 WT, S241T and I828T were 11.0±0, 29.0±0.4 and 26.3±1.3 Hz, respectively (n=4). The NaV1.7 gain-of-function mutations reduce the minimum current required for AP spiking and increase the neuronal AP firing frequency relative to NaV1.7 WT, reflective of the differences in ion channel properties for the NaV1.7 mutants and consistent with published findings. These findings demonstrate the feasibility of using a real-time dynamic clamp system to generate *in silico* neurons that display characteristic AP behavior. Such simulations can determine how ion channel mutations affect neuronal function, particularly in neurological disorders. Ion channel currents expressed in living cells can be input into the *in silico* neurons for cell-based electrophysiology research. Effects of drugs targeting the expressed ion channels can be assessed with neuronal function and AP behavior as the measurable endpoints. These studies were supported in part by NIH R44 MH119842.

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Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.13

Topic: I.06. Computation, Modeling, and Simulation

Support: CIHR Grant

Title: Implications of incorrectly estimating channel activation properties when fitting channel conductance densities in computational models

Authors: *J. YANG[1], S. A. PRESCOTT[2];
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Abstract: Optimization algorithms find combinations of ion channel conductance densities that allow model neurons to reproduce various output features. Importantly, the set of successful solution comprises many different parameter combinations. In other words, each successful model produces the same output on the basis of different ion channel density (g_{max}) combinations—the models are degenerate. Using more features to fit (constrain) the model will yield fewer solutions that should, in principle, more accurately reflect true g_{max} values. However, other channel parameters, such as voltage at half-maximal activation ($V_{1/2}$) vary due to measurement errors and/or biological factors, such as genetic variations, beta subunit association, or modulation (e.g. cyclic AMP for H current). Here, we demonstrate the potential pitfalls of modeling $V_{1/2}$ as a fixed parameter. We have previously shown using simple geometry that the dimensionality of the solution space is determined by the difference between the number of free-to-vary conductances (n_{in}) and the number of constraining features (n_{out}). Using the same modified Morris-Lecar model, we performed a grid search of g_{Na} and g_M with different $V_{1/2}$ values to show that the conductance combinations, i.e. the slope of correlation, producing the same firing rate, differ depending on $V_{1/2}$. Hence, when trying to recover g_{max} values of a reference model, setting $V_{1/2}$ for channel X to a wrong value corrupts estimates of g_{max} for channel X and for other channels that co-vary with channel X. When $V_{1/2}$ is also allowed to vary (n_{in} = 3), the 2D iso-firing rate contour expands into a 3D surface. The “experimental range” of g_M was then simulated by superimposing a normal distribution, which, in turn, corresponds to a wide range of $V_{1/2}$. Treating $V_{1/2}$ as a fittable parameter does not enable definitive inference of $V_{1/2}$ since $V_{1/2}$ values co-vary with g_{max} values. Past demonstrations of optimization algorithms have used ground truth for $V_{1/2}$ (known from the reference model) to recover the correct g_{max} values. But in reality, $V_{1/2}$ values are not known with high confidence; if they are misestimated, then g_{max} values are misestimated. “Corrupted” models still reproduce the desired output, but on the basis of different parameters, which may cause them to respond to perturbations differently than the reference model. Fitting $V_{1/2}$ values adds extra degrees of freedom but allows for fuller consideration of alternative models that include correct $V_{1/2}$ values.

Disclosures: J. Yang: None. **S.A. Prescott:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.14

Topic: I.06. Computation, Modeling, and Simulation

Title: Markov Chain Monte Carlo and detailed electrical models of neurons: correlations and generalisation

Authors: *A. ARNAUDON[1], M. REVA[2], M. ZBILI[3], L. KANARI[3], W. VAN GEIT[3], H. MARKRAM[4];
[1]Brain Mind Institute, École Polytechnique Fédérale De Lausan, Brain Mind Institute, École Polytechnique Fédérale De Lausan, Lausanne, Switzerland; [2]École polytechnique fédérale de Lausanne, EPFL, Geneva, Switzerland; [3]Ecole Polytechnique de Lausanne, Lausanne, Switzerland; [4]EPFL, Blue Brain Project, EPFL, Blue Brain Project, Lausanne, Switzerland

Abstract: Detailed electrical models of neurons consist of several ion channels in various compartments yielding models with as many as 30 free parameters. These parameters, such as ion channel conductances, have to be fitted to experimental data via optimization of a cost function. However, little is known about the robustness of the model, an essential property of biological cells. To address this problem, we go beyond methods based on optimization and use Markov Chain Monte Carlo sampling of the parameters of the models with probability proportional to the cost function. We show that this approach provides detailed information about the properties of the parameter space, such as the shape of the high dimensional subspace of valid models, or, equivalently, correlations between parameters. For example, the maximum conductance of sodium in the axon initial segment and soma must not both be small or the threshold current will dramatically increase. Thus, the shape of parameter space allows us to assess stability under perturbations such as morphological changes or current blockades. We then propose algorithms for selecting a population of robust models. In addition, we show that by adjusting the size of the axon initial segment and the soma to match their respective input resistance with the input resistance of the dendrites, we further improve the generalisability and consistency of the models. We apply this procedure to various electrical types as a means to produce a heterogeneous and robust population of neuron models, which, in network simulations, can provide further insight into how neuronal variability impacts circuit computations.

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Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.15

Topic: I.06. Computation, Modeling, and Simulation

Title: Modeling microglial stress based on engineering predictions of metal fatigue

Authors: *K. MCGLOTHEN[1], E. CUTLER[1], R. M. HINES[2], D. J. HINES[3];
[1]Univ. of Nevada Las Vegas, Las Vegas, NV; [2]Psychology, Univ. of Nevada Las Vegas, Henderson, NV; [3]Psychology, Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: Metal is a commonly utilized material in engineering structures and the fatigue of metal is the most frequent failure outcome of metal components. Instances of fatigue occur when a material is subjected to fluctuating stress cycles resulting in an accumulation of fractures. Predictive models of metal fatigue are often implemented to avoid or delay the failure of metal exposed to accumulating cycles of stress. Comparably, increases in the cycles of cellular stress within the brain may result in irreparable damage in the form of chronic disease states. Inflammatory responses in the brain are a result of the interactions between the central immune and nervous systems cycling throughout the lifetime of an individual. Microglia are resident immune cells in the brain that undergo dynamic morphological changes in response to nervous system stress. Estimations of increasing inflammation have historically relied on relatively limited measures and would benefit from a novel predictive model of microglial stress separating the boundary between acute and chronic disease states. Increased brain inflammation and the subsequent cellular responses of microglia are being considered for their roles in the pathogenesis of inflammatory diseases of the brain, however, the transition between the acute to chronic states of neuroinflammation are still poorly understood. In the present theoretical model, the relationship between stress and microglial morphology change is predicted by applying models commonly used to predict the fatigue and deformation of metal. Models of metal fatigue are paralleled with the acute and chronic reoccurring amounts of stress applied to microglia. We hypothesize that as microglia are introduced to increasing cycles of stress it creates irreversible deformations that make the cell less capable of enduring future stress. The dynamic nature of microglial processes makes it difficult to distinguish when they are healing or harming a system and at what point they catapult a system into an irreversible inflammatory state. Adapting a model of metal fatigue to fit models of microglial stress provides a novel method for to predicting acute to chronic inflammation in the brain. The predictive model of microglial stress applied in this study will provide insight on the transition between acute to chronic inflammation and aid in the advancement of novel therapeutic diagnostics for neuroinflammatory diseases.

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Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

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Program #/Poster #: 330.16

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH/NINDS R01NS118562
NSF Grant No. IOS-1921065

Title: Automating ion channel model tuning and building a Drosophila ion channel model database

Authors: *K. KULLU[1,2], A. MORENO-SANCHEZ[1], A. N. VASSERMAN[3], C. R. VON REYN[1,3], J. AUSBORN[1];
[1]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Computer Engin., Ankara Univ., Ankara, Turkey; [3]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA

Abstract: Computational modeling and simulation of neurons and circuits help researchers test mechanistic hypotheses and propose new ones. Biophysically accurate models in turn require modeling and simulating ion channels. Voltage-gated ion channels are commonly modeled with Hodgkin-Huxley (HH) style models. HH models involve several parameters that need to be determined and adjusted in order for them to successfully mimic the biological channels. This often involves time-consuming manual tuning to match the model outputs to electrophysiology recordings. There have been some efforts to tackle this using computational tools such as evolutionary optimization algorithms or artificial neural networks. We set out with two aims, (1) to construct a framework for automating this task as much as possible, and (2) to build a database of biophysically accurate *Drosophila* ion channel models. This database will later be used in creating multi-compartment models of *Drosophila* neurons and circuits. For (1), we used the Covariance Matrix Adaptation Evolutionary Strategy (CMA-ES) and a *Drosophila* T-type Calcium (Ca) channel (Ca-α1T) for which we found published electrophysiology data. We developed a semi-automated pipeline as a proof-of-concept that allowed us to determine and fine-tune the model parameters based on existing standard voltage clamp protocols. We modeled a one-compartment, isopotential cell with the ion channel in question and leak current. CMA-ES optimization was employed to simultaneously search for 17 parameters in a HH model (max. Ca conductance, leak and Ca reversal potentials, and 14 values appearing in activation, inactivation, and time constant equations). The optimization targeted minimizing the mean square error (MSE) between a set of current recordings (in nA) from a voltage clamp protocol and the simulated versions of these recordings from the model. We reached MSE values as low as 374.059, meaning that on average, the difference between simulated and experimental currents at any instant was $\sqrt{374.059} \approx 19.341$ nA. Numerous computational experiments were carried out to test the pipeline, including starting the search with an approximate centroid vector to represent having no initial estimates for the parameters. Despite taking more time, the optimization was able to converge on a solution even without the estimates. Our efforts to utilize this framework to fit models for other voltage-gated *Drosophila* ion channels continue. The final *Drosophila* ion channel model database will fill an existing gap and facilitate the creation of biophysically accurate cellular models by complementing the anatomical and electrophysiological data available today.

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Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.17

Topic: I.06. Computation, Modeling, and Simulation

Support: NRF (nos. 2020R1A2C2010285, I21SS7606036)
Ministry of Health & Welfare and Ministry of Science and ICT (no. HU22C0115)

Title: Construction of Physiological Cerebral Environment Augmented with a Brain Decellularized Extracellular Matrix - Matrigel Hybrid

Authors: H. NGO[1], M. BAE[2], Y. KANG[1], J. JANG[2], D.-W. CHO[2], H. CHO[1];
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Abstract: The brain extracellular matrix (ECM) anchors cellular components and helps maintain brain homeostasis from development to adulthood. However, specific biochemical components participating in regulation of cellular behaviors in both healthy and diseased conditions are yet to be elucidated due to the lack of ex vivo models that can faithfully recapitulate the combinatorial biophysical and biochemical components of the brain ECM. Here, we used the porcine brain decellularized ECM (P-BdECM) supplemented with Matrigel (MTG) (P-BdECM - MTG or Hybrid) to create a human brain-mimicking microenvironment ex vivo, with human brain cell cultures, enabling the discovery of biochemical factors regulating brain physiology and pathology. We encapitulated human brain cells, including neural progenitor cells (NPCs), astrocytes (ACs) and microglia (MG), into P-BdECM, Matrigel (MTG) or Hybrid. The results showed that Hybrid promoted the neural stemness characteristic of NPC, evidenced by the increase in Pax6 marker (2-fold). Hybrid condition also enhanced the differentiation of NPC into Neuron (Tuj1, 2.3-fold) and Astrocyte (Aldh1l1, 3-fold). We also characterized ACs phenotypes in Hybrid, which showed that Hybrid maintained ACs in their homeostatic state, with increased homeostatic marker Aldh1l1 (1.2-fold) and reduced basal reactivity level compared to MTG (GFAP 0.7-fold vs MTG). We found that Hybrid maintained ACs in resting state (A0) via BdECM-derived ANNEXIN A1 protein (ANXA1). Supplementing MTG with the same amount of ANXA1 contained in Hybrid (0.74μg/ml) showed the increase in homeostatic marker Aldh1l1 (1.2-fold) and the reduction in reactivity (GFAP) to the same level of Hybrid (Aldhl11 1.2-fold vs MTG, GFAP 0.7-fold vs MTG). In the presence of LPS, Hybrid dramatically shifted ACs into an inflammatory state with greater increase in GFAP level (2.7-fold vs 1.3-fold of MTG) and ROS level (5.3-fold vs 2.7-fold of MTG). Hybrid also kept MGs in resting state with low reactivity level, including CD86 (0.5-fold vs MTG), ROS (0.4-fold vs MTG), iNOS (0.8-fold vs MTG) and NO (0.3-fold vs MTG). Similar to AC, MG in Hybrid also dramatically responded to LPS, with greater increase in proinflammatory MG (M1) marker, including CD86 (3.8-fold vs 1.6-fold of MTG), ROS (8.3-fold vs 3.3-fold of MTG), iNOS (1.9-fold vs 1.3-fold of MTG) and NO (3.9-fold vs 1.5-fold of MTG). Taken together, our study showed that P-BdECM - MTG

Hybrid have provided a very useful tool in ex vivo brain modeling that can be applied to neuroscience research, material-based neuroregeneration and translational medicine.
Keywords—Brain decellularized extracellular matrix, Neurogenesis, Neuroinflammation

Disclosures: **H. Ngo:** None. **M. Bae:** None. **Y. Kang:** None. **J. Jang:** None. **D. Cho:** None. **H. Cho:** None.

Poster

330. Computational Models at the Cellular Level

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Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 330.18

Topic: I.06. Computation, Modeling, and Simulation

Support: ERC Grant 714892

Title: Modeling electrodiffusive, osmotic, and hydrostatic interplay in astrocyte networks

Authors: M. SÆTRA, A. J. ELLINGSRUD, M. E. ROGNES;
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Abstract: During high neuronal activity, the intra- and extracellular ion concentrations change. These changes affect the osmotic pressure gradients across the membranes of both neurons and astrocytes, leading to water movement and cellular swelling. We asked: Can swelling generate a hydrostatic pressure gradient of sufficient magnitude to drive non-negligible fluid flow within astrocytes or the extracellular space [1]? As it is currently infeasible to measure such intracellular pressure gradients in vivo, computational modeling emerges as a viable alternative to study the interplay between osmotic and hydrostatic forces at the microscale. In this study, we present a computational model of ionic electrodiffusion, hydrostatic pressures, and transmembrane- and intracompartmental fluid flow in a homogenized astrocytic syncytium surrounded by extracellular space. The model builds on previous models of ionic electrodiffusion [2,3], and potassium buffering [4]. Our findings show that increases in extracellular potassium concentrations in response to neuronal activity induce swelling and hydrostatic pressure gradients within the intra- and extracellular spaces. The fluid flow induced by these hydrostatic pressure gradients alone did not have a significant effect on the transport of potassium within any of the compartments. However, when also accounting for fluid flow induced by osmotic gradients within the astrocytic syncytium, convection played a considerable role in potassium clearance. These findings point at a mechanistic understanding of how astrocytic permeability may impact fluid flow in the brain.
[1] Halnes, G., Pettersen, K. H., Øyehaug, L., Rognes, M. E. & Einevoll, G. T. Astrocytic ion dynamics: Implications for potassium buffering and liquid flow. In Computational Glioscience, 363-391 (Springer, 2019). [2] Mori, Y. A multidomain model for ionic electrodiffusion and osmosis with an application to cortical spreading depression. Phys. D: Nonlinear Phenom. 308, 94-108 (2015). [3] Zhu, Y., Xu, S., Eisenberg, R. S. & Huang, H. Optic nerve microcirculation:

Fluid flow and electrodiffusion. Phys. Fluids 33, 041906 (2021). [4] Halnes, G., Østby, I., Pettersen, K. H., Omholt, S. W. & Einevoll, G. T. Electrodiffusive model for astrocytic and neuronal ion concentration dynamics. PLoS computational biology 9, e1003386 (2013).

Disclosures: **M. Sætra:** None. **A.J. Ellingsrud:** None. **M.E. Rognes:** None.

Poster

330. Computational Models at the Cellular Level

Location: SDCC Halls B-H

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Program #/Poster #: 330.19

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH MH122023
NSF OAC-1730655

Title: Inference of neuron morphology and orientation from LFP data

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Abstract: Neurophysiological data are being generated at increasing levels of precision and at multiple scales, e.g., electrophysiological data at single neuron and network levels. However, schemes to effectively mine such data are only beginning to be developed, slowing down their utilization in hypothesis-driven research both on experimental and computational fronts. Fortunately, advances in machine learning algorithms and the development of automated parameter estimation schemes are being reported in many domains, many of which have potential to assist with mining such data. We propose computational algorithms for inference related to single neurons using experimental multi-electrode probe data. Specifically, we infer neuron location and morphology using in vivo extracellular action potential recordings in an inverse modeling scheme. For this, we designed a generic biophysical single neuron model with stylized morphology that had adjustable parameters for the dimensions of the soma, basal and apical dendrites, and the position and orientation of the neuron relative to the multi-electrode probe. Using training data from this computational model, we develop a machine learning approach that uses model extracellular action potential output to infer the parameters of the neuron that generated it. Initial results suggest that the proposed methodology can infer the key location and morphology parameters of layer 5 cortical pyramidal neurons reliably from the model data. On-going work focuses on validating the inference algorithm using both model data from neurons with detailed morphology and in vivo recordings, and then generalize to various types of neurons. Future study will explore the accuracy and sensitivity of the inference due to the reduction of morphology and the related biophysical dynamics, e.g., back propagation of somatic action potential, and electrotonic distance of dendrites.

Disclosures: **Z. Chen:** None. **D. Dopp:** None. **S.S. Nair:** None.

Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 331.01

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH BRAIN Initiative Project NS109553
NSF GRFP

Title: Effects of electrical stimulation across species

Authors: *B. MARSH, E. HALGREN, M. BAZHENOV;
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Abstract: Determining the effects of electrical stimulation on individual neurons is a non-trivial problem. Even when experimental data on individual neurons is available, this data is most often from rodent sources; determining the effects of stimulation on individual human neurons is an even more elusive problem. In this project, we aim to combine physics, biology, and computer science to simulate the effect of various levels of electrical stimulation on individual neurons. Specifically, we use morphological reconstructions of pyramidal cells and interneurons across multiple cortical layers from rats, mice, and humans. These neuron reconstructions are individually subjected to a range of currents from a (simulated) square cortical electrode, and average activation probabilities are calculated across a range of horizontal and vertical distances (within biological layer bounds) from the electrode. This allows us to compare response probabilities across cell types, layers, and species; importantly, we can do this very quickly and iterate over a large combination of possible stimulation parameters for a given cell. Preliminary results show that cell response probability for a given stimulation strength decreases with increasing layer depth and species size, as well as generally stronger responses to anodal than cathodal stimulation of the same strength. Additionally, rat and human neurons show qualitatively similar response patterns while mouse cell response patterns are significantly different. Rat neuron reconstructions simulated with human parameters (cortical depths, current amplitudes, etc.) further provide a reasonable estimate of the responses of human neurons. If this proves to be a generalizable trend, this may allow for much more accessible rat cells to be used to accurately predict human cell response probabilities under a variety of experimental conditions. Our approach could further be used by experimental scientists to test species-specific hypotheses in-silico to select the appropriate subset of parameters for the expensive (and time-consuming) in-vivo work. In summary, this project both builds new knowledge of cross-species cell response probabilities and contributes to tool building for more informed & efficient experimental protocols.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 331.02

Topic: I.06. Computation, Modeling, and Simulation

Title: Quick and accurate prediction of neural responses to electrical stimulation: the Generalized Activating Function

Authors: *J. GARCIA ORDOÑEZ[1,2], T. H. NEWTON[3], N. KUSTER[3,4], E. NEUFELD[1,3]; [1]ZMT Zurich MedTech, Zurich, Switzerland; [2]Inst. of Neuroinformatics (INI), Univ. of Zurich (UZH) and ETH Zurich, Zurich, Switzerland; [3]IT'IS Fndn., Zurich, Switzerland; [4]ETH Zurich, Zurich, Switzerland

Abstract: Controlling interactions between electrical stimulation (i.e. electromagnetic fields, EM) and neurons is key to most neural interfaces for neuroprosthetics and electroceutical therapies. In this context, simulations are an efficient and flexible method to evaluate safety, optimize stimulation conditions and improve device design. While coupled EM-NEURON simulations have been extremely valuable to gain mechanistic understanding and personalize therapies, e.g., to restore locomotion in paraplegics through spinal-cord stimulation (SCS) (Rowald et al., Nature Med, 2022) or drive translational bioelectronics research at the level of the peripheral nervous system (Gupta et al., Comms Biology, 2020), they are computationally too expensive for meta-modeling applications such as optimization, rare event detection, and uncertainty quantification, which are critical to regulatory applications. However, when detailed knowledge of the subthreshold dynamics is not required, the activating function (AF) (Rattay, Neuroscience, 1999) provides a useful predictor of extracellular-field-induced neuronal firing, greatly reducing computational costs. Despite its usefulness, the AF compromises accuracy, fails to predict stimulation in end-node compartments and does not account for the stimulation pulse-shape. Here, we extend the AF concept by introducing a “generalized” activating function (GAF) based on the Green’s function for ionic diffusion. The GAF models the subthreshold dynamics of neural membranes and accounts for the dynamics resulting from stimulation with an arbitrary pulse shape, overcoming the limitations of the AF mentioned above. We find that the GAF significantly outperforms classical AF in its stimulation prediction accuracy, at a much lower computational costs than with state-of-the-art neural dynamics simulations. Furthermore, we demonstrated its suitability for the fast and efficient optimization of stimulation configurations in high-dimensional parameter spaces, using the highly detailed EM model of the SCS from Rowald et al. (Nature Med, 2022) as an example application. The GAF exhibits superior accuracy and efficiency in predicting neurostimulation, thus enabling meta-modeling applications such as those demonstrated for optimizing SCS to restore locomotion in paraplegics.

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funds); Shareholder of NFT Holding AG. **E. Neufeld:** A. Employment/Salary (full or part-time):; IT'IS Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Shareholder and Board Member of TI Solutions AG.

Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 331.03

Topic: I.06. Computation, Modeling, and Simulation

Title: Improving selectivity of vagus nerve stimulation through realistic nerve modeling

Authors: *N. KATIC[1,2], F. CIOTTI[2], R. JOHN[2], P. BENLLOCH[2], T. SUDAN[2], V. TOTH[3], N. JAYAPRAKASH[3], S. ZANOS[3], S. RASPOPOVIC[2];
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Abstract: The vagus nerve constitutes one of the main components of the parasympathetic nervous system, which oversees a number of crucial body functions, including control of mood, immune response, digestion, breathing and heart rate. It establishes one of the connections between the brain and the gastrointestinal tract and conveys information between inner organs and the brain. It has a very complex structure with several branches and different types of fibers conveyed. Cervical vagus nerve (cVN) contains more than 100 thousand afferent and efferent nerve fibers from almost all thoracic and abdominal organs, making it a promising target for different neuromodulating applications. Vagus nerve stimulation has been approved by the FDA for treating drug-resistant epilepsy and depression. However, devices currently employed for vagus nerve stimulation are not achieving satisfactory spatial and functional selectivity while eliciting nerve fibers. This deficit is belived to be the cause for a multitude of side effects during the vagus stimulation, such as voice alterations, coughing, dyspnea, nausea and headaches, which are currently limiting the application and benefit of long-term VNS therapy. To make a first step in overcoming this limitation, we constructed a novel anatomically detailed and biophysically plausible computational model of the cervical vagus nerve. Utilizing histological images obtained at multiple cross sections along the pig cVN, we reconstructed the nerve taking into account specific fascicular geometry, including three-dimensional curvatures and branching. We employed distinct models for myelinated type A and type B fibers, as well as unmyelinated type C fibers. Furthermore, we implemented their precise position, obtained by applying computer vision techniques to the experimentally collected data. We found that it is essential to employ highly realistic computational models rather than the simplified extruded models which are commonly being used. Finally, we also developed a complete computational tool for cVN that enables testing of complex stimulation paradigms and arbitrary electrode geometries. In future research the insights gained from this computational model will be utilized to improve the

fiber selectivity of vagus nerve stimulation, both through the development of new stimulation strategies as well as the design of a sophisticated novel electrodes.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

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Program #/Poster #: 331.04

Topic: I.06. Computation, Modeling, and Simulation

Support: NSF Grant GRFP DGD174501
NSF Grant LEAP HI CMMI-1953323

Title: Mutually Inhibitory Circuit Using Neuron-Inspired, Biomolecular Neuristors

Authors: *A. S. MOHAMED[1], A. S. LIAO[2], Y. J. ZHANG[2], V. A. WEBSTER-WOOD[2], J. S. NAJEM[1];
[1]Mechanical Engin., The Pennsylvania State Univ., State College, PA; [2]Mechanical Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Using a system of voltage-gated ion channels, neurons form connections with neighboring cells, creating complex circuits that allow for a myriad of functions, such as information processing and movement control. However, replicating these neural circuit systems *in vitro* by culturing neurons can be very challenging due to the need for precise intra- and extracellular signaling and maintenance of specific culture conditions. Alternatively, Hodgkin-Huxley-inspired [1] synthetic biomolecular neuristors are easy to fabricate and closely mimic the spiking behavior and lossless signal transmission of biological neurons. Moreover, they can be assembled into desirable circuitry, making them ideal candidates for studying neural mechanisms *in vitro* and creating biocompatible controllers for biohybrid systems. To investigate the viability of designing neural control circuits using biomolecular neuristors, we used computational models to compare the membrane potentials of a single, bursting Izhikevich neuron [2] and a single neuristor, and their membrane potentials in a four-unit mutually inhibitory configuration. Models enable tuning different experimental parameters to design a neuristor that behaves similarly to a neuron of interest. A biomolecular neuristor is comprised of two dynamical lipid-bilayer-based memristors that exhibit volatile memory and negative differential resistance due to the dynamics of voltage-gated ion channels and the ion gradients across the lipid membranes. By tuning the types and concentrations of the elements constituting each memristor, the neuristor's firing frequency and pattern can be modulated to meet the targeted neural circuit requirements. The neuristor and neuron models demonstrated an alternating bursting behavior in the mutually inhibitory configuration. However, using experimentally feasible parameters, the neuristor firing frequency was lower than that of the neuron in both the single-cell and mutually inhibitory

circuit. The mutually inhibitory neuristor circuit achieved a firing frequency of ~45 Hz compared to ~100 Hz for the Izhikevich model circuit. The alternating behavior indicates the potential for setting the neuristors up in a neuron-like circuit. Still, the lower firing frequency suggests the need for further parameter optimization of the neuristor model, which corresponds to changes in experimental parameters such as droplet size, lipid type, and peptide and salt concentrations [3][4]. References: [1] Hodgkin and Huxley. *J. Physiol*. (1952). [2] Izhikevich. *IEEE Transactions on Neural Networks*. (2003). [3] Eisenberg, *et al*. *J. Membrane Biol*. (1973). [4] Najem *et al*. *ACS Nano*. (2018).

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Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 331.05

Topic: D.07. Visual Sensory-Motor Processing

Title: Deriving testable predictions to distinguish candidate principles linking neural network structure and function

Authors: *T. BISWAS[1], T. L. LI[2], J. E. FITZGERALD[1];
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Abstract: Uncovering principles that guide how neurons are connected to generate their functional responses is a challenging but aspirational goal in neuroscience. Degeneracy is a major difficulty that limits the discovery of robust structure-function links, as many different networks can produce the same responses to the limited number of stimulus conditions that are typically measured experimentally. In previous work, we addressed this challenge by developing a geometric framework that allowed us to analytically characterize the solution space of synaptic weight matrices consistent with specified steady-state stimulus responses (Biswas and Fitzgerald, *Phys Rev Res*, 2022). This analysis mimicked the situation in whole-brain imaging by assuming access to all neurons in the network and focused on threshold-linear recurrent networks, which offered analytical tractability and produced numerous insights. However, it remains unclear how this solution space can be best used to identify experimentally testable predictions that distinguish between candidate principles. Here we introduce a unified mathematical formalism for identifying predictions from a wide class of ensemble modeling and optimization principles. For instance, if we assume that the L_2 norm of the incoming synaptic weight vector to all the neurons obeys a biological bound, then we can obtain an ordered list of the synapses that are most certain to exist across the ensemble, and we can also predict their sign. This result extends our previous analytic approach to arbitrary stimulus patterns and introduces novel numerical methods. If we instead postulate that the L_2 norm of the weight vector is minimized, then we can

determine the weight vector uniquely, even in a nonlinear network. The optimal weight vector changes if we combine the L_2 and L_0 norms to penalize both the strength and number of synapses, and we have developed approximate methods to identify maximally sparse weight vectors. As a final example, our formalism also allows us to find the weight vector in the solution space that is closest to any given weight vector or ray of weights, which may productively combine brain-wide imaging and connectomics data. Each of these candidate principles provides distinct predictions at the level of synapse occurrence and sign, as well as for neural responses to hitherto unseen stimulus pattern. We thus hope that our analysis will not only provide a way to obtain information about structure from functional data, but also help us identify and test the underlying theoretical principles and modeling frameworks.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

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Program #/Poster #: 331.06

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH 1 R03 HD099426-01A1

Title: Function defines neural damage: A simulation study

Authors: Y. PRYYMA[1], *S. YAKOVENKO[2];
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Abstract: Complex biological systems evolved to control dynamics in the presence of noisy and often unpredictable inputs. The staple example of this is the locomotor control, which is vital for survival and spans multiple systems - from passive dynamics of inverted pendulum representing body movement to coupled neural oscillators that integrate sensory inputs at different levels of the neuraxis. The full analytical description of this system is currently beyond our scientific scope; yet, the data-driven approximations could be “good enough” solutions with practical applications. Machine learning tools developed for simple integrate-and-fire neurons or spiking neural nets (SNN) offer a new biomimetic formulation that can be used in place of approximations. In this study, we converted an analytical rate model of the central pattern generator (CPG) using the SNN approach (Nengo). The network approximation faithfully recreated the input-output relationship of the model describing the modulation of locomotor phase characteristics. Moreover, it allowed us to conduct “lesion” experiments on different elements of this network. A novel finding is that the functional identity of lesioned flexor or extensor neurons with an identical structural organization affects differentially the performance of the damaged system. Also, the CPG input increasing the general excitability within the network can compensate for the loss of function after progressive lesions. This observation may

explain the response to spinal stimulation and propose a novel theoretical framework for research and applications.

Disclosures: **Y. Pryyma:** None. **S. Yakovenko:** None.

Poster

331. Computational Models of Neurons, Circuits, and Networks

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Program #/Poster #: 331.07

Topic: I.06. Computation, Modeling, and Simulation

Support: NIH NINDS R01NS118188
DC000566
NIH U01NS099577
NSF CBET-1631912

Title: Drumbeat Optogenetics: Improving the firing frequency of channelrhodopsin-2

Authors: *T. A. WELTON[1], A. FONTAINE[1,3], J. H. CALDWELL[2], S. F. LITTICH[1], R. F. WEIR[1,3], D. RESTREPO[2], E. A. GIBSON[1];
[1]Bioengineering, [2]Cell & Developmental Biol., Univ. of Colorado Denver - Anschutz Med. Campus, Aurora, CO; [3]Rocky Mountain Regional VA Med. Ctr., Aurora, CO

Abstract: In optogenetics, Channelrhodopsin-2 (ChR2) is a cation channel that is commonly used to excite action potentials in response to light stimulation. The experimental usefulness of ChR2 is inherently limited by its slow kinetics which makes triggering action potentials at frequencies ≥ 40 Hz very challenging. We investigated whether it was possible to increase the firing frequency of a nerve by stimulating two separate populations of ChR2 along the length of the nerve asynchronously. This would allow the opsin at one location to recover while action potentials are excited at the other. Initially, a computational model was used to evaluate this "Drumbeat" stimulation scheme. A Wang-Buzsaki model was coupled with a three and four state opsin model to simulate the neuronal response of a single compartment, fast spiking interneuron to ChR2 stimulation. This model was extended to allow for two different populations of opsin to be stimulated with the same light protocol that was shifted by a time delay. The results of this simplistic computer model indicate that it is theoretically possible to double the firing frequency of a nerve when the stimulation of both populations is offset by the correct time delay. We also present some of our preliminary experimental findings from *in-vitro* recordings of compound action potentials (CAP) and loose patch recordings of single axons in the sciatic nerve of CHAT-ChR2-YFP mice. Optogenetics is a powerful tool to study the nervous system, but its utility is fundamentally limited by the kinetics of the opsin themselves. Perhaps the key to unlocking the full potential of optogenetics lies in both the engineering of new opsin variants and the creation of innovative stimulation procedures.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

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Program #/Poster #: 331.08

Topic: I.06. Computation, Modeling, and Simulation

Support: This work is supported by a Foundation Grant from the Canadian Institutes of Health Research

Title: Effects of ephaptic coupling on action potential propagation in peripheral axons

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Abstract: Neurons encode information using the rate and/or timing of their spikes. Spike arrival times at post synaptic neurons are important for temporal coding. In primary somatosensory neurons, tactile stimuli can evoke spikes synchronously across neurons, but variations in conduction velocity risk corrupting that synchrony by the time spikes arrive at the CNS. However, recordings from somatosensory cortex have shown highly synchronized spiking, indicating that synchrony is not lost. This raises the question of how synchronization is maintained despite variations in conduction velocity. Experimental and theoretical work has demonstrated that when an action potential propagates down an axon, it affects the extracellular electric field, which in turn influences the membrane potential of nearby axons - an effect is known as ephaptic coupling. Ephaptic coupling has been shown to affect conduction velocity and prevent dispersion of spike arrival times in myelinated axons, but the effect has only been studied in pairs of axons and appears to be very weak. Assuming a Gaussian distribution of conduction velocities across a large population of axons, it is important to consider if and how the majority of axons with a typical conduction velocity influence the minority of axons with slower or faster velocities. We built a detailed biophysical model of a bundle of primary afferents in a fascicle including myelinated Aβ, Aδ and unmyelinated C fibers to study how ephaptic coupling affects spike propagation in primary sensory axons. Simulations showed that ephaptic coupling generated by a spike in one Aβ fiber causes subthreshold voltage deflections in nearby fibers. The voltage deflection depends on how misaligned the nodes are, with less misalignment leading to higher voltage deflection. We also studied the insulating effects of perineurium, which bundles axons into fascicles. Simulations revealed that a highly resistive boundary, which prevents the extracellular field from dispersing, increases voltage deflection and can cause

ectopic spike generation. Our results show that ephaptic coupling resulting from the activity of synchronously activated Aβ fibers speeds up and homogenizes conduction velocity, thus improving synchronization.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

Location: SDCC Halls B-H

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Program #/Poster #: 331.09

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Title: The NetPyNE multiscale modeling tool: latest features and models.

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Abstract: NetPyNE is an NIH-funded tool for data-driven multiscale modeling of brain circuits. It enables users to consolidate complex experimental data from different brain scales into a unified computational model. NetPyNE builds on top of NEURON, one of the most widely used neural simulation engines. NetPyNE is unique integrating all major steps of the modeling workflow under a single framework. The core of NetPyNE consists of a standardized JSON-like declarative language that allows the user to define the model across scales, from molecules to neurons to circuits. The NetPyNE API can then be used to automatically generate the corresponding NEURON network, run parallel simulations, optimize and explore parameters, and visualize and analyze the results through a wide range of built-in functions. NetPyNE facilitates model sharing by exporting/importing to the NeuroML and SONATA standardized

formats. All functionality is also available via a state-of-the art web-based graphical application, which now includes management of simulations and automated exploration of parameters. This is the only graphical tool that allows users to define parameters values to explore, run the corresponding simulations and visualize the results. Additionally, the web app is fully integrated with the Open Source Brain (OSB) platform, providing users with an online persistent workspace, file management, access to online resources and interactive jupyter notebooks. NetPyNE has been interfaced with CoreNEURON, and several large-scale models were benchmarked on GPUs for the first time, obtaining impressive 40x speedups. The new interface with the LFPykit tool allows NetPyNE to generate dipole current moments for any arbitrary model, and simulate EEG signals at electrodes placed along a head volume conduction model. The new co-simulation interface between NetPyNE and The Virtual Brain (TVB) achieves a new milestone for multiscale modeling: linking molecular chemical signaling (via RxD) to whole-brain network dynamics. NetPyNE is now also available as a service on the Human Brain Project EBRAINS platform, including example use cases. The SciUnit tool has been adapted to work with NetPyNE, resulting in the NetPyNEUnit package which facilitates model reproducibility, validation and evaluation. At least 25 publications describe models or tools that have made use of NetPyNE, including our recent detailed models of the motor, auditory and somatosensory thalamocortical circuits, and of spinal cord circuits. Others have developed NetPyNE models to study Parkinson's disease, schizophrenia, ischemic stroke and epilepsy.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

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Title: Neuroscience Gateway - modeling, data processing and software dissemination

Authors: *A. MAJUMDAR[1], S. SIVAGNANAM[4], K. YOSHIMOTO[2], M. KANDES[3], S. H. YEU[3], D. CHOI[3], N. T. CARNEVALE[5];
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La Jolla, CA; [3]San Diego Supercomputer Ctr., Univ. of California San Diego, LA JOLLA, CA; [4]UCSD, UCSD, La Jolla, CA; [5]Yale Univ., Yale Univ., New Haven, CT

Abstract: The Neuroscience Gateway (NSG) enables neuroscientists to easily utilize high performance computing (HPC), high throughput computing (HTC) and cloud computing resources for large scale modeling, data processing, and AI/ML work. NSG provides close to 20 neuroscience modeling, data processing and AI/ML tools optimally made available on supercomputer resources. Via NSG's easy to use web-based and programmatic interface neuroscientists can upload input file or data to the NSG, chose the appropriate tool, specify the tool and computing related parameters and submit the computing workload to a supercomputer. Upon completion of the processing, users can download the output results. NSG eliminates administrative and technical barries to utlize supercomputing resources for modeling, data processing and data analytics work in neuroscience. NSG also provides a software developer platform which neuroscience software developers can use to test, benchmark and scale their software and then release the software on supercomputers where NSG acquires yearly allocation for the neuroscience community. NSG provides a platform for dissemination of neuroscience software. NSG is free and open to any neuroscientist researchers and students. Yearly training and workshops are hosted by the NSG team where NSG users and software developers discuss their research results and software. NSG is utilized by other neuroscience projects, such as the NeuroElectroMagnetic data Archive and tools Resource (NEMAR), the Open Source Brain, as the computational engine for processing of data and simulations. NSG is used in classroom teaching for undergraduate and graduate students. The presentation at SfN will describe the tools that NSG provides, how they are utilized on supercomputers, statistics of NSG's growth, research enabled by NSG and external projects that are utilizing NSG as the compute engine.

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Poster

331. Computational Models of Neurons, Circuits, and Networks

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Support: NSF NeuroNex Award DBI-1707398
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Simons Foundation Collaboration on the Global Brain

Title: Controlled switching linear dynamical systems: a framework for perturbative interrogation of RNNs

Authors: *A. NEJATBAKHSH, M. BEIRAN, L. LOGIACO;
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Abstract: A powerful approach to understanding how the dynamics of biological neural networks control animals' behavior during a task consists of building models that solve the same task while reproducing some features of neural data. Building these models has become relatively easy thanks to recent machine learning advances for training Recurrent Neural Network models (RNNs) with gradient descent. However, challenges remain. First, even though the resulting RNNs are simpler than biological networks, we still have difficulties understanding how they work - notably, we do not know how to act on these networks to get a desired outcome. Second, it is unclear whether the activity of these RNNs just correlates with the data they have been trained to emulate, or whether they capture a more causal and robust network mechanism. The latter would require these models to behave like the original network beyond the training conditions - for instance under new input perturbations. Here, we take novel steps to address these challenges by combining a switching-linear approximation for the RNN's dynamics with a causal interrogation of these dynamics informed by control theory. By probing how the fitted and original systems agree on the directions of larger variability at critical timepoints, this methodology helps capture the phase portrait of the original system in a meaningful part of the activity space. The resulting switching-linear model reveals interpretable mechanisms governing the RNN's activity beyond fixed points and beyond the input-driven response. In addition, this method can uncover the RNN's generalization properties beyond the conditions experienced during training.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 332.01

Topic: I.08. Methods to Modulate Neural Activity

Support: FDA/CDRH/OSEL/Division of Biomedical Physics lab fund

Title: Amplitude and frequency-dependent activation of layer II/III neurons by intracortical microstimulation

Authors: G. K. WU[1], C. MASTRACCHIO[1], J. KENT[1], ***M. YE**[2];
[1]U.S. Food and Drug Admin., Silver Spring, MD; [2]FDA, FDA, Silver Spring, MD

Abstract: Intracortical microstimulation has been used in a number of preclinical studies and clinical trials to provide sensory feedback to the motor system for the development of a closed-loop brain machine interface. However, little is known of its safe levels of stimulation, which creates tremendous uncertainties in the therapeutic design. The current gold standard reference for safety thresholds stems from early animal and computational studies on macroelectrodes, for example, a threshold charge density of 30 μC/cm^2. The effective and safe range of stimulation is foreseeably different for microelectrodes with a geometric surface areas (GSA) much less than 2000 μm^2. In this study, we chronically implanted 16-channel microelectrode arrays (GSA of 177 μm^2 or 703 μm^2 per individual electrode) in the motor/somatosensory cortices of transgenic mice (Thy1-GCaMP6s), and used a combination of optical coherence tomography and two-photon calcium imaging to evaluate the stimulation effect for activating layer II/III neurons. Monopolar stimulation was delivered at various levels of current amplitude (3-100 μA) with cathodic-first biphasic currents at a frequency range of 10 to 200 Hz. We defined an activation threshold (at least one neuron that can be activated) and a saturated threshold (maximal neuronal population activated despite increasing stimulation amplitude). Near the stimulating electrode (<250 μm distance), the activation threshold for neurons is lower than that for locations distal to the stimulating electrode (>500μm). When ramping up the stimulation amplitude, the neurons near the electrode quickly saturated, whereas in the location distal to the stimulation electrode, only a subset of neurons responded and remained constant. The neuronal response to stimulation amplitude 10 μA above activation threshold is frequency dependent. Slow build-up responses were observed at lower stimulation frequency stimulation (10 or 20 Hz). The strongest and sustained responses were evoked by 50 and 100 Hz stimulation frequency. But neuronal responses showed large transient and quick decay when higher stimulation frequency was applied. Our results suggest a narrow range of stimulation amplitude should be considered to ensure the spatial specificity of neuronal activation for safety considerations, and a proper range of stimulation frequency should also be considered to provide effective neuronal activation. Future studies will be needed to understand the mechanisms of stimulation-amplitude related activation specificity and stimulation-frequency related neuronal responses.

Disclosures: G.K. Wu: None. **C. Mastracchio:** None. **J. Kent:** None. **M. Ye:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.02

Topic: I.08. Methods to Modulate Neural Activity

Title: Novel design of polyimide-based buckling-resistant intracortical microelectrode array

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Abstract: Intracortical microelectrodes arrays (MEAs) offer immense utility in brain-machine interface applications and basic neuroscience research towards treatment of neurological disorders and understanding of the nervous system. Over the last few decades, silicon-based microelectrode arrays became a major platform for recording neural activities at high spatial and temporal resolutions. However, penetration of a stiff silicon probe into the brain damages the native brain cells along the implant trajectory and triggers foreign body response (FBR). The FBR can be characterized by neuronal loss, cell death, and glial reactions around the implant. Moreover, the stiffness of silicon irritates the surrounding brain tissue and exacerbates the FBR during micromotions of the brain. More recently, soft polymer-based probes have emerged as the technology that can attenuate the FBR caused by mechanical mismatch between the ultra-soft brain tissue and stiff silicon materials. However, the caveat is that polymer-based MEAs are difficult to insert due to their mechanical compliance and often buckle before penetrating the brain. Alongside the development of flexible MEA technology, many assistive insertion strategies were also developed to overcome the insertion challenge. The insertion strategies include shuttles, guides, stiffening coatings, magnetic guidance, and microfluidic actuation among other. Although they have been used to successfully implant flexible probes, these workaround methods have drawbacks, for instance, the shuttle method requires more complicated surgical procedure and also causes larger insertion injury to the brain by the shuttle. In this work, we developed a novel design of flexible polyimide-based probe that can be inserted into the rat brain without secondary assistive methods. We leveraged the known mechanics of column buckling and designed the probe such that the shank can resist buckling under compressive loading during insertion into the brain. Preliminary results show that the new design can have 10 times higher buckling strength compared to a current state-of-the-art probe design when controlled for total volume/amount of tissue displaced. Using Finite Element Analysis, we also found that the novel probes cause minimal strain on the surrounding brain tissue due to micromotion which is comparable to current standard polyimide probes and almost half as much tissue strain as NeuroNexus silicon probes. Future work will investigate the tissue response and recording quality of the designed arrays in comparison to the standard polyimide and silicon probes.

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Poster

332. Electrical Stimulation: Animal Models

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant R01DK129194
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Title: Biphasic kHz-frequency field stimulation excites CA1 pyramidal neurons in slices

Authors: *S. V. KARNUP[1], N. YOSHIMURA[2], W. C. DE GROAT[1], J. M. BECKEL[1], C. TAI[2];
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Abstract: Electrical stimulation in the kilohertz-frequency range has been successfully used for treatment of various neurological disorders. Nevertheless, the mechanisms underlying this stimulation method are poorly understood. To study the effect of kilohertz-frequency electric field stimulation (kHz-FS) on neuronal membranes, we measured membrane parameters and responses of single hippocampal CA1 pyramidal neurons to kHz-FS in submerged slices. We aimed to reveal the neural activity during kHz-FS, whereas most of the published studies consider post-effects of kHz-FS. A slice was placed in the chamber (~1 ml volume) and continuously perfused with ACSF (saturated with carbogen, O_2 95% + CO_2 5%) at the rate 3–5 ml/min at room temperature. Recorded neurons were located between two 2 mm long platinum-iridium stimulating electrodes (0.1 mm diameter) separated by 1 mm touching the surface of a slice. The stimulating electrodes were parallel to the CA1 pyramidal cell layer but were beyond areas occupied by apical (*stratum radiatum* or *lacunosum moleculare*) and basal dendrites/axons (*stratum oriens* or *alveus)* of CA1 pyramidal cells; imaginary isopotential lines between electrodes did not overlap the CA3 subfield. Whole-cell recordings were performed before, during and after stimulation with a biphasic charge-balanced electric field at 2kHz, 5kHz or 10 kHz. Only one cell per slice was recorded and exposed to a few 5-10 min long kHz-FS sessions. Total 27 neurons were recorded for one hour or longer. Changes of the membrane characteristics (V_m, R_{in}, R_h and in some cases SpFR - spontaneous firing rate) during kHz-FS were always abrupt, repeatable, significant ($p>0.95$) and dependent on an amplitude and/or frequency of kHz-FS. Pyramidal neurons demonstrated excitatory responses at all frequencies during kHz-field stimulation. It was found that the rheobase only decreased and spontaneous firing either was initiated in silent neurons or became more intense in previously spontaneously active neurons. Response thresholds were in the range of 0.5-2 mA and were higher at higher frequencies. Overall, the largest percentage of neurons responding to stimulation intensity lower than 2 mA occurred during 2 kHz-FS (100%), whereas this percentage was the lowest at 10 kHz-FS (18%) and intermediate at 5 kHz-FS (45%). Blockade of glutamatergic synaptic transmission did not alter the magnitude of responses. We conclude that a kHz-frequency current applied in brain tissue has an excitatory effect on pyramidal neurons during stimulation, which could be involved in the kHz stimulation-induced improvement of neurological disorders.

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Poster

332. Electrical Stimulation: Animal Models

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Boston University Center for Systems Neuroscience

Title: Calcium imaging analysis of the cellular mechanisms of deep brain stimulation

Authors: *C. RAVASIO, K. KONDABOLU, S. ZHOU, E. LOWET, R. MOUNT, X. HAN; Biomed. Engin., Boston Univ., Boston, MA

Abstract: Deep Brain Stimulation (DBS) is an effective treatment for motor disorders like Parkinson's disease, dystonia, and essential tremor, and preclinical studies show promising neuroprotective effects for the treatment of Alzheimer's Disease (AD). However, the underlying therapeutic mechanism is largely unknown. This knowledge gap leads to varied responsiveness in patients. Clinically, DBS is typically delivered at high frequencies of 130-200Hz, as lower frequency stimulations produce no consistent therapeutic effects. To understand the cellular and network effect of DBS, we performed large scale calcium imaging analysis in awake mice while delivering DBS at 40Hz or 140Hz. We analyzed individual neurons expressing the genetically encoded calcium sensor GCaMP7f in the motor cortex and the hippocampus throughout 5-second-long electrical stimulation delivered via a locally implanted DBS electrode. We found that calcium activity in a large fraction of neurons was significantly modulated, activated or inhibited, by DBS delivered at either 40Hz or 140Hz in both the motor cortex and the hippocampus. Most of the modulated neurons exhibited significant inhibition or a reduction in their intracellular calcium concentration by DBS throughout the 5 second stimulation period. Interestingly, the neurons that are inhibited by DBS often showed an initial transient activation within the first second of DBS onset, which was followed by a profound ramping inhibition which reaches its peak a couple of seconds later. Together, our results demonstrated that even though DBS creates transient neuronal activation within the first second of stimulation onset, sustained DBS creates prominent neuronal inhibition in both the hippocampus and the motor cortex regardless of stimulation frequency. While DBS delivered at 40Hz and140Hz has been suggested to have distinct therapeutic benefit, the fact that both stimulation patterns create prominent local neuron inhibition highlights that therapeutic DBS relies on additional network mechanisms in addition to inhibiting local neural activity.

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Poster

332. Electrical Stimulation: Animal Models

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Title: Deep brain stimulation creates information lesion through membrane depolarization

Authors: *S. L. ZHOU, E. LOWET, K. KONDABOLU, R. MOUNT, C. RAVASIO, Y. WANG, X. HAN;
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Abstract: Deep brain stimulation (DBS) is a promising neuromodulation therapy administered through direct electrical stimulation of brain tissue via intracranial electrodes in patients. However, the neurophysiological mechanisms of DBS remain largely unknown. Here, we performed high-speed membrane voltage fluorescence imaging of individual hippocampal CA1 neurons expressing the genetically encoded voltage indicator SomArchon, while delivering DBS in awake mice. Because fluorescence imaging is free of electrical interference, we were able to detect concomitant membrane voltage changes during DBS. We found that DBS, delivered at either 40Hz or 140Hz, reliably and rapidly depolarized somatic membrane potentials. Further, DBS enhanced spike rates and paced subthreshold membrane voltage and spike timing at the stimulation frequency, though more prominent at 40Hz than 140Hz. To determine how DBS induced membrane voltage depolarization impacts a neuron's ability to process inputs, we delivered 40Hz or 140Hz DBS in conjunction with 8Hz optogenetic CoChR activation. We found that DBS disrupted neurons responding to optogenetic stimulation, leading to a reduction in optogenetically evoked spike rate, subthreshold membrane voltage power, and spike entrainment. These effects lasted beyond the stimulation period, particularly for 140Hz DBS. Finally, the suppression of optogenetically evoked responses was found to be correlated with the strength of DBS induced membrane depolarization, highlighting a key role of membrane depolarization in mediating the cellular effects of DBS. Together, these results demonstrate that DBS produces powerful membrane depolarization that interferes with neurons' ability to process inputs thereby creating neural circuit information lesion.

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Poster

332. Electrical Stimulation: Animal Models

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Topic: I.08. Methods to Modulate Neural Activity

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Title: Neuromodulatory intervention using MRI-compatible carbon electrodes in a self-administration model of alcohol disorder in Wistar rats.

Authors: *A. LOPEZ-CASTRO[1], D. ANGELES-VALDEZ[1,2], G. ROJAS-PILONI[1], E. A. GARZA-VILLARREAL[1];
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Abstract: *Background:* Neuromodulation interventions as a complementary treatment for neuropsychiatric conditions have increased over the years. Alcohol use disorder (AUD) is a neuropsychiatric condition in which neuromodulation is not yet approved, although it can induce neural plasticity in the frontostriatal system, increasing the days of abstinence and decreasing the amount of alcohol use. Preclinical rodent models of AUD self-administration may help us to know the effects of neuromodulation therapies in different pathologies, as well as the neural mechanisms behind the positive effects. Deep Brain Stimulation (DBS) is a neuromodulation technique with implanted electrodes, which would benefit from the use of Magnetic Resonance Imagen (MRI) to study the longitudinal effects and mechanisms of stimulation as well as novel targets. This work describes chronic, MRI-compatible, carbon electrode implantation, and the use of focal electrical stimulation on a preclinical model of AUD with a longitudinal follow-up. *Methods:* Twelve adults (male n = 6) and (female n = 6) Wistar rats (Rattus norvegicus albinus) received 20 sessions of the Intermittent Access Two Bottle Choice (IA2BC) self-administration protocol and after 10 days of abstinence were randomly divided into sham (placebo) (n = 6) and active (n = 6) stimulation groups. The stimulation protocol consisted of ~10 sessions of high-frequency (20 Hz) focal electrical stimulation protocol on Area 32 (PrL) according to the Paxinos and Watson atlas. Upon reinstitution of IA2BC, intake measures were obtained to determine the effect of stimulation. *Results:* Our results suggest the possibility of a decrease in alcohol consumption after the stimulation protocol. We obtained that 66% of the rats in the stimulated/active group had a positive effect of the stimulation/surgery, while 50% of the rats in the sham group also showed a reduction in alcohol consumption. *Conclusion:* We propose that this technique can also be used to investigate the neurophysiological basis of neuromodulatory treatments in other preclinical models of substance use disorders (SUD).

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Poster

332. Electrical Stimulation: Animal Models

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Program #/Poster #: 332.07

Topic: I.08. Methods to Modulate Neural Activity

Title: Antidepressant-like effects of transcutaneous auricular vagus nerve stimulation in an animal model of depression

Authors: *P. S. CHONG, S. C. CHAU, Y. LIU, C. H. POON, M. L. FUNG, L. W. LIM; Sch. of Biomed. Sci., The Univ. of Hong Kong, Pokfulam, Hong Kong

Abstract: Depression is a highly prevalent psychiatric disorder and is one of the top leading cause of disability worldwide. Approximately 20%-30% of patients are refractory to pharmacotherapy, and the possible complicated pathophysiology could be due to dysfunction of the monoaminergic neurotransmission and the hypothalamic-pituitary-adrenal axis. Thus, an alternative therapeutic strategy is needed to target multiple depression-related mechanisms for improving the treatment efficiency. Transcutaneous auricular vagus nerve stimulation (taVNS) is a novel and promising non-invasive technique for treating depression. Here, we investigated the antidepressant-like activities of taVNS and its potential mechanisms related to the hippocampal neuroprotection, neurotransmission, and regulation of hypothalamic-pituitary-adrenal axis in chronic restraint stress (CRS) mouse model of depression. 10-weeks-old male C57BL/6J were subjected to 6 h daily restraint stress for 3 weeks. Animals with taVNS (n=8-10/group) received 30 min of taVNS at stimulation parameters (frequency: 20 Hz; pulse width: 300 μs; amplitudes: 250, 500 or 1000 μA) for 3 weeks, while the taVNS-sham received no electrical stimulation. CRS-mice (n=10) were intraperitoneally injected with vortioxetine for 3 weeks daily to compare its antidepressant-like effects with taVNS. All groups were compared to non-CRS-sham group to validate the CRS model and to examine the treatment effects in rescuing the depressive-like symptoms induced by CRS. Behavioral tests were conducted after 3 weeks of taVNS and we found that 250 μA taVNS induced remarkable anxiolytic and antidepressant-like effects in CRS mouse model. Strikingly, we demonstrated that taVNS reduced the levels of corticosterone and adrenocorticotropic hormones, and these findings were further supported by normalization of levels of various monoamine neurotransmitters (e.g., dopamine, serotonin and norepinephrine) and metabolites related to essential amino acid and glucose metabolism functions. These results suggest that taVNS could potentially alleviate depression through regulating the hypothalamic-pituitary-adrenal axis, neurotransmission, and specific metabolic signaling.

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Poster

332. Electrical Stimulation: Animal Models

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Title: Differential activation of Paraventricular Nucleus neurons using select paradigms of vagus nerve stimulation

Authors: *E. BEAUMONT[1], M. OWENS[1], A. FARRAND[1], M. ZAHNER[2];
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Abstract: The paraventricular nucleus of the hypothalamus (PVN) is known to be a key regulator of homeostasis, contributing to neuroendocrine output and controlling autonomic nervous system responses to numerous visceral functions. Vagus Nerve Stimulation (VNS) is used as a treatment for heart failure and for several neurological conditions including epilepsy and depression. The PVN contributes to the results of VNS in the brain and is a key regulator of autonomic control, but the vagus stimulation parameters that are the most advantageous have yet to be determined. Thus, this study evaluates neuronal responses from 11 VNS paradigms using standard frequencies (5-30 Hz) and bursting frequencies (250-350 Hz). The stimulation paradigms consisted of 5 consecutive cycles that were delivered randomly to chloralose-anesthetized rats (n=6) through a bipolar cuff electrode attached to the left cervical vagus nerve. The activity of single PVN neurons was recorded via stereotaxically-placed, high-impedance tungsten electrodes. Recorded neurons (n=30) were classified as non-responders, negative responders, or positive responders based upon changes in firing rate during VNS, compared to their baseline level. Interestingly, the first cycle of VNS did not change neuronal activity in all neurons in PVN, but the maximum response over 5 VNS cycles indicated that 33% of PVN neurons responded to VNS ($p<0.05$). The VNS settings that best targeted PVN neurons were the standard frequencies 20 and 30 Hz and also a burst setting at 300Hz ($p<0.05$). Moreover, these results indicate that the VNS parameters that are the most effective in activating higher order PVN neurons are also the most effective in activating brainstem neurons (Locus Coeruleus and Nucleus of the solitary tract). In conclusion, functionally understanding the neuronal interaction between the vagal afferents up to PVN neurons during vagal nerve stimulation is critical in optimizing VNS therapy. Once properly understood, VNS can mechanistically be used to mitigate the progression of chronic diseases like epilepsy, heart failure and depression.

Disclosures: E. Beaumont: None. **M. Owens:** None. **A. Farrand:** None. **M. Zahner:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.09

Topic: I.08. Methods to Modulate Neural Activity

Support: FWO-project grant G0B4520N

Title: Enhancement of passive avoidance learning with tDCS in rats: a cerebral or peripheral mechanism?

Authors: *L. VAN BOEKHOLDT, S. KERSTENS, M. MC LAUGHLIN;
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Abstract: Transcranial direct current stimulation (tDCS) is widely used by neuroscientists and clinicians to modulate brain activity and treat a wide range of disorders. It is assumed that effects are achieved through direct membrane polarization of neurons, but recent research suggests that peripheral nerve activation may be an alternative mode of action. On a level of cognition and learning, evidence of tDCS effects is scarce, but a notable exception is the effect of tDCS on passive avoidance (PA) learning in rats. Two different groups have reported that 30 minutes of 0.25mA DC stimulation enhances PA learning. In these experiments, the anodal electrode was placed on the skull (over hippocampus), while the cathodal electrode was positioned on the chest. We set out to investigate whether this increased PA learning was caused by the cerebral or peripheral electric field. For brain stimulation, we implanted disc electrodes on the skull (over hippocampus and cerebellum), while peripheral stimulation was achieved with subcutaneous carbon electrodes in the neck (around occipital nerve) and posterior back. Four groups of rats (n=14 per group) were used: skull-only, skin-only, skull-skin, and sham stimulation. In the skull-skin condition with one electrode over the skull (over hippocampus) and one in the neck (around occipital nerve), there is an electric field in both the brain and the skin. Skull-only and skin-only stimulation conditions separate these fields by generating electric fields exclusively in the brain or skin. After 30 minutes of 0.25mA (or sham) stimulation, the PA task was performed: rats were placed in a light room, and received an electric shock when entering a connected dark room. 24 hours later, rats were placed in the light room again, and the latency to enter the dark room was recorded. Preliminary analysis (n=10 at time of writing) showed increased latency of all groups between training and testing days ($p < 0.0001$). Moreover, preliminary results showed a trend of increased learning in skull-only and skull-skin stimulation groups, compared to sham group (average time difference (seconds) of testing day - training day: sham 95.2; skull-skin 142.7; skull-only 164.2; skin-only 80.8). After all testing is completed, data analysis will reveal whether these differences are significant. If so, this would provide evidence that 0.25mA DC stimulation enhances PA learning in rats via direct effects on the brain. It should be noted that, compared to human tDCS, the used 0.25mA generates a much higher cerebral, but lower peripheral electric field. Future experiments are needed to investigate the effect of electric field amplitudes closer to those used in humans on learning.

Disclosures: L. van Boekholdt: None. **S. Kerstens:** None. **M. Mc Laughlin:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.10

Topic: I.08. Methods to Modulate Neural Activity

Support: DAPRA Grant N65236-19-C-8017

Title: Focused steerable non-invasive brain stimulation using ultra high density pulsed electrical stimulation (uHD-PES) in rodent model

Authors: *V. JAIN, M. FORSSELL, D. Z. TANSEL, C. GOSWAMI, G. K. FEDDER, P. GROVER, M. CHAMANZAR;
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Abstract: Objective: Electrical stimulation mediated neuromodulation has been widely used for mitigation of different neurological disorders. Non-invasive transcranial electrical stimulation has gained much attention in the last couple of decades due to its potential therapeutic benefits. However, this method lacks high spatial resolution for targeted brain stimulation. One of the reasons for this shortcoming is that large electrodes (> 1 cm) are typically used to deliver electrical currents. In the present study we demonstrate a novel stimulation approach to focus and steer currents within the brain using ultra-high-density pulsed electrical stimulation (uHD-PES). **Methods**: A high-density array of flexible surface electrodes was placed on mice skull above the motor cortex and pulses of currents were injected to elicit neural activity followed by a behavioral response. Motor evoked potentials (MEPs) in forelimbs were measured using bipolar needle electrodes and multi-unit activity (MUA) in brain was measured using a linear microelectrode array with 64 channels covering the entire cortical column. A confined stimulation volume was achieved by injecting a specific pattern of currents through individual surface electrodes. **Results**: The results show uHD-PES approach evokes preferential contralateral MEP responses when steered to target the motor cortex in either hemisphere. Steerability was demonstrated using intracortical recordings that show uHD-PES can evoke neural response at sub-millimeter resolution. Steerability and focality were further validated by *c-fos* immunostaining as a marker of neuronal activation which shows high correlation between shifting of cluster of *c-fos* positive cells with stimulation focus. **Conclusions**: These findings demonstrate the potential of uHD-PES for non-invasive electrical stimulation of the intact brain for chronic studies in experimental models. In the future, this approach could be extended to clinical applications as a therapeutic for different neurological disorders by providing high resolution targeted stimulation of brain regions involved in pathophysiology.

Disclosures: V. Jain: None. **M. Forssell:** None. **D.Z. Tansel:** None. **C. Goswami:** None. **G.K. Fedder:** None. **P. Grover:** None. **M. Chamanzar:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.11

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH U24NS113647-01

Title: A generic Parylene microelectrode array for brain recording in behaving animals

Authors: *H. XU, K. SCHOLTEN, W. JIANG, Z. LU, E. MENG, D. SONG;
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Abstract: Long-term neural recording with high spatio-temporal resolution from behaving animals is crucial for studying brain functions. However, localized brain tissue inflammation often impairs recording yield in implanted devices over time due to the stiffness mismatch between brain tissue and neural implant. Microelectrodes arrays (MEAs) constructed with flexible polymers can greatly reduce such mismatch and mitigate the immune responses to implanted devices. This advantage renders polymer MEAs (pMEAs) a promising candidate for chronic monitoring of neuronal activities. Unfortunately, access to pMEAs is limited to a small number of groups that have the capability of fabricating their own devices. To promote the development of pMEAs and provide researchers an easy access to pMEAs, the Polymer Implantable Electrode (PIE) Foundry was launched under the support of NIH. The PIE Foundry is a shared resource center dedicated to the standardization and customization of pMEAs for the neuroscience and neuroengineering community. In the PIE Foundry, a standardized penetrating pMEA with a generic layout was developed for recording from rodent brains. In this generic pMEA, 64 electrodes (15 or 30 μm diameters) were evenly distributed on the tips of four shanks (5 mm long). With 500 μm spacing between shanks and 120 μm spacing between electrodes, this pMEA can span large brain areas such as multiple sub-regions of the hippocampus or multiple layers of the cortex. The pMEA was dip-coated with polyethylene glycol (PEG) and implanted into the rat brain. Both spiking activities and local field potentials were recorded in the hippocampus and the somatosensory cortex of anesthetized rats. Over 40 units were recorded in the hippocampus. Hippocampal spikes were recorded with high signal-to-noise ratio (greater than 3) for over two months. These preliminary evaluations indicate that this generic pMEA has the potential to be used as a standard tool for chronic recording of the rodent brain.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.12

Topic: I.08. Methods to Modulate Neural Activity

Title: A 64-Channel Multi-Shank Silicon Probe with High Stiffness-to-Volume Ratio for Exploring Hard-to-Reach Regions of the Rat Brain

Authors: *B. ROSTAMI[1], V. HETRICK[2], O. J. AHMED[2], K. NAJAFI[1];
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Abstract: Neural probes with high electrode density and count are necessary for studying neuronal circuits on a large scale with high resolution. In conventional planar probes, adding more recording sites to a single probe shank requires larger shank widths, resulting in larger volume, added tissue displacement, and long-term damage. One of the key challenges for chronic experiments utilizing implanted probes is neuronal loss and scar formation as a result of larger probe shank dimensions. Reducing probe dimensions while maintaining a large electrode count poses several technical challenges. The probe shank needs to be narrow to minimize tissue damage but accommodate desired number of electrodes. Its thickness should be small, yet large enough to provide sufficient mechanical stiffness to allow easy tissue penetration with minimal dimpling. Materials need to be compatible with long-term implantation and standard microfabrication techniques. Ideally, probe shanks should have sufficient stiffness to precisely reach the targeted region. Probe shanks also need to have sufficient compliance after insertion to minimize trauma to the surrounding tissue due to constant brain motion. In this research, we have developed a device structure and fabrication approach that provides narrow shank widths, reduces shank stiffness-to-volume ratio (SVR), maintains the ability to maximize mechanical stiffness, and improves compliance. Each probe shank can have a custom-designed cross-section as a T-bar or a Pi-bar, with independent control over the width and height. We have developed and fabricated a four-shank silicon probe with 64 sites suitable for high-resolution extracellular studies. The four planar silicon shanks and their tips are angled depending on the size and geometry of the targeted region. For example, four shanks, each supporting 16 electrodes, are arranged at a 45° angle to study midline regions with co-planar targeting of specific layers. These shanks are 4.5-5 mm long, only 43 µm wide, 3 µm thick, and contain one or two vertical stiffeners that are 4 µm wide and 10 µm tall. Toward the tip, the stiffener's width narrows, leaving a sharp tip for easier insertion. Each shank is only 170-210 μm^2 in cross sectional area, which is <50% of the volume of a probe with uniform thickness. An integrated Parylene-C ribbon cable, containing 64 10 µm-wide interconnect lines, connects the 4-shank probe to a small backend that is mounted on the skull and wire-bonded to a percutaneous connector. In this work, we present super-fine planar probes that are capable of penetrating with minimal brain tissue damage and provide a high SVR for high-resolution extracellular recordings and stimulations.

Disclosures: **B. Rostami:** A. Employment/Salary (full or part-time):; University of Michigan. **V. Hetrick:** A. Employment/Salary (full or part-time):; University of Michigan. **O.J. Ahmed:** A. Employment/Salary (full or part-time):; University of Michigan. **K. Najafi:** A. Employment/Salary (full or part-time):; University of Michigan.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.13

Topic: I.08. Methods to Modulate Neural Activity

Support: Abbott Neuromodulation

Title: Assessing Thresholds for Activation of the Medial and Lateral Pain Pathways in a Rodent Model of Spinal Cord Stimulation

Authors: Y. YOUN[1], B. S. COVENTRY[2], **J. K. TREVATHAN**[2], B. KNUDSEN[2], A. BRZECZKOWSKI[1], K. CHENG[2], H.-J. PARK[3], W. LAKE[1], K. A. LUDWIG[2], E. K. ROSS[3], A. J. SUMINSKI[1];
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Abstract: Neuromodulation approaches like spinal cord stimulation (SCS) are increasingly being applied as alternatives to the traditional pharmacologic treatments of chronic pain. Electrical stimulation of the spinal cord is most often applied through epidural electrodes. Traditional tonic stimulation waveforms activate the dorsal column sensory axons, and thus induces paresthesia. On the contrary, burst stimulation waveforms (i.e. BurstDR) do not cause paresthesia, and shows superior pain migitation effect compared to tonic stimulation waveform. Unfortunately, the exact mechanism of action for paresthesia free SCS paradigms is not well understood. One important distinction between SCS methodologies may be lie in differences in activity evoked within the lateral and medial pain pathways which encode the discriminatory and motivation/affective/attentional aspects of pain, respectively. Here, we present a combination of stimulation and recording methods that enable an interrogation of the effects of different SCS paradigms on the medial and lateral pain pathways based on evoked activity in the cortex. In urethane anesthetized Long Evans rats, we implanted two-contact paddle electrodes for the application of SCS at the T13/L1 and L4/L5 spinal levels. Additionally, we implanted a 2 shank, 32 contact microelectrode array (NeuroNexus; Ann Arbor, MI) in the prefrontal cortex (PFC) and anterior cingulate cortex (ACC), the site of cortical projections from the medial pain pathway. We also implanted a custom 16 channel μECoG array on the dorsal surface of the somatosensory cortex, the site of cortical projections from the lateral pain pathway. Lastly, we utilized EMG of the tail extensors to determine thresholds for motor activity elicited by stimulation as an important indicator of side-effect. Evoked cortical potentials were observed in both the PFC/ACC and the somatosensory cortex and their magnitude varied with changes in stimulation amplitude. This preliminary data shows proof-of-concept for the development of a high through-put method for assessing differences in thresholds for activation in the medial and lateral pain pathways in response to different simulation paradigms. The ability to combine target measures from multiple cortical areas with measures of side-effects could be an important paradigm for understanding the differences in therapeutic effect between traditional tonic stimulation and new ‘paresthesia-free’ stimulation paradigms that are being applied in clinical practice.

Disclosures: Y. Youn: None. **B.S. Coventry:** None. **J.K. Trevathan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neuronoff Inc.. **B. Knudsen:** None. **A. Brzeczkowski:** None. **K. Cheng:** None. **H. Park:** A. Employment/Salary (full or part-time):; Abbott Neuromodulation. **W. Lake:** None. **K.A. Ludwig:** 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.; Abbott Neuromodulation. F. Consulting Fees (e.g., advisory boards);

Abbott Neuromodulation. **E.K. Ross:** A. Employment/Salary (full or part-time):; Abbott Neuromodulation. **A.J. Suminski:** 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.; Abbott Neuromodulation.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.14

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1R43DK122846-01

Title: Novel Bio-electronic Dual Neuromodulation of Vagal Celiac Fibers and Vagal Hepatic Fibers Demonstrated a Decrease in Fasting Plasma Glucose in an Alloxan Induced Type 2 Diabetes Swine Model

Authors: R. R. NIHALANI[1], ***J. WAATAJA**[1], C. J. BILLINGTON[2];
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Abstract: Despite diet modifications, medication and surgery; type 2 diabetes mellitus (T2DM) remain a challenge to treat effectively. Modulation of nerves innervating organs that regulate plasma glucose (PG) may be a novel method for treating T2DM. Standalone vagotomy/treatments and stimulation of the Vagus nerve has mixed/undesirable results. Dual Vagus neuromodulation consisting of stimulation and block is a new therapeutic concept that we have successfully tested in animal models of T2DM. The neuromodulation comprises of stimulation (1 Hz) of celiac fibers innervating the pancreas w/ simultaneous reversible electrical blockade of neuronal hepatic fibers innervating the liver, by applying a 5000 Hz alternating current. Previously we have studied area under the curve of oral glucose tolerance tests (OGTTs) to calculate glycemic control. However, measuring fasting plasma glucose (FPG) is another method of accessing glycemic control. In this study a male alloxan induced swine model of type 2 diabetes (n=4) was utilized to determine if stimulation and block had effects on FPG. We observed a sustained decrease in FPG following 4 hrs applications of simultaneous stimulation and block. For the 3 consecutive post implant control experiments FPG remained constant between experiments, with an average of 147±45 mg/dL. Results following the 1st application of 4 hrs of stimulation and block plasma glucose decreased to 68±5 mg/dL. Interestingly, following 2 days after the cessation of stimulation and block the FPG remained depressed, with an average of 71±6 mg/dL. The depression of FPG compared to control was consistent and significantly different (ANOVA $p<0.05$) throughout six 4 hr applications of stimulation and block with 2 days washout between experiments (i.e. there was not a steady decrease in FPG over time) with the last FPG measuring at an average of 71±4 mg/dL. No hypoglycemia was observed. This finding of lasting plasticity is interesting to us and we believe that this effect may further enhance the

therapeutic benefit of our stimulation and block technique to be used as a bio-electronic device for a treatment of type 2 diabetes.

Disclosures: **R.R. Nihalani:** A. Employment/Salary (full or part-time):; ReShape Lifescinces Inc., Full-time. **J. Waataja:** A. Employment/Salary (full or part-time):; ReShape Lifescinces Inc./Full-time. **C.J. Billington:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 332.15

Topic: I.08. Methods to Modulate Neural Activity

Support: Air Force Office of Scientific Research grant 20RHCOR04
DARPA HIRT (FA8650-14-D-6500)

Title: Vagus nerve stimulation paired with training enhances recognition memory

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Abstract: Recognition memory, a fundamental component of cognitive health, suffers when biological limitations are reached due to stress, ageing, or neurodegenerative disease. FDA approved vagus nerve stimulation (VNS) has been reported to enhance multiple elements of cognition (including arousal, attention, and decision-making), but knowledge is limited regarding the efficacy of VNS to improve recognition memory. This study investigated the ability of VNS paired training to enhance recognition memory in healthy adults and rodents. All human work was approved under an IRB protocol. Healthy human subjects (N = 44, mean age = 26 ± 6 years) were randomly assigned to sham or non-invasive cervical transcutaneous VNS (ctVNS). To determine the cognitive effects of ctVNS paired training (25 Hz for 2 min per side before and after training), all participants underwent training and testing on a target search/change detection task using Synthetic Aperture Radar images. Stimulation paired training (n = 22) improved target identification ($p < 0.05$ vs sham) and target change detection accuracy ($p < 0.01$ vs sham). These findings were recapitulated in a rodent model of VNS. All animal activities were approved under an IACUC protocol. Healthy male Sprague-Dawley rats (N = 16) were implanted with a platinum/iridium electrode cuff around the unsheathed left cervical VN. Successful implantation was confirmed using the cessation of breathing test. At 10-12 weeks of age, direct cervical VNS (administered as fifteen 100 μs biphasic pulses at 30 Hz, 0.8 mA constant current every 18

seconds for 30 minutes) was paired with the training phase of the Novel Object Recognition (NOR) task. Stimulated rats (n = 7) exhibited enhanced performance in NOR, with more time spent exploring the novel object ($p < 0.05$ vs familiar) and improved Novel Object Preference Score ($p < 0.05$ vs sham). This study establishes that VNS can enhance recognition memory in healthy adults and that this phenomenon can be recapitulated in a rodent VNS model. These results suggest that a targeted approach to VNS can mediate task specific cognitive performance and support using the rodent VNS model for mechanistic investigation. No DoD endorsement implied.

Disclosures: **L.K. Olsen:** A. Employment/Salary (full or part-time):; Oak Ridge Institute for Science and Education. **S.H. Jung:** A. Employment/Salary (full or part-time):; Oak Ridge Institute for Science and Education. **L.K. McIntire:** A. Employment/Salary (full or part-time):; Infoscitex, Inc. **A.A. McKinley:** A. Employment/Salary (full or part-time):; Air Force Research Laboratory/Applied Neuroscience Branch. **R.J. Moore:** A. Employment/Salary (full or part-time):; Infoscitex, Inc. **C.N. Hatcher-Solis:** A. Employment/Salary (full or part-time):; Air Force Research Laboratory/Applied Neuroscience Branch.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.16

Topic: I.08. Methods to Modulate Neural Activity

Support: Mayo Clinic

Title: Intravascular electrical stimulation of the vagus nerve in swine requires precise placement of stimulation electrodes: design considerations for electrodes and tools for targeting electrode position

Authors: ***E. N. NICOLAI**[1,2], J. L. ARTURO LARCO[4], S. I. MADHANI[4], I. KIM[4], S.-Y. CHANG[4], W. BRINJIKJI[4], R. KADIRVEL[4], D. F. KALLMES[4], S. J. ASIRVATHAM[4], K. A. LUDWIG[3], L. E. SAVASTANO[4], G. A. WORRELL[4];
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Abstract: Vagus nerve stimulation has been proposed, and is used clinically, for a wide variety of indications. Despite impressive outcomes across indications, on-target effects are sporadic and off-target effects occur in the majority of patients. Recent studies of vagus nerve anatomical structure suggest this is due to inhomogenous organization of the nerve fibers within the vagus and the proximity of motor nerve fibers at the cervical vagus region, respectively. In this study we investigated intravascular vagus nerve stimulation, which may allow for activation of the nerve at locations where the fibers of interest can be selectively targeted. We electrically stimulated at intravascular electrodes within the nearby internal jugular vein while recording

vagus nerve compound action potentials, neck muscle motor evoked potentials, and heart rate in a swine model of vagus nerve stimulation that we previously characterized. We found that the stimulation electrode position within the jugular vein is critical for efficient activation of the vagus nerve. We determined anatomical factors that contribute to intravascular stimulation efficacy via histology of whole carotid sheathes - vagus nerve, internal jugular vein, and carotid artery. Finally, we demonstrate use of imaging modalities including cone beam computed tomography and high frequency ultrasound to determine the position of the intravascular stimulation electrode with respect to the nerve, vein, and artery. At the most effective stimulation locations tested, thresholds for nerve activation for all measures were several times higher than direct stimulation of the nerve using a cuff electrode. Thus, while this work demonstrates feasibility of intravascular vagus nerve stimulation and provides tools to target intravascular stimulation electrode positions, additional work is required to optimize delivery of electrical stimulation across the vein wall to the nerve. Given the conductivity of blood, increased distance between the electrode and nerve, and resistance of the vein wall, we do not expect intravascular stimulation to reach the efficiency of cuff electrode stimulation, but further discussion on tradeoffs between stimulation efficiency and invasiveness are warranted.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 332.17

Topic: I.08. Methods to Modulate Neural Activity

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RIS Grant 2021RIS001
RIS Grant 2021RIS0428

Title: Alleviation of trigeminal neuralgia by optogenetic inhibition of posterior hypothalamus in CCI-ION rat with and without α-CGRP knockdown

Authors: *J. ISLAM[1], E. KC[1], K. HYUN[2], Y. Y. PARK[2], Y. S. PARK[2];
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Abstract: In trigeminal autonomic cephalalgias, the posterior hypothalamus (PH) has been found to be hyperactive, which is an important part of the descending pain processing pathway. Therefore, we aimed to ascertain whether precise modulation of PH activity by optogenetic inhibition approach could affect the outcomes of a chronic constriction injury in the infraorbital nerve (CCI-ION) rat model of trigeminal neuralgia (TN). We used female Sprague-Dawley rats and they were randomly divided into three groups: the TN, sham, and naive-control groups. CCI-ION surgery was performed to mimic TN symptoms, and either optogenetic (AAV-hSyn-eNpHR3.0-EYFP) or null virus (AAV-hSyn-EYFP) was injected into the ipsilateral PH. *In vivo* single-unit extracellular recordings were obtained from both the ipsilateral ventrolateral periaqueductal gray (vlPAG) and contralateral ventral posteromedial (VPM) thalamus in optogenetic inhibition "OFF" and "ON" conditions. Alterations in behavioral responses during the optogenetic inhibition -OFF and -ON states were also examined. Immunofluorescence staining was performed on brain and trigeminal ganglion sections to observe the expression of optogenetic virus, c-Fos, active CGRP. We observed that inhibiting PH activity in a TN animal model had an analgesic effect by modulating PH-vlPAG projections. Optogenetic inhibition of the PH considerably improved behavioral responses in optogenetic virus-injected animals. We also found increased and decreased neural activity in the vlPAG and VPM thalamus, respectively, during optogenetic inhibition of the PH. In fine, the inhibition of the PH hyperactivity attenuated trigeminal pain signal transmission in CCI-ION rat model by modulating the activity of the vlPAG and trigeminal nucleus caudalis, thereby providing evidence of the therapeutic potential of PH in TN management.

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Poster

332. Electrical Stimulation: Animal Models

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Program #/Poster #: 332.18

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH NIBIB Grant 1-U18-EB029251-01

Title: Frequency-dependent reduction of Aα/Aβ fiber recruitment during continuous dorsal root ganglion stimulation in rats

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Abstract: Chronic pain is a debilitating condition that affects approximately 100 million adults in the United States. Dorsal root ganglion stimulation (DRGS) has been shown to be an effective therapy for patients who have exhausted pharmacological options. DRGS induces paresthesias in the target dermatome, indicating recruitment of Aα/Aβ sensory neurons, but the mechanisms of action by which DRGS leads to analgesia are not well understood. One potential therapeutic mechanism of DRGS involves frequency-dependent suppression of action potentials at the T-junction of afferent fibers in the dorsal root ganglion (DRG), creating a low-pass filtering effect described as T-junction filtering. Previous studies have shown that filtering primarily affects C and Aδ fibers during DRGS; however, these small-diameter fibers have a higher threshold for activation by electrical stimulation than larger diameter Aα/Aβ fibers. In this study, we aimed to characterize afferent fiber recruitment during 180 s of continuous DRGS to explore the effects of frequency on the persistence of Aα/Aβ fiber responses to DRGS. We exposed the L5 DRG via a partial laminectomy in Sprague-Dawley rats (n=3). Trains of DRGS pulses (300 μs pulse width, current amplitude = 120% of Aα/Aβ recruitment threshold) were applied continuously at rates of 5, 20, 50, or 100 Hz for 3 minutes. We measured evoked compound action potentials (eCAPs) in the sciatic nerve using a tripolar cuff electrode. The eCAP responses were averaged during 1 s periods immediately after stimulation onset (0-1 s) and at 15 s intervals throughout each trial. The magnitude of the Aα/Aβ portion of the eCAP was measured as the area under the curve in an epoch corresponding to the Aα/Aβ response latencies. A progressive decline in eCAP amplitude occurred within 30 s of stimulation onset, decreasing exponentially at a rate that varied with stimulation frequency. The eCAP amplitudes remained depressed for the duration of the pulse train. The largest reductions in amplitude occurred with DRGS at 50 and 100 Hz, where eCAP amplitudes decreased to ~8% of baseline levels. The eCAP amplitudes were diminished to ~30% of baseline levels during 10 and 20 Hz stimulation and to ~60% during 5 Hz stimulation. Additionally, the rate of amplitude decay was higher for DRGS at frequencies of 50 and 100 Hz than for frequencies of 5, 10, and 20 Hz. Notably, the eCAP recovery time was similar across frequencies (~120 s). These results demonstrate that DRGS frequencies influence the stability of Aα/Aβ fiber recruitment and are consistent with frequency-dependent filtering effects previously observed in small-diameter afferent fibers.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.19

Topic: I.08. Methods to Modulate Neural Activity

Support: Swiss National Science Foundation DOC.Mobility Grant P1FRP3_188027

Title: Amplitude modulated high-frequency stimulation elicits asynchronous axonal spiking activity

Authors: B. BARRA[1], R. KUMAR[2,1], C. GOPINATH[1,3], E. MIRZAHKALILI[5,6], S. F. LEMPKA[5,6,7], R. A. GAUNT[1,2,3], M. CAPOGROSSO[1,2,4], ***L. FISHER**[1,2,3];
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Abstract: Regaining sensory feedback is a priority for people with limb amputation, and electrical stimulation of the peripheral nerves can produce sensory percepts that feel like they emanate from the missing limb. However, subjects often describe these sensations as unnatural. Traditional electrical stimulation paradigms activate neural structures using charge-balanced rectangular pulses at frequencies lower than 100 Hz. This simplistic approach elicits synchronous responses in all recruited fibers raising the possibility that this unnatural synchronicity is responsible for the unnatural sensory percepts that occur in amputees (e.g. tingling). Recently, Formento et al. proposed that amplitude modulated high-frequency trains of square pulses (BioS) could cause asynchronous firing of axons. However, the biophysical mechanisms behind this effect are not understood and the efficacy of this technique in realistic geometries has not been investigated. Here, we combined computational modeling and in-vivo recordings in an anesthetized cat, to dissect how amplitude modulation, high-frequency and waveform shape modulate neural activity. First, we modeled a set of parallel multicompartmental axons and inserted them in an infinite homogeneous space, where two ideal current sources were inserted parallel to the axons. We computed the responses of the modeled axons to stimulation using the NEURON simulation environment. Second, we validated the computational results by stimulating the sciatic nerve of an anesthetized cat with a multi-contact cuff, while recording with two micro-electrode arrays (Blackrock Microsystems, Utah) implanted in the L6 and L7 dorsal root ganglia (DRG). We investigated the firing patterns produced by three different stimulation waveforms: (1) BioS, (2) an amplitude modulated high-frequency sinusoidal waveform (AMHFS) and (3) a simple high-frequency sinusoid. The use of amplitude modulation, high frequency, and sinusoidal waveforms all moderately affected ion-channel behavior towards the achievement of asynchronous firing. However, the AMHFS waveform, which combined amplitude modulation, high-frequency and a sinusoidal waveform was the most effective at causing asynchronous firing of primary afferent in the DRG. These results represent an important step towards the development of biomimetic stimulation strategies that could be applied for the restoration of sensory feedback.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.20

Topic: B.04. Synaptic Transmission

Support: NIH Grant P20GM103650

Title: Ultrashort nanosecond electric pulses: a novel stimulus for modulating peripheral nerve activity

Authors: F. HOSSAIN[1], V. MANSUROV[1], D. HEREDIA[2], T. GOULD[2], ***J. ZAKLIT**[1]; [1]Dept. of Electrical and Biomed. Engin., Univ. of Nevada, Reno, Reno, NV; [2]Dept. of Physiol. and Cell Biol., Univ. of Nevada, Reno Sch. of Med., Reno, NV

Abstract: Electroceutical stimulation has gained attention as a therapeutic for conditions in which conventional pharmaceutical therapies are ineffective or not tolerated by patients. Pulsed power technologies have produced a new type of electroceutical stimulus, nanosecond-duration electric pulses (NEPs). Because of their potential to achieve non-invasive, spatially targeted excitation, NEPs represent an emerging alternative to ms- and µs-duration electric pulses presently in clinical use. For example, in patients with respiratory dysfunction, implanted conventional electrodes target the phrenic nerve to stimulate the diaphragm. Therefore, using an *ex vivo* neuromuscular preparation consisting of the phrenic nerve and diaphragm of mice expressing the genetically-encoded Ca^{2+} indicator GCaMP6f in muscle, we compared the effects of nerve stimulation with NEPs vs. conventional-duration EPs (CEPs) on intramuscular Ca^{2+} transient responses and muscle fiber shortening. First, using a suction electrode designed and fabricated in our laboratory, we identified the threshold at which individual NEPs excite the phrenic nerve (1.5±0.6 kV for 40 ns pulse-width, 1.1±0.4 kV for 60 ns, and 0.9±0.3 kV for 80 ns; $n = 3$ male). Similar to previous studies of NEPs delivered by wire electrodes to excitable cells, we observed that as pulse duration increased, the electric field amplitude required to evoke a diaphragm contraction and intramuscular Ca^{2+} transient decreased. Next, we compared the muscle response to high-frequency stimulation (HFS) trains of NEPs and CEPs delivered to the phrenic nerve. We found that trains of NEPs (20 ns pulse-width) and CEPs (0.3 ms pulse-width) applied at 20 Hz for 30 s evoked contractions whose peak shortening and fatiguability were similar (peak shortening = 612±156 µm vs. 746±58 µm, for NEPs vs. CEPs, respectively; $p > 0.05$; $n = 3$ male). These results demonstrate that stimulation of the phrenic nerve with NEPs is as effective as CEPs in stimulating the phrenic nerve. Therefore, these studies open the door to the development of NEPs as a non-invasive mode of electroceutical stimulation of peripheral nerves.

Disclosures: F. Hossain: None. **V. Mansurov:** None. **D. Heredia:** None. **T. Gould:** None. **J. Zaklit:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.21

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 5T32GM108539-08

Title: Wireless, battery-free, fully implantable electrical spinal cord neuromodulation in freely moving rats

Authors: *A. J. WIDMAN[1], A. BURTON[2], T. BASHAR[2], D. MARSHALL CLAUSEN[2], D. K. WOLF[1], J. FOLARIN-HINES[1], M. PAYNE[1], K. MEACHAM[1], J. A. ROGERS[3], P. GUTRUF[2], R. W. GEREAU, IV[1];
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Abstract: Electrical stimulators implanted in the spinal cord are a valuable treatment option for people living with chronic pain. Traditional spinal cord stimulation (SCS) is given at 50Hz frequency over the affected area of the spinal cord and produces paresthesia. In recent years, novel stimulation paradigms have been deployed for spinal cord stimulation, and some of these are reported to show significant analgesia without unpleasant paresthesia. While SCS shows great promise to help many patients, there are some challenges for long-term use in patients. For example, for many patients, efficacy of chronic SCS for pain is limited due to habituation over time. Furthermore, the mechanisms by which the various stimulation parameters produce analgesia are not well understood, hampering attempts to make improvements. Mechanistic studies in small animals on chronic stimulation are hindered by tethered systems that restrict animal movement and battery systems that require interruptions in stimulation to recharge the system. To circumvent these challenges, we created a fully implantable, wireless, battery-free device capable of delivering SCS in rodents with stimulation parameters, including a wide range of frequencies, controlled in real time. These devices implanted into the spinal epidural space do not have significant changes in gait implying there are no gross motor impairments, and minimal tissue damage is observed in histological studies suggesting the devices are well-tolerated and minimally invasive. Using constant voltage biphasic pulses, motor responses were elicited at low frequencies. Additionally, the device significantly decreased spared nerve injury hyperalgesia with 60 minutes of 50Hz frequency SCS replicating previous findings with tethered systems and providing evidence for potential use of these wireless devices in SCS research with a variety of behavior applications.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.22

Topic: I.08. Methods to Modulate Neural Activity

Support: EBT Medical Inc.

Title: The Bladder Inhibitory Effects of Saphenous Nerve Stimulation are Mediated via a Supraspinal Pathway in Anesthetized Rodents

Authors: *G. GRUENSPAN[1,3], J. WANG[4], L. HACHEM[4], J. ZARIFFA[1,3], M. FEHLINGS[4], P. YOO[1,3,2];
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Abstract: Electrical stimulation of the saphenous nerve (SN) is a novel therapeutic approach aimed at treating patients with idiopathic overactive bladder (OAB). Preclinical studies in rats have shown that electrical stimulation of this purely sensory nerve can elicit bladder inhibitory responses characterized as (1) a decrease in bladder contraction rate (BCR) and (2) overflow incontinence (OI) episodes [1,2]. Percutaneous SN stimulation in a feasibility study involving OAB patients showed significant improvement in bladder symptoms along with clinically meaningful improvements in quality-of-life measures [3]. The goal of this study was to better understand the mechanism of action involved in the neuromodulatory effects of SN stimulation. Non-survival experiments were performed in Long Evans rats, which were separated into a control group (healthy, 246 ± 2 g, n = 10) and a chronic spinal cord injury (SCI) group (complete transection at T7, 274 ± 24 g, n = 14). Under urethane anesthesia, each animal was instrumented with a suprapubic catheter to provide continuous infusion of saline (rate = 0.1 ml/min). A pair of stainless steel wires were inserted paraurethrally to target the urethral sphincter muscle and a bipolar nerve cuff electrode was implanted on the surgically dissected SN. Once stable reflex bladder contractions were confirmed (baseline), SN stimulation was applied intermittently in 40 min cycles (30 Min ON + 10 min OFF) and changes in bladder function were recorded. Reflex bladder contractions were achieved in 10 control and 10 SCI animals during baseline. Repeated SN stimulation elicited OI in 60% of experiments, along with a 7% decrease in BCR in the control (healthy) animals (stimulation vs. baseline, t-test, $P<0.05$). In contrast, SN stimulation was unable to elicit OI episodes in chronic SCI rats and stimulation also failed to elicit any significant changes in BCR (stimulation vs. baseline, t-test, $P=0.22$). The results of this study provide first preclinical evidence that the bladder inhibitory effects of SN stimulation involve a supraspinal mechanistic pathway in rodents, therefore suggesting a functional connection between the lumbar spinal cord and the brainstem (e.g., pontine micturition center). 1. Moazzam Z, Yoo PB,. *Neurourol Urodyn*. 2018;37(2):592-599. 2. Franz KS, Yoo PB. *Auton Neurosci*

Basic Clin. 2020: https://doi.org/10.1016/j.autneu.2020.102672 3. MacDiarmid SA, John MS, Yoo PB. *Neurourol Urodyn*. 2018;37(5):1815-1820. **Funding:** Research Contract - EBT Medical Inc

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.23

Topic: I.08. Methods to Modulate Neural Activity

Title: Decoding and modulating spiking activity of the sciatic nerve in an awake and moving rodent

Authors: *E. T. ZHAO, Z. N. MAAN, K. S. FISCHER, J. BARRERA, G. GURTNER, N. A. MELOSH;
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Abstract: Conventional prostheses do not interface directly with neural signals but use either body motion or muscle activity as a proxy. Consequently, these prostheses are difficult to use, tiring, have limited function, and provide no sensory feedback. For the development of an advanced anthropomorphic prosthetic arm that can recapitulate the degrees of freedom and sensory feedback of the human hand, it is critical to design a low damage, high fidelity, and stable peripheral nerve interface (PNI). Here, we fabricate arrays of individual 1 μm thick, and 4 μm wide polymer electrodes, each with a 15 μm diameter recording/stimulation pad, mimicking the dimensions, compliance, and spatial distribution of axon bundles in the peripheral nerve. An electrochemically etched, 80 μm tungsten microwire was threaded through a hole on the device and drawn through the sciatic nerve of a C57BL/6J mouse. A custom designed circuit board was mounted on a 3D-printed backpack to facilitate chronic recording and stimulation.
Mice ran voluntarily on a cylindrical treadmill in a head strained condition, while we recorded the neural activity with our device. Spiking activity was readily observed in our electrodes, where we isolated 6 single units across our 16 electrodes. Using markerless pose estimation, we extracted the joint angles innervated by the sciatic nerve and found a robust correlation between spiking activity and gait. The ultra-small size of the electrodes and their proximity to individual axons permitted extremely local stimulation - down to eliciting movement in a single toe. This represents a substantial advance, in that we overcame a series of challenges involving: (1) Microfabrication of ultra-thin electrodes to mirror the surrounding biomechanical environment to minimize the foreign body response, (2) Device robustness under substantial movement and strain, (3) Microsurgical device implantation to minimize acute insertion damage. Future work will involve electrical modulation to augment movement in peripheral nerve neuropathies and translation into larger animal models with increased channel counts for movement decoding.

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Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.24

Title: WITHDRAWN

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

Time: Monday, November 14, 2022, 8:00 AM - 12:00 PM

Program #/Poster #: 332.25

Topic: I.08. Methods to Modulate Neural Activity

Support: NRF (Korea) 2022M3E5E9016884
IDEC Chip Fabrication Support

Title: Current steering microstimulators for implantable multi-node retinal prostheses

Authors: C.-E. LEE[1,2], Y. JUNG[1], *Y.-K. SONG[3];
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Abstract: Retinal prostheses are implantable devices that primarily aim to restore vision in blind patients suffering from retinal degeneration by electrically stimulating the remaining retinal neurons. Recently, some retinal prostheses have successfully reached the clinical trial stage. However, those devices can only partially restore vision and are insufficient to allow the patient to independently perform daily activities. The limitation of visual acuity is attributed to both engineering and physiological problems, such as the low spatial resolution of the devices or lack of understanding on the physiology of retinal network. To overcome these problems and further improve the visual resolution of retinal prostheses, the geometrical optimizations of electrode arrangement and stimulation pulse parameters have been proposed. In this study, we aim to determine a microstimulator design suitable for retinal prostheses and validate the optimal electrical stimulation parameters. Multi-node stimulators implemented in the TSMC 180nm 1P6M RF CMOS process forms flexible microsystem hardware in which several sub-millimeter-sized single node micro-stimulators interact through an external interrogator. Each node possesses an 8-channel stimulation circuit, a wireless data receiver that processes stimulation commands converted from visual information, and a wireless power supply. Low power

consumption of under 1mW in 3.3 V supply voltage minimizes heat dissipation. Furthermore, the bipolar stimulation structure is paired with 8 active electrodes and 1 reference electrode to steer the direction of the current flow according to the active channels. The core stimulation circuit is designed based on the optimal stimulation parameters obtained in the previous experiments, analyzing the electrical response to light stimulation in the retinal ganglion cells of wild-type mice. The pulse frequency was 5-20 Hz, and the phase width was 0.2-1 ms. Also, the threshold current range from 120-160 μA. Both 7-week-old male and female mice are used. The results indicate that, as the amount of the charge injected to evoke AP increases, the frequency range remains the same. To strictly compare the effectiveness of stimulation according to the amount of charge reaching the target cell, the charge dissipation in the electrolyte is calculated using the circuit models of the electrode-tissue interface with the capacitance and a constant phase element. The system can resolve the limitation of the conventional multichannel stimulators by increasing flexibility and stimulation efficiency.

Disclosures: C. Lee: None. **Y. Jung:** None. **Y. Song:** None.

Poster

332. Electrical Stimulation: Animal Models

Location: SDCC Halls B-H

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Program #/Poster #: 332.26

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF CAREER 1653080

Title: Characterizing the neural output of physiologically-induced and stimulation-induced action potential interactions in vivo

Authors: *L. R. MADDEN, S. IVANESCU, R. S. LIU, S. J. SHRIFT, T. M. BRUNS; Biomed. Engin., Univ. of Michigan, Ann Arbor, MI

Abstract: Electrical neuromodulation of the peripheral nervous system is an available treatment for various neurological conditions, such as bladder disorders and chronic pain. These therapies often deliver continuous stimulation to neural structures that have ongoing physiological activity present. Computational studies have suggested that different interactions between physiologically-induced and electrical stimulation-induced action potentials can occur. They also found that differences in the relative frequencies of physiologically-induced and stimulation-induced action potentials can lead to differences in endpoint neural output, and therefore different potential therapeutic effects. The goal of this study is to characterize neural output in response to different stimulation frequencies imposed on ongoing physiological activity within the pudendal nerve of an *in vivo* feline model. Anesthetized felines were implanted with a stimulating cuff electrode around one pudendal nerve and recording microelectrode arrays in the ipsilateral S1 and S2 dorsal root ganglia. Physiological action potentials were induced via cutaneous brushing of the perineal region, administered by a computer-controlled actuator.

Electrical stimulation was administered via the cuff electrode at 10 different frequencies spanning logarithmically from 0.5 Hz to 30 Hz. Ten trials, one for each stimulation frequency, were recorded with electrical stimulation applied at the motor threshold amplitude during cutaneous brushing. Ten additional cutaneous brushing trials were recorded with electrical stimulation at twice the motor threshold amplitude. Recordings were also performed during brushing without electrical stimulation. Spike sorting analysis was performed on the raw data and further analysis was performed on sorted results to extract interspike intervals, number of neural units recorded, and overall number of spikes from each neural unit from the data. Preliminary results indicate a non-monotonic trend between mean neural output interspike interval and electrical stimulation frequency. The outcomes of this study will inform development of continuous and closed-looped neuromodulation systems and shed light on how the relative stimulation frequency, compared to physiological activity frequencies, can influence therapeutic outcomes. These results may also have implications for neuromodulation of peripheral motor and central nervous systems as well.

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Poster

332. Electrical Stimulation: Animal Models

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Program #/Poster #: 332.27

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF Career 1844762
NIH R01 DK120824

Title: Robust visceromotor responses to colorectal distension in mice anesthetized by closed-loop control of urethane infusion

Authors: *S. ZHANG, L. CHEN, B. FENG;
Biomed. Engin., Univ. of Connecticut, Storrs, CT

Abstract: Background: To develop and optimize novel neuromodulatory protocols for treating visceral pain requires testing those protocols in animal models of behavioral visceral pain, the most widely used metric of which is visceromotor responses (VMR) to graded balloon distension of the colorectum in rodents, i.e., colorectal distension (CRD). The VMR is often objectively quantified by electromyography recordings from abdominal oblique musculature. The invasive nature of neuromodulation requires the assessment of VMR in rodents under anesthesia, which often affects the brainstem leading to complete suppression of VMR to CRD. In this study, we demonstrate that anesthesia by intraperitoneal (i.p.) infusion of urethane in mice at high and low levels can recapitulate two distinct states of sleep, i.e., rapid eye movements (REM) sleep and non-REM sleep, respectively. The VMR to CRD is preserved in the non-REM anesthesia and

abolished in the REM anesthesia. We then developed a closed-loop control system of urethane i.p. infusion to maintain non-REM anesthesia in mice for up to three hours and tested the robustness and repeatability of VMR to CRD. **Methods**: C57BL/6 mice of both sexes (8-16 weeks, 25-40 g) were anesthetized by 2% isoflurane inhalation for surgical implantation of three pairs of EMG electrodes sutured to the bilateral abdominal oblique musculatures and one side of the intercostal muscle for measuring the VMR and respiratory rate, respectively. We then switched the mice to urethane anesthesia by an initial i.p. injection of 0.4 g/kg. Mice were allowed 30 min to completely recover from isoflurane and received a continuous i.p. infusion of urethane, the rate of which is controlled by a programmable syringe pump with respiratory rate as the feedback control parameter. A cylindrical polyethylene balloon was inserted trans-anally to deliver graded CRD. **Results:** Increased urethane infusion rate resulted in a biphasic change in the respiratory rate, i.e., an initial decrease followed by a gradual increase. The initial decreased phase recapitulated non-REM sleep in which mice demonstrated suppressed respiratory rate. The increased phase demonstrated similar features as REM sleep with increased respiration. Maintaining non-REM anesthesia through precise control of the respiratory rate allowed robust recordings of VMR to CRD for up to 3 hours. **Conclusion**: We have established a robust and repeatable behavioral test bench of visceral pain by the feedback control of the depth of anesthesia in mice receiving i.p. urethane infusion. This behavioral assay of VMR to CRD will allow objective assessment of the effect of peripheral neuromodulation in suppressing visceral pain.

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