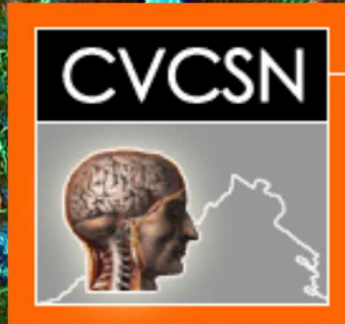


Annual Central Virginia Chapter of the Society for Neuroscience Conference

March 29th, 2025



Imaging in Neuroscience

Hosted By:

VIRGINIA COMMONWEALTH UNIVERSITY

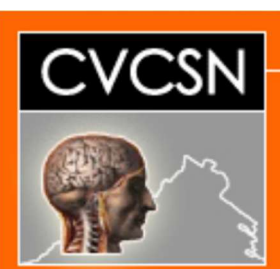


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About CVCSN



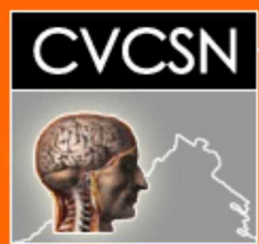
The objective is to advance the understanding of the brain and nervous system by encouraging collaboration among scientists from diverse backgrounds. This involves integrating research across various biological levels and promoting translational efforts to develop better treatments and cures for diseases. We support neuroscientists at all career stages including undergraduates, graduates, and postdoctoral fellows by providing professional development opportunities and educational resources, while emphasizing the importance of diversity in the field. We aim to enhance public understanding of scientific discoveries and communicate the implications of recent neuroscience research. We also facilitate discussions on the ethical considerations in neuroscience research and inform policymakers about new findings and their significance for public policy and scientific advancement. Additionally, CVCSN seeks to foster collaboration among neuroscientists in Virginia.



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Welcome Message:

Dear attendees,

Welcome to the annual meeting of the Central Virginia Chapter of the Society for Neuroscience Conference, 2025! We are thrilled to gather with you all to explore the latest advancements in neuroscience and foster connections among passionate minds in our field. This year's program features esteemed speakers, engaging panels, and opportunities for collaboration that reflect the vibrancy of our community. We encourage you to share your insights and make the most of this unique opportunity. Together, let's inspire one another and further our understanding of the complexities of the brain. Thank you for being here, and we hope you enjoy the conference!

We are looking forward to seeing you again.

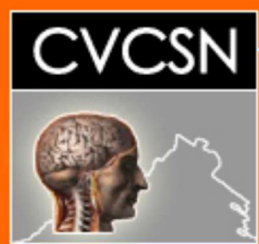


Jeffrey Dupree, PhD

Co-Chair of the CVCSN Organizing Committee

Associate Professor, Department of Anatomy and Neurobiology,
Virginia Commonwealth University

Deputy ACOS for Research, Richmond Veterans Affairs Medical Center



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CVCSN 2025 Conference Organizing Committee:

Jeffrey Dupree, PhD: Associate Professor, Department of Anatomy and Neurobiology, VCU, and Deputy ACOS for Research, Richmond VA Medical Center

Rory McQuiston, PhD: Professor, Department of Anatomy and Neurobiology, VCU

Kimberle Jacobs, PhD: Associate Professor, Department of Anatomy and Neurobiology, VCU

Usha Mahawar, PhD: Post Doctoral Researcher, Department of Cellular Molecular and Genetic Medicine, VCU

Jessica Lynn Maltman: Neuroscience PhD Candidate, Department of Physiology and Biophysics, VCU

Belle Buzzi: Neuroscience PhD Candidate, Department of Pharmacology and Toxicology, VCU

Aaron Wallace, MS: CVCSN Treasurer, Department of Anatomy and Neurobiology, VCU

Lisa Green, MS: Administrative Support, Department of Anatomy and Neurobiology, VCU

CVCSN 2025 Conference Volunteers from VCU:

Alayna Palamar, Amy Wegener, Corinne Smith, Dana Kneisley,

Alexia Zylko, and Sumit Saha.



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Special Guest:

John J. Ryan, PhD

Associate VP for Research Development
Professor
Department of Biology
Virginia Commonwealth University



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Conference Program at a Glance:

- | | |
|---|-------------------------|
| ○ Check-in/breakfast/poster set up | 8:30 a.m. – 10:00 a.m. |
| ○ Welcome speech | |
| ➤ John J. Ryan, PhD | 10:00 a.m. – 10:15 a.m. |
| ○ Morning Keynote Speaker: James Otis, PhD | 10:15 a.m. – 11:15 a.m. |
| ○ Break | 11:15 a.m. – 11:30 a.m. |
| ○ Student Speed Round | 11:30 a.m. – 12:30 p.m. |
| ○ Break | 12:30 p.m. – 12:45 p.m. |
| ○ Postdoctoral Speed Round | 12:45 p.m. – 01:45 p.m. |
| ○ Lunch | 01:45 p.m. – 02:15 p.m. |
| ○ Poster sessions | 02:15 p.m. – 04:15 p.m. |
| ➤ Odd numbered posters | 02:15 p.m. – 03:15 p.m. |
| ➤ Even numbered posters | 03:15 p.m. – 04:15 p.m. |
| ○ Afternoon Keynote Speaker: Edward Nieh, PhD | 04:15 p.m. – 05:15 p.m. |
| ○ Awards Ceremony/closing remarks | 05:15 p.m. – 05:30 p.m. |



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Keynote Speakers



Morning Keynote Speaker

James M. Otis, PhD

Associate Professor

Department of Neuroscience

Medical College of South Carolina

Title of talk: Computational underpinnings of relapse and risk taking in addiction



Afternoon Keynote Speaker

Edward Nieh, PhD

Assistant Professor

Department of Pharmacology

University of Virginia

Title of talk: Unraveling the neural code by understanding population neural dynamics



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Student Speed Round (11:30 a.m. – 12:30 p.m.)

(4-minute talk and 1 minute for Q & A)

11:30 a.m. : **Does astrocytic S1P-S1PR1 signaling regulate VAPA dependent ER- organelle membrane contact sites?**

Jean Patrik Gonzales, Virginia Commonwealth University

11:35 a.m. : **Non-retinal derived sonic hedgehog is required for the maintenance of prethalamic cytoarchitecture**

Parsa Khaksar, Virginia Tech

11:40 a.m. : **Patterns of Cortico-Tectal Input Across Species: An Evolutionary Comparative Analysis**

Christopher Turner, University of Virginia

11:45 a.m. : **Frequency-Coupled Low-Intensity Focused Ultrasound Modulation of Neuronal and Astrocytic Activity in the Prelimbic Cortex**

Greatness Olaitan, University of Virginia

11:50 a.m. : **The Impact of Gut Health on Brain and Muscle Decline**

Hunter A. Moore, Christopher Newport University

11:55 p.m. : **The Critical Intersection of Race, Football and Brain Injury: The Disproportionate Risk of CTE In Black Male Athletes**

Alexis Alston, James Madison University / Massachusetts College of Pharmacy and Health Sciences

12:00 p.m. : **Unleashing Sphingolipid Production in Oligodendrocytes**

Matthew Peart, Virginia Commonwealth University

12:05 p.m. : **Characterizing the Novel Quipazine Analog VCU-1012: Unveiling a New Class of Psychedelic Compounds**

Jessica Lynn Maltman, Virginia Commonwealth University



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Post Doctoral Fellows Speed Round (12:45 a.m. – 01:45 p.m.)

(5-minute talk and 1 minute for Q & A)

- 12:45 p.m. : **Cross-species Compatibility of Novel AAV-R2e-MAC to Cross Blood-brain Barrier and Transduce Myeloid Cells in the Central Nervous System**
Xinxu Yuan, Virginia Commonwealth University
- 12:51 p.m. : **Climbing Fiber signaling is essential for the development of motor control but not social behaviors**
Jonathan Coello, Fralin Biomedical Research Institute
- 12:57 p.m. : **Effect of chronic adolescent stress and morphine dependence on T cell profile in male and female rats**
Hannah D Fulenwider, Virginia Commonwealth University
- 01:03 p.m. : **Repetitive Mild TBI in juvenile mice causes T-Cell-mediated White Matter Disruption and Anxiety**
Kirill Shumilov, Virginia Commonwealth University
- 01:09 p.m. : **Morphological network alterations in Autism Spectrum Disorder and sex-related differences**
Nooshin Safari, University of Virginia
- 01:15 p.m. : **Cross-Species Fecal Microbiota Transplant Reduces Ethanol Consumption in Mice**
Lauren May, Virginia Commonwealth University
- 01:21 p.m. : **Congenital myotonic dystrophy patient derived hiPSCs generate premature neurons**
Surya Chandra Rao Thumu, Virginia Commonwealth University
- 01:26 p.m. : **Tight Junction Disruption and Elevated Macrophage Activity in the Choroid Plexus Linked to Posthemorrhagic Hydrocephalus**
Rajiv Swarup, Virginia Commonwealth University



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Abstracts



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Abstract: S1**Title: Does astrocytic S1P-S1PR1 signaling regulate VAPA-dependent ER-organelle membrane contact sites?****Gonzales JP¹, Tuck C¹, Rao S¹, Singh SK¹;**¹Department of Biochemistry & Molecular Biology, VCU.**Abstract:**

Astrocytes and neurons exhibit cellular crosstalk to bidirectionally provide homeostatic support, regulate development and maturation, and shape neuronal circuitry; however, little is known about the mechanisms which influence astrocyte morphology and intercellular communication. Previously, our lab has demonstrated that neuronal contact stimulates expression of S1PR1, the astrocytic GPCR which binds the bioactive metabolite sphingosine-1-phosphate (S1P), and that S1PR1 drives astrocyte morphology. We also found that S1P-S1PR1 signaling increases the expression of astrocyte-secreted synaptogenic factors in a contact-dependent manner, positing a novel role for astrocytic S1PR1 signaling as a mediator of bidirectional astrocyte-neuron crosstalk. Nevertheless, the downstream mechanisms by which S1PR1 signaling influences astrocyte development and crosstalk are still a mystery. To further investigate the role of astrocytic S1PR1 signaling in the brain, we utilized transgenic mice (S1PR1 Δ Ast) to knockout both S1PR1 alleles in GFAP+ astrocytes (S1PR1^{fl/fl};GFAP-Cre) and performed bulk RNA sequencing. Remarkably, we found a consistent 50% reduction at the mRNA level and later, at the protein level for VAPA, an ER-resident protein that forms ER-organelle membrane contact sites (MCSs). Further investigation determined that the downregulation of VAPA was due to a hemizygous gene deletion of VAPA present in unfluxed GFAP-Cre (77.6) mice likely via the insertion of the GFAP-Cre sequence into the coding sequence of VAPA at one allele. Despite this finding, we serendipitously discovered that astrocytic S1PR1 regulates VAPA expression, as the 50% reduction was seen throughout the brain except in S1PR1 Δ Ast astrocytes. Using an AAV approach to deliver Cre into S1PR1^{fl/fl} mice postnatally, we observe a significant increase in VAPA expression in astrocytes. Current investigations are underway to determine whether MCSs or their roles are influenced in S1PR1 Δ Ast astrocytes. MCSs are specialized regions where the membranes of two organelles come into close proximity without fusing, facilitating rapid, non-vesicular exchange of lipids, ions, and metabolites. Despite astrocytes' key roles in CNS lipid metabolism and calcium signaling, the relevance of astrocytic MCSs in brain homeostasis remains unexplored. Therefore, our current data places a novel role for S1P-S1PR1 signaling as a regulator of MCS formation and function.



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Abstract: S2**Title: Non-retinal derived sonic hedgehog is required for the maintenance of prethalamic cytoarchitecture****Khaksar P**^{1,2}, Stebbins K^{1,2,3}, Su J¹, Fox MA^{1,4,5,6}¹Fralin Biomedical Research Institute at Virginia Tech Carilion, Roanoke, Virginia 24016, USA²Virginia Tech Carilion School of Medicine, Roanoke 24016, USA³Graduate Program in Translation Biology, Medicine, and Health, Virginia Tech, Blacksburg, Virginia 24061, USA⁴School of Neuroscience, College of Science, Virginia Tech, Blacksburg, Virginia 24061, USA⁵Department of Biological Sciences, College of Science, Virginia Tech, Blacksburg, Virginia 24061, USA⁶Department of Biology, College of Natural Sciences, University of Massachusetts Amherst, Amherst, Massachusetts 01003, USA**Abstract:**

In the visual system, information from the outside world is captured by retinal ganglion cells (RGCs) in the retina and transmitted to distinct regions of the brain, where it is processed and ultimately perceived as a unique visual experience. One essential region is the ventral lateral geniculate nucleus (vLGN), which is associated with non-image-forming vision and can be divided into two domains: the retinorecipient external vLGN (vLGNe) and the non-retinorecipient internal vLGN (vLGNi). Through both activity- and morphogen-dependent mechanisms, retinal inputs play important roles in the development of vLGNe. One particular signaling molecule, sonic hedgehog (SHH), has been shown to be essential for interneuron recruitment into the developing vLGN. However, the impact of retinal and non-retinal sonic hedgehog underlying the development of principal neurons in vLGN remains unknown. We identified and characterized retinal and nonretinal-derived morphogens that influence the formation and maintenance of vLGNe laminae, hypothesizing that SHH is necessary for lamination of vLGNe. Immunohistochemistry and in situ hybridization were performed to label cell types in mice with intact (C57) or altered (Math5^{-/-}, Shhfl/flNesCre, Shhfl/flCalb2Cre) retinal activity. Area, cell count, proportion, and cell density of vLGNe and vLGNi were quantified to demonstrate changes in principal neurons of vLGN. Our findings revealed that retinal-derived SHH is not required for laminar formation within vLGNe. Loss of retinal SHH causes a significant reduction in area and neuronal cell count, suggesting a unique role in RGC-derived SHH in the formation of vLGN. Observed changes in specific proportions and cell density of principal neurons unique to vLGNe provides additional evidence of SHH involvement in maintaining the cytoarchitecture of vLGN. We conclude that nonretinal-derived morphogens, such as SHH, play a crucial role in the proper lamination and cytoarchitecture of vLGN, thus demonstrating the necessity of such genes in governing the development of ventral thalamic regions responsible for vision. While it can be concluded that retinal-derived SHH plays a role in interneuron recruitment in vLGN, the influence of SHH on projection neurons necessitates further investigation.



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Abstract: S3**Title: Patterns of Cortico-Tectal Input Across Species: An Evolutionary Comparative Analysis****Christopher Turner CT¹, Arda Kipcak AK¹, Dr. Alev Erisir AE¹**¹University of Virginia, Department of Psychology**Abstract:**

The superior colliculus (SC) is a highly conserved midbrain structure within the tectum that integrates multisensory information to generate spatial maps of salient stimuli, facilitating orienting behaviors critical for survival. While the SC exhibits distinct morphology, connectivity, and function across species, the circuit and cell-type specific mechanisms that underlie the differential visuospatial reflexive behavior remain incompletely understood. In this study, I catalogue the corticotectal projections across the mouse, cat, tree shrew, and monkey. These species were selected based on their evolutionary divergence from primates (mouse), complex cortical integration and phylogenetic proximity to primates (tree shrew), and highly developed sensorimotor and visuospatial reflexive systems (cat and monkey). To investigate projection intensity from discrete cortical regions to SC, I performed a data-mine and analysis of the Allen Mouse Brain Connectivity Atlas anterograde tracer experiments. This analysis revealed 20 unique cortical regions projecting to the SC in functionally organized, yet distinct patterns across the anterior-posterior, medial-lateral, and superficial-deep gradients. In the tree shrew, I conducted an rgAAVtdtomato tracer injection into the SC of one animal, identifying 27 unique cortical regions expressing fluorescent signal in cell bodies. Cat and monkey input patterns were obtained through a literature review. These findings reveal species-specific differences in corticotectal circuits that may underlie variations in visuospatial processing and sensorimotor adaptations. By comparing these connectivity patterns, this study provides insight into the evolutionary divergence of SC circuitry and its implications for reflexive behavior across mammals.



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Abstract: S4**Title: Frequency-Coupled Low-Intensity Focused Ultrasound Modulation of Neuronal and Astrocytic Activity in the Prelimbic Cortex****Olaitan GO¹, Okojie AK², Lynch WJ², Venton BJ¹**¹Chemistry Department, UVA; ²Department of Psychiatry and Neurobehavioral Sciences, UVA.**Abstract:**

Synchronous oscillations of cortical neurons play a crucial role in cognitive function, influencing both inhibitory and excitatory downstream neurotransmission and signaling. Disruption of temporal coupling in prefrontal cortex oscillations has been linked to the dysregulation of dopamine in the nucleus accumbens core. Previous research has demonstrated the effect of Low-intensity focused ultrasound (LIFU) intensity on downstream dopamine neurotransmission. In this study, we investigated the effects of different frequency-coupled LIFU sonication parameters applied to the prelimbic cortex (PLC) on neuronal and astrocytic activity. We examined the impact of theta-modulated beta (16:2 Hz) and gamma (50:5 Hz) LIFU waves on c-Fos expression, a neuronal activation marker, and glial fibrillary acidic protein (GFAP), an astrocyte activation marker. Our results revealed that LIFU sonication at a frequency of 16:2 Hz led to increased neuronal activation, as evidenced by elevated c-Fos expression, while simultaneously reducing astrocytic activity, indicated by decreased GFAP levels. Conversely, the application of 50:5 Hz waves resulted in increased astrocytic activity, demonstrated by enhanced GFAP expression. These findings suggest that frequency modulation of LIFU parameters could potentially be utilized in future studies for circuit-specific treatment of various phases of psychiatric disorders. The differential effects observed on neuronal and astrocytic activation highlight the importance of precise parameter selection in LIFU neuromodulation. Further research is warranted to elucidate the specific activation patterns of GABAergic and glutamatergic neurons that may contribute to the observed effects. Additionally, investigating the behavioral consequences of these changes on neural circuit dynamics will be crucial for fully understanding the potential therapeutic applications of LIFU. Understanding these mechanisms could pave the way for the development of novel LIFU-based therapies for neurological and psychiatric disorders, offering a non-invasive approach to targeted neuromodulation.



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Abstract: S5**Title: The Impact of Gut Health on Brain and Muscle Decline****Moore H¹, Auby C¹, Sebastian E¹, Salazar A².**¹Neuroscience Program, CNU²Department of Molecular Biology and Chemistry, CNU**Abstract:**

Many neurodegenerative diseases, such as Parkinson's Disease and Alzheimer's Disease, increase with age and are associated with elevated protein aggregate levels. Previous research in fruit flies has discovered a relationship between increasing age and a deterioration in gut health, with developing intestinal barrier dysfunction tightly linked to mortality. Because of this tight association between organismal health and gut function, several markers of aging have been investigated in order to determine the role of barrier integrity on aging phenotypes. This study examines whether intestinal barrier deterioration can impact protein aggregation within the brain and muscles, a specific aging phenotype associated with several diseases of aging. This research utilizes the knockdown and overexpression of the protein Snakeskin (Ssk) in the midgut of *Drosophila melanogaster*, with Ssk knockdown inducing intestinal barrier permeability and Ssk overexpression reducing intestinal barrier permeability. Ssk is a septate junction protein, equivalent to mammalian tight junctions, important for maintaining the integrity of the connections between adjacent gut epithelial cells. Ssk mislocalizes in aging flies, perturbing these junctions and causing intestinal permeability. Knocking down its expression in the gut, using the GeneSwitch Gal4/UAS system, causes a decrease in barrier function and a severely reduced lifespan, in addition to other markers of aging. Conversely, overexpressing Ssk reinforces barrier function, extends lifespan, and reduces aging symptoms. These experiments are investigating protein aggregation in both the Ssk knockdown model and Ssk overexpression model in order to understand whether unhealthy or aging guts exhibiting barrier dysfunction may be associated with neurodegenerative diseases linked with protein aggregation in the brain and muscle. Ideally, these insights will fuel a focus on intestinal health in the studies of several diseases with accompanying protein aggregation.



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Abstract: S6**Title: The Critical Intersection Of Race, Football and Brain Injury: The Disproportionate Risk of CTE In Black Male Athletes.****Alexis Alston¹**¹James Madison University / Massachusetts College of Pharmacy and Health Sciences
Neuroscience**Abstract:**

Departments Abstract: Chronic Traumatic Encephalopathy (CTE) is a neurodegenerative condition linked to repeated head injuries. Despite their significant participation in high-risk sports like American football, Black male athletes remain underrepresented in CTE research. This study examines how race, socioeconomic factors, and healthcare access intersect in addressing both CTE risks and management, while acknowledging the current limitations in formal diagnosis. The primary objectives were to assess the prevalence of CTE-associated risk among Black male athletes and evaluate how factors such as first-generation college status, limited healthcare access, and socioeconomic challenges contribute to these elevated risks. I collected data from 263 athletes across 30 institutions, including 10 Division I, 12 Division II, 8 Division III universities, and 4 high schools, using a 17-item survey. The survey examined football experience, institutional support, and socioeconomic background. Ethical guidelines ensured participant anonymity. Statistical analysis focused on correlations between race, socioeconomic factors, and CTE risk. Results revealed that 73% of Black respondents identified as first-generation college students compared to 27% of their non-Black peers. Of Black athletes, 47% reported learning about CTE through independent research, while 53% of non-Black athletes, including 32% White players, received education from healthcare providers. Concussion history showed 63% of Black athletes reported 1-2 diagnosed concussions, 35% experienced 3-4, and 2% reported 5 or more. Comparatively, 33% of non-Black athletes reported 1-2 concussions, 12% had 3-4, and 0.3% reported 5 or more. Additionally, 63% of Black athletes felt pressured to return to play before recovering, compared to 37% of non-Black athletes. Importantly, 41% of Black respondents felt race influenced brain injury management. This research highlights the need to address disparities in concussion education, healthcare access, and support for Black athletes. By identifying these gaps, the aim to inform strategies for early diagnosis, equitable treatment, and long-term care to protect vulnerable athletes.



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Abstract: S7**Title: Unleashing Sphingolipid Production in Oligodendrocytes****Peart M¹, Mahawar U¹, Wattenberg B¹.**¹ Department of Cellular Molecular and Genetic Medicine, VCU.**Abstract:**

Myelin is a multilayer membrane produced by specialized glial cells named oligodendrocytes that allows for rapid conduction of action potentials along neuronal axons. A diverse profile of lipids contributes to the structure and function of myelin; one group within this profile is the sphingolipids, which have canonical roles in lipid bilayer structure and cellular signaling. Complex sphingolipids such as galactosylceramide, sulfatide, and sphingomyelin account for a substantial proportion of the myelin membrane. As with all sphingolipids, the initiating and rate-limiting step in the production of these molecules is catalyzed by Serine Palmitoyltransferase (SPT). SPT is anchored to the lipid bilayer of the endoplasmic reticulum, where it is regulated by its transmembrane subunits, the ORMDLs. We have conducted experiments demonstrating that a conditional knockout of one isoform of ORMDL in mouse oligodendrocytes results in an increased myelin thickness and axon diameter, demonstrating that increasing sphingolipid production in oligodendrocytes allows them to produce larger myelin membranes. Additionally, this knockout of one ORMDL isoform leads to an upregulation of myelin associated proteins, hinting at a mechanism within oligodendrocytes that links sphingolipid levels to myelin protein gene expression. Based on these initial findings, we hypothesized that deregulating SPT would provide oligodendrocytes with enhanced ability to repair damaged myelin during a demyelinating disease. We tested this using an inducible and conditional knockout of ORMDL3 and the Experimental Autoimmune Encephalomyelitis (EAE) model of Multiple Sclerosis (MS) in mice. Consistent with our hypothesis, we found that female mice with the knockout had significantly reduced neurological symptoms throughout the course of the experiment compared to wild-type littermates—interestingly, we saw no differences in males. Our experimental data serves as evidence that increasing SPT activity in oligodendrocytes allows for increased lipid incorporation into the myelin membrane and drives myelin protein gene expression. This enhanced ability to produce the lipids and protein in myelin appears to protect against the destruction of myelin in a mouse model of MS. These discoveries identify sphingolipid production as a potential candidate for therapeutic intervention in MS.



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Abstract: S8**Title: Characterizing the Novel Quipazine Analog VCU-1012: Unveiling a New Class of Psychedelic Compounds**

***Jessica Lynn Maltman**^{1,3}, Jason Younkin¹, Michael Fiorillo^{2,1}, Alaina M Jaster^{2,1}, Archana Paymode⁴, Somdatta Saha¹, Justin Silverman^{1,2}, Ajay Bansode⁴, George D Miller¹, Roya Abedi¹, James Kang², Richard A Glennon⁴, Hamid I Akbarali², I. Scott Ramsey¹, Malgorzata Dukat⁴, Javier González-Maeso¹

¹Department of Physiology & Biophysics, Virginia Commonwealth University, Richmond VA, USA,

²Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond VA, USA,

³Neuroscience Graduate Program, Virginia Commonwealth University, Richmond VA, USA, 4.

Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA, USA

Abstract:

Classical/serotonergic psychedelics are currently categorized into 3 broad classes based on chemical structure (tryptamine, phenethylamine, and ergoline). Each class and its respective compounds display unique binding interactions with 5-HT_{2A}R (a G protein-coupled receptor which heavily mediates hallucinogenic effects), and distinct pharmacological and therapeutic profiles. A current goal in psychedelic research is to uncover the mechanisms underlying the therapeutic effects of psychedelics observed in both preclinical and clinical research for a variety of mental and physical conditions, and to design ligands that more specifically elicit therapeutic over adverse effects. Quipazine, a drug originally intended as an anti-depressant which also displayed hallucinogenic and adverse gastrointestinal effects, was hypothesized to represent a new class of psychedelic due to its different chemical structure. The goal of our current work was to design quipazine analogs that would lack the adverse gastrointestinal effects mediated by 5-HT₃R while maintaining agonist activity at 5-HT_{2A}R and therapeutic benefits. VCU-1012 is one of the resulting quipazine analogs with 5-HT_{2A}R binding affinity and agonist activity, no adverse 5-HT₃R-mediated gastrointestinal effects, and hallucinogenic, neuroplastic, and anti-depressant-like effects in mice. Mutation studies further revealed unique 5-HT_{2A}R residues deep in the binding pocket mediating this analog's agonist activity. These results suggest that VCU-1012 and potentially other quipazine analogs represent an entirely new structural class of psychedelic with therapeutic potential that can be further investigated for intentional drug design.

Methods:

- Binding displacement assays with [³H]ketanserin in HEK-293 cells stably expressing h5HT_{2A}R cDNA.
- Agonist-induced Ca²⁺ mobilization assay in HEK-293 cells stably expressing h5HT_{2A}R cDNA and also with alanine mutations in binding pocket residues.
- Head-twitch response in adult male C57BL/6 mice.
- Whole-cell voltage clamp electrophysiology in a tetracycline-inducible Flp-In-293 T-REx cell line.
- Gut motility assay in adult male C57BL/6 mice.
- Forced swim test in adult male C57BL/6 mice.
- Frontal cortex dendritic spine density in adult male C57BL/6 mice.



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Abstract: PD1**Title: Cross-species Compatibility of Novel AAV-R2e-MAC to Cross Blood-brain Barrier and Transduce Myeloid Cells in the Central Nervous System**

Xinxu Yuan¹, Danmeng Zhang¹, Ruotong Zhao², Grey De La Torre³, Dabbu Jaijyan¹, Abdul Rasheed Baloch^{1,2}, Jian Xu¹, Yuekun Li¹, Xufeng Qu⁴, Bing Wang⁵, Fang Li^{1,2}, Hong Wang², Qizhao Wang⁵, Jeffrey L. Dupree¹, Jinze Liu⁴, Binhua Ling³, and Wenhui Hu^{1,2}

¹Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, Richmond, VA 23098

²Center for Metabolic Disease Research, Department of Pathology and Laboratory Medicine, Temple University Lewis Katz School of Medicine, 3500 N Broad Street, Philadelphia, PA 19140

³Host Pathogen Interactions Program, Texas Biomedical Research Institute, San Antonio, TX 78227

⁴Department of Biostatistics, Virginia Commonwealth University School of Public Health, Richmond, VA 23098

⁵AAVnerGene Inc, 9620 Medical Center Dr, Suite 100, Rockville, MD 20850.

Abstract:

Myeloid cells, including resident microglia and central nervous system (CNS)-associated macrophages, play crucial roles in both healthy and diseased conditions within the CNS. Gene therapy targeting myeloid cells holds significant promise for treating CNS diseases and injuries. Extensive research has identified AAV9, AAV8, and AAVrh10, along with their derivatives, as capable of crossing the blood-brain barrier (BBB) in both neonatal and adult animals. These serotypes, classified as AAV-B, are known for their high efficiency in transducing neurons, astrocytes, oligodendrocytes, and blood vessels. However, these serotypes exhibit limited or no ability to transduce brain myeloid cells in vivo. Conversely, several AAV serotypes, such as AAV1, AAV2, AAV5, and AAV6, have demonstrated potent tropism for myeloid cells (AAV-M). To develop novel AAV serotypes (AAV-BM) that combine the BBB-crossing ability of AAV-B with the myeloid cell transduction capability of AAV-M, we performed epitope insertional hybrid screening. This involved transferring a BBB-crossing epitope from AAV-B to the variable region VIII of the AAV-M capsid. Among the eight AAV-M serotypes validated in our preliminary studies, we selected AAV2 for initial screening due to its high transduction efficiency in human, monkey, and mouse primary microglia, well-documented study history, and natural binding to heparan sulfate proteoglycans (HSPGs) on many cell types. AAV-CAP-MAC, AAV-9P31, AAV-PhP.eB, AAV-cc47, AAV-CPP16, AAV-CPP21, AAV-Pal1, and AAV-MDV1A were chosen for the first trial due to their efficiency in crossing the BBB at relatively low dosages in multiple species. This trial identified R2-MAC as the primary AAV-BM, derived from AAV CAP-MAC and natural AAV2. By introducing quadruple tyrosine-mutants (Y272F, Y444F, Y500F and Y737F) and one threonine mutant (T491V) to the R2-MAC capsid, we created an enhanced version, R2e MAC, which showed higher transduction efficiency in cultured microglia. To test R2e-MAC, we packaged CMV-EGFP using the AAVone system and administered doses ranging from 1×10^{10} to 5×10^{12} genome copies (GC) per mouse intravenously (IV). R2e-MAC demonstrated dose-dependent BBB crossing and neural cell transduction across various mouse strains, including C57BL/6, BALB/c, and FVB/N. The capsid also proved effective in rhesus macaques (3×10^{13} GC/kg, IV) and human organoid BBB models. Both male and female C57BL/6 mice showed comparable transduction efficiency, while lipopolysaccharide (LPS) treatment robustly enhanced neural cell transduction. Bioinformatics analysis of single-cell RNA sequencing from fluorescence-activated cell sorting (FACS)-sorted EGFP-positive cells of the C57BL/6 mouse brains (5×10^{12} GC, IV) revealed 30% myeloid cells, 64% endothelial cells, 3.9% astrocytes, 0.6% mature neurons, and 1.4% oligodendrocytes. Hybridization Chain Reaction RNA Fluorescence In Situ Hybridization (HCR-RNA-FISH) validated the transduction in TMEM119 and HexB positive microglia. In conclusion, the hybrid AAV-R2e-MAC capsid outperforms the parent AAV-CAP MAC in BBB crossing and AAV2 in myeloid cell transduction across different species, offering a potential gene therapy approach for CNS diseases and injuries. Acknowledgements: This study is supported by NIH R01MH130193 and R01DA056876 funding.



Abstract: PD2**Title: Climbing Fiber signaling is essential for the development of motor control but not social behaviors****Coello J¹**, Dao B¹, Lyon A^{1, 2}, van der Heijden M^{1, 2}¹Fralin Biomedical Research Institute**Abstract:**

The cerebellum plays a pivotal role in motor learning, coordination, balance, and other behaviors. It achieves these functions through Purkinje cells (PCs), which receive excitatory input from granule cells (GCs) and climbing fibers (CFs) of the inferior olive. Previous studies have shown that selectively disrupting granule cell signaling impairs motor learning during development without affecting social behaviors. Here, we investigate the developmental role of CF signaling in developmental motor reflexes, motor control, and social behaviors using genetic mouse models. Our observations of pup mice reveal that CF signaling is essential for motor coordination and balance, as demonstrated by impaired performance on the negative geotaxis and righting reflex tests. During development, CF signaling is also necessary for social communication, as shown by ultrasonic vocalization (UV) during social isolation tests. At three and six weeks of age, we used the rotarod test to assess motor learning impairments due to disrupted CF signaling but found no behavioral changes in the open field test, three-chamber assay, and UV tests at later developmental stages. These findings establish that disrupting either GC or CF signaling impairs motor learning, coordination, and balance but does not affect social behaviors. This suggests that during development, Purkinje cells can compensate for the loss of either excitatory input, emphasizing the importance of investigating downstream output in regulating complex behaviors.



CVCSN



Abstract: PD3**Title: Effect of chronic adolescent stress and morphine dependence on T cell profile in male and female rats**

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Abstract:

Opioid use disorder (OUD) is an extremely prevalent and debilitating condition, characterized by relapsing cycles of uncontrolled use, craving, and withdrawal. While many factors contribute to an individual's risk for developing OUD, early life stress (ELS) – physical or psychosocial stress exposure during infancy, childhood, or adolescence –, is perhaps one of the most prevalent. For example, a “dose-response” relationship has been repeatedly between ELS and OUD, in that increasing levels of ELS are associated with more severe OUD symptoms in adulthood. A potential mediator for this increased risk may lie within the immune system, as ELS exposure can lead to long-lasting impairments in immune function, evidenced by accelerated aging of immune cells, worsened responses to infection, and excessive inflammation in certain individuals. Of particular interest in these immune processes are regulatory T cells (Tregs), a T cell subtype critical in mediating immunosuppressive processes. Treg dysfunction has been observed in individuals with ELS histories and in those with OUD, making this specific cell type a promising target for therapeutic intervention in patients with OUD and ELS histories. Additional work is needed, however, to understand the effects of ELS on opioid-induced Treg dysfunction and immune dysregulation and how these alterations affect OUD pathology. Further, the effect of biological sex on these processes has yet to be determined. Therefore, the goals of this work were to examine the effects of ELS and morphine dependence on Treg profile in males and females. Rats of both sexes were exposed to chronic psychosocial stress during adolescence, followed by chronic morphine treatment in adulthood. Blood draws were conducted before and after stress exposure, at onset and conclusion of morphine treatment, and during spontaneous withdrawal. Samples were then processed for flow cytometry analysis to assess T cell profile. We found that, males and females exposed to chronic adolescent stress exhibited significant decreases in functional Treg levels, demonstrating a stress-induced impairment in immunosuppression. Data analyses for the remaining experimental timepoints are ongoing, the findings from which will provide the basis for future studies aimed at evaluating immunotherapies as possible interventions for OUD patients with histories of developmental stress exposure.



CVCSN



Imaging in Neuroscience

Abstract: PD4**Title: Repetitive Mild TBI in juvenile mice causes T-Cell-mediated White Matter Disruption and Anxiety**

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²Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA, USA

Abstract:

Pediatric repetitive mild traumatic brain injury (rmTBI) is a major public health concern with links to cognitive dysfunction, anxiety, and depression. Despite its high incidence, the precise mechanisms underlying pediatric rmTBI leading to poor neurobehavioral outcomes remains enigmatic, hindering the development of effective treatments and optimal clinical management. To examine the fundamental neurodevelopmental impact of peripheral immune cell infiltration and the subsequent neuroinflammation on white matter (WM) disruption and neurobehavior outcomes in pediatric rmTBI. Our Central Hypothesis is that pediatric rmTBI alters brain development and long-term neurobehavior through peripheral immune cell infiltration impacting the functionality of myelinating oligodendrocyte lineage cells (OLC). Two groups of juvenile (n=23; postnatal day 21) mice were injured using a repetitive impact acceleration model. 25g weight suspended at 50 cm was impacted 3 times at weekly frequency. Seven days after the last mTBI, flow cytometry of cortical tissue and blood samples was performed to quantify infiltration of peripheral immune cells, as well as immunohistochemistry of brain slices to address gliosis and oligodendrocyte density. Thirty days after injury, diffusion tensor Imaging was used to analyze WM structural disruption, and light-dark box behavioral test was performed to evaluate anxiety-like behavior. One week after rmTBI, acute peripheral immune cell infiltration in the cortex, including CD4⁺ T-cells (p=0.0179) and proinflammatory M1 macrophages (p=0.0357), and increased density of astrocytes (p=0.0471) occurred in the corpus callosum (CC). One month after rmTBI, injured animals exhibited anxiety-like behavior, and continued to have increased astrocyte density (p=0.0159), decreased OLC density (p=0.0079), and decreased Fractional Anisotropy (FA) (p<0.05) in the CC. These findings suggest that rmTBI triggers WM disruption and long-term neurobehavioral impairments, probably associated with the infiltration of CD4-activated T-cells and persistent activation of astrocytes. Our future goal is to develop an immunotherapeutic tool targeting CD4⁺ T-cells to prevent WM disruption and neurobehavioral impairments, leading to improved treatment and reducing the long-term burden of children with brain trauma.



CVCSN



Imaging in Neuroscience

Abstract: PD5**Title: Morphological network alterations in Autism Spectrum Disorder and sex-related differences.
Safari N¹**

¹Postdoctoral research associate at Dr. Javier Rasero's lab, University of Virginia

Abstract:

Autism spectrum disorder (ASD) is characterized by heterogeneous neurodevelopmental alterations, complicating the identification of reliable neural biomarkers. In this study, we apply the Morphometric INverse Divergence (MIND) network approach to examine morphology-based connectivity in ASD using data from 317 individuals (152 females) aged 8 to 18 years, including 166 autistic individuals. T1-weighted MRI data were processed with FreeSurfer to extract cortical features, including cortical thickness, volume, surface area, mean curvature, and sulcal depth. Morphometric similarity was then estimated via symmetric Kullback-Leibler (KL) divergence. Network-based statistics (NBS) were applied to identify significant morphology-based connectivity differences while accounting for covariates, including age, sex, cohort, total intracranial volume (TIV), site of data collection, and the interaction between sex and cohort. By comparing morphology-based connectivity patterns, we aim to identify morphological connectivity alterations to better characterize/understand ASD and its sex-related differences. Our findings reveal significant morphology-based connectivity alterations in key regions across multiple large-scale brain networks. Notably, the entorhinal cortex and fusiform gyrus exhibited the most alterations, highlighting their central role in ASD-related neurobiology. The entorhinal cortex is crucial for memory processing and spatial navigation, while the fusiform gyrus is strongly associated with face perception and social cognition—both of which are commonly affected in ASD. These regions, along with the inferior temporal cortex, are linked to the default mode network (DMN) and salience network (SN), suggesting disruptions in social cognition, memory, and sensory integration. We also observed connectivity variations in the precentral and postcentral gyri, which are involved in sensorimotor processing, the rostral middle frontal cortex and superior parietal lobule, which are part of the central executive network (CEN), and the precuneus and cingulate cortex, which play a major role in self-referential thought and social processing.

Notably, we also observed significant connectivity alterations in cohort and sex interaction, most prominently in the supramarginal and parahippocampal gyri. Overall, our study highlights significant structural connectivity differences in ASD across major brain networks. This approach offers insights into ASD's neural heterogeneity and potential biomarkers for improved classification and intervention.



CVCSN



Abstract: PD6**Title: Cross-Species Fecal Microbiota Transplant Reduces Ethanol Consumption in Mice**
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²VCU Alcohol Research Center, Virginia Commonwealth University, Richmond, Virginia, 23298

³Department of Internal Medicine, Division of Gastroenterology, Hepatology, and Nutrition, Virginia Commonwealth University, Richmond, Virginia, 23298

Abstract:

There is a need for new approaches to treating alcohol use disorder (AUD) beyond the currently available pharmacologic therapies. One such approach is manipulation of the host gut microbiome. Alcohol consumption changes the host microbiome by changing the bacterial diversity, and it has additional effects on the gut, including changes in mucosal immunity and intestinal permeability. Previous studies have shown that manipulation of the host microbiome with a fecal microbiota transplant (FMT) can ameliorate AUD-related behaviors, including alcohol consumption, as well as potentially preventing further liver damage in patients with AUD and alcoholic liver disease (ALD). Thus, the goal of this study is to use stools from healthy human donors in a mouse model of alcohol consumption to determine what characteristics, (i.e. bacterial diversity, engraftment efficacy) in donor stools translate to the greatest reduction in ethanol consumption. Female C57BL/6 mice were treated with a course of antibiotics prior to FMT administration to encourage engraftment. Three donor stools were used, each with different amounts of *Lachnospiraceae* and *Ruminococcaceae*, two bacterial families common in the gut that produce short-chain fatty acids (SCFAs). Following FMT administration, mice underwent six weeks of drinking with a two-bottle choice model, as well as behavioral testing to measure locomotive and anxiety-like behaviors. We report changes in ethanol drinking behavior over the course of human FMT engraftment and reduction and correlate these changes to efficiency of human microbiome engraftment and alterations in the mouse microbiome diversity. Additionally, we report no changes in anxiety or locomotor behavior following FMT engraftment. Future studies will investigate the use of diet modulation in increasing the diversity and length of human FMT engraftment. This model, using immunocompetent mice, will provide a basis for further studies on modulating the gut-brain axis via FMT in a model of chronic ethanol consumption.



CVCSN



Imaging in Neuroscience

Abstract: PD7**Title: Congenital myotonic dystrophy patient derived hiPSCs generate premature neurons**

Thumu, S.C.R.,¹ Enricks, D.,² Hale, M.,² Carrell, S.,² Gonzales, J.P.,¹ Tuck, C.,¹ Munir, S.,¹ Dominguez, O.,¹ Johnson, N.E.,² Singh, S.¹

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Abstract:

Myotonic Dystrophy type 1 (DM1) is an autosomal multisystem disorder manifested due to unstable CTG nucleotide repeat expansion within the 3'-untranslated region of the dystrophin myotonia protein kinase (*DMPK*) gene. Although progress towards understanding of molecular pathogenesis in muscle and heart has been made, the pathways that affect the brain in DM1 is fundamentally unknown. Despite the wealth of existing cellular and animal models, iPSCs based studies are being fostered as they replicate the human model more closely to the disease. In view of this context, we intended to study the neural induction in DM1 iPSC lines (JB6 and CDM-57) derived from congenital DM1 patients along with control line (CTL 1-9). Surprisingly, we found through immunofluorescence studies that the mutant iPSCs generate premature neural progenitor cells (NPCs) within 1 week by their expression of neuronal markers TUJ1 and Map2. These cells augmented and outnumbered the control cell line along with extended processes and neurite length in the subsequent induction. Investigation of MBNL1 expression during the neural induction revealed its enhanced expression and nuclear aggregation (a hallmark of the DM1 disease) in the mutant lines. RNA sequencing and qPCR analysis unexpectedly revealed overexpression of pioneer transcription factors including, *Ascl1*, *NeuroG2*, *NeuroD1* and *Lhx2* that play key roles in neuronal development. Our results revealed novel paradigms accountable for neurological pathogenesis in DM1 patients. Mechanistic studies are underway to understand the disease pathogenesis that would open new avenues in the field of gene therapy for DM1 individuals.



CVCSN



Imaging in Neuroscience

Abstract: PD8**Title: Tight Junction Disruption and Elevated Macrophage Activity in the Choroid Plexus
Linked to Posthemorrhagic Hydrocephalus**

Rajiv Swarup¹, Maria Garcia-Bonilla¹, Owen W. Limbrick², Marie Michenkova¹, Konrad McKalip¹, William Bernhardt¹, Kirill Shumilov¹, Ayodamola Otun², Jayne Crouthamel², Krikor Dikranian³, James P. McAllister II¹, David D. Limbrick Jr¹.

¹Department of Neurosurgery, Virginia Commonwealth University, Richmond, VA, USA

²Department of Neurosurgery, Washington University in St. Louis, St. Louis, MO, USA

³Department of Neuroscience, Washington University in St. Louis, St. Louis, MO, USA

Abstract:

Introduction: Posthemorrhagic hydrocephalus (PHH) following intraventricular hemorrhage is a severe neurological complication occurring in almost 35% of preterm neonates. The choroid plexus (ChP) is a secretory tissue responsible for producing cerebrospinal fluid (CSF) that consists of epithelial cells joined by tight junction (TJ) proteins. The ChP also acts as a reservoir of immune cells, including border-associated macrophages (BAMs). BAMs are key players in neuroinflammation by the expression of matrix metalloproteinases, or MMPs. We hypothesized that alterations in the junctional biology of the ChP may be associated with the immune response of BAMs in the ChP. **Objectives:** To test this hypothesis, we analyzed ChP TJ proteins, Claudin-1 and Zonula Occludens-1, and quantified the expression of BAMs in ChP TJ disruption in PHH. **Methods:** Postnatal day 4 (P4) mice were given intraventricular injections of lysed blood (n=11) or saline (n=7). 7 days post-induction, MRI neuroimaging and transmission electron microscopy (TEM) were performed, ventricular volumes of the lateral and third ventricles were assessed, and histology, immunohistochemistry, and flow cytometry were performed. Further, ChP were extracted from P4 mice and exposed to lysed blood (n=7) or saline (n=6) and co-cultured with splenic monocytes for 24 hours. **Results:** PHH mice exhibited significantly larger (p=0.0025) ventricles, ZO-1 and Claudin-1 density in the ChP was significantly reduced (p=0.0048 and p=0.033 respectively), and the number of GFAP+ astrocytes (p=0.0144), Iba1+ macrophages (p=0.0346) and BAMs (p=0.0177) in the ChP in PHH. Iba1+ BAMs expressed MMP9, contributing to an increase (p=0.0278) in total levels of MMP9 in PHH. Additionally, TEM suggested BAM activation, characterized by endocytic activity via phagosomes and lysosomes in the cytoplasm. In-vitro studies showed a decrease (p=0.0256) in ZO-1 TJs in PHH when ChP organoids were exposed to lysed blood and co-cultured with monocytes. **Conclusion:** Our findings illustrate macrophage-mediated ChP TJ disruption and neuroinflammation through MMP9 in experimental PHH and opens avenues for exploring novel immunomodulatory treatments aimed at preventing the pathogenesis and neurodevelopment impairments common in PHH.



Abstract: 1**Title: Harnessing spatial imaging mass cytometry to explore Lacritin's potential in restoring Alzheimer's disease homeostasis****Thonda S¹, Laurie WG^{1,2}**¹Department of Cell Biology, UVA; ²Ophthalmology and Biomedical Engineering, UVA.**Abstract:**

Treatment options for Alzheimer's disease (AD) are limited despite recent advances. The ocular surface tear protein 'lacritin', recently discovered to be distributed in the cerebral spinal fluid (CSF) and plasma, may be beneficial. Under the conditions of immune or protein aggregate stress, lacritin transiently accelerates autophagy to restore oxidative phosphorylation through mitochondrial fusion. Via a largely different signaling pathway, it regenerates neurons. In preliminary studies, we have identified the presence of lacritin monomer and the transglutaminase 2 inactivated dimer form of lacritin within human CSF samples. Intracerebroventricular administration of the C-terminal lacritin synthetic peptide ('N-104') resulted in the stimulation of autophagic flux and a reduction in imaging mass cytometry (IMC)-detectable A β levels in mRFP-eGFP-LC3B/5xFAD mice. Our working hypothesis is that lacritin is an unappreciated dual homeostasis and restorative activity in the CNS. The presence of bioactive monomeric lacritin may be selectively deficient in the cerebrospinal fluid of individuals with AD. In these instances, the use of replacement therapy is potentially transformative. Our immediate goal is to utilize the IMC antibody panel to decipher single-cell and spatial proteomic profiles with 'N-104' peptide treatment in different-aged mRFP-eGFP-LC3B/5xFAD mice and then seek to validate its therapeutic benefit in AD human brain slice cultures. Our long-term goal is to harness this information towards the development of an early-onset AD therapeutic.



Abstract: 2**Title: Modeled Wildfire Smoke Triggers Neurodegenerative Processes in the Aged Mouse Brain****Mohammad K. Siddiqi¹**, Temm P. Phan¹, Joseph Wang¹, Sarah E. Timis¹, David Scieszka², Matthew J. Campen², Andrew K. Ottens¹.¹Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA 23298²Department of Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM 87131**Abstract:**

Wildfires have intensified in recent years, contributing to an estimated 54,000 premature deaths over a decade in California alone. Wildfire smoke (WFS) particulates penetrate deep into the lungs, causing respiratory complaints and comorbid cardiovascular events with increased hospitalizations in impacted areas. Yet, little is known as to the effects of wildfire smoke inhalation on the brain. Epidemiological literature shows that older adults living in areas prone to WFS significantly increases the incidence of dementia, more than traffic pollution; yet, how this is connected with the pathogenesis of related neurodegenerative disease remains unknown and this study addresses this gap. This study employed a neuroproteomic investigation into the pathobiological outcomes following 2-weeks of 4h/day exposure to inhaled modeled WFS particulate in 18-month-old mic. Findings were then followed up with targeted assays to affirm results. Of 6558 proteins assayed, WFS significantly impacted 390 proteins at the 21-month timepoint, independent from normal aging, in abundance. The long-term impact of WFS was significantly associated with neurotransmission-related proteins involved in synaptic vesicle trafficking, the neurotransmission release cycle, transmission across chemical synapses. Further downstream effects analysis in Ingenuity Pathway Analysis predicted that these changes were tied to a significant increase in neurodegeneration ($p=0.000166$) as a disease process, a significant decrease in neurotransmission ($p<0.0001$) and an activation of apoptosis ($p=0.00363$). Results included notable biomarkers of neurodegenerative disease pathology amyloid precursor protein, tau and synucleins along with decreased proteostasis proteins. Ongoing confirmatory evidence shows an increase in beta-amyloid hippocampal pathology, increased phosphorylated tau in CA1 region, decreased glutamatergic synapses and activated caspase-3 staining, particularly in hippocampal input areas, corroborating neuroproteomics findings. Finding from this study underscores WFS as a new and growing environmental factor in increasing neurodegenerative processes in the aged mammalian brain, associated with Alzheimer's and related dementia.



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Imaging in Neuroscience

Abstract: 3**Title: Purkinje Cell Spike Patterns Do Not Predict Nuclei Cell Spike Patterns in Mouse Models of Cerebellar Disease****Lyon A.^{1,2} , van der Heijden M.^{1,2,3}**¹Graduate Program in Translational Biology, Medicine, and Health, Virginia Tech, Blacksburg, VA²Fralin Biomedical Research Institute at Virginia Tech Carilion, Roanoke, VA³School of Neuroscience, Virginia Tech, Blacksburg, VA**Abstract:**

Cerebellar dysfunction causes various movement disorders, including ataxia, dystonia, and tremor. Often these changes arise from neural dysfunction in the cerebellar cortex, through misfiring, miswiring, or degenerating Purkinje cells. Even though Purkinje cells form the sole output from the cerebellar cortex, their information is relayed to other regions of the motor network via cerebellar nuclei cells. Purkinje cells make inhibitory synapses onto cerebellar nuclei cells, and it is often assumed that changes in Purkinje cell spike patterns result in inverse changes in nuclei cell spike patterns. Here, we test this hypothesis by answering the question of whether a systemic relationship between Purkinje cell and nuclei cell spike patterns exists. Single-cell, in vivo electrophysiology recordings of both cell types from six mouse models for cerebellar movement disorders were analyzed according to parameters relating to spike rate and irregularity. First, we compared spike parameters to those observed in healthy controls, revealing a range of changes in Purkinje and nuclei cell spike patterns in the disease models. Next, we investigated whether Purkinje cell spike patterns predicted nuclei cell spike patterns. We found that parameters for spike irregularity were positively correlated between Purkinje and nuclei cells but no (especially no inverse) relationship was observed between Purkinje and nuclei cell spike rate. Overall, this study begins to illuminate the true relationship between Purkinje cells and nuclei cell spike activity in a disease state. The data does not suggest a distinct systemic relationship between these neuron types, meaning Purkinje cell spike activity changes cannot accurately predict nuclei cell changes. This information is essential for identifying how Purkinje cells can be targeted for future diagnostics and therapeutics.



CVCSN



Imaging in Neuroscience

Abstract: 4**Title: When Fish Flounder: A Zebrafish Model for Choice Paralysis Using a Discrete Choice Instrumental Response Task**

Hiltz E¹, Puhlick M¹, Brennan L², Buck R¹, Durante D², Dwyer M³, Glather R⁴, Krebs J⁵, Porner A¹, Scaia M¹, Thai-Nguyen A², Thayer Z¹, Landry K², Flores-Vaccari M² & Velkey A (PI)²

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Abstract :

Zebrafish (*Danio rerio*) have shown increasing utility as a research model for investigating the fundamental aspects of vertebrate behavior, as their neurological pathways and brain chemistry are similar to other vertebrates, including humans, in a number of ways. The phenomenon of choice paralysis can occur under two conditions; choice overload when an excessive number of options are presented, or the similarity effect when two options that are so similar that differentiation between each potential reward is difficult. Choice paralysis manifests under the similarity effect in notably delayed execution of choice responding despite the functional equivalence of each reward. In human research, this phenomenon is often explained by invoking higher-order cognitive functions (e.g. counterfactual reasoning, cognitive fatigue, executive dysfunction, etc), but an animal model in a simpler organism would provide a more fundamental approach to understanding the phenomenon. In the present study, choice paralysis due to the similarity effect in zebrafish is examined in an attempt to develop an animal model for the phenomenon. Subjects were housed in a submerged T-maze and underwent thrice-daily trials (09:00, 12:00, and 15:00) of instrumental responding on a discrete choice task. During each trial, subjects were provided the choice between different pairs of rewards involving a visual interaction via a live conspecific (high-fidelity stimulus) or a mirror presentation (low-fidelity stimulus). Choice conditions included: mirror vs mirror, mirror vs same-sex conspecific, mirror vs opposite-sex conspecific, and same-sex conspecific vs opposite-sex conspecific. Preferences were considered stabilized after 15 trials if subjects consistently selected the same option in 8 of the 10 most recent trials. Subjects in the mirror-mirror condition showed no instrumental reinforcement or preference, whereas subjects provided with more distinct choice conditions demonstrated instrumental reinforcement and were more likely to develop a preference. Regardless of preference or not, however, patterns emerged indicating zebrafish can demonstrate the choice paralysis effect due to option similarity. Additional replications and refinements will be obtained in future research. Finally, video analyses of subjects' behavior in response to varying stimulus fidelity could provide further understanding of zebrafish social preferences and the mechanisms underlying choice paralysis.



CVCSN



Imaging in Neuroscience

Abstract: 5**Title: Hippocampal Bioenergetic Responses & Changes in Neuronal Architecture in Mouse Brains Following Wildfire Smoke Exposure****T. P. Phan¹**, M. K. Siddiqi¹, S. E. Timis¹, D. Scieszka², M. J. Campen², and A. K. Ottens¹.¹Virginia Commonwealth University, Richmond, VA²University of New Mexico, Albuquerque, NM.**Abstract:**

Wildfire smoke (WFS) has been linked to an increase in dementia in WFS-prone regions, raising concern that WFS is a significant environmental modifier of Alzheimer's disease and related dementia (ADRD) pathogenesis. Thus, there is a need for accessible biomarkers to study at-risk individuals and WFS's broader health profile. We applied serum peptidomics as a biomarker discovery platform to evaluate early-onset peptide indicators. Putative biomarkers were evaluated against brain protein origins and associated with neurodegenerative processes in the hippocampus, a key site of early AD.

A neuropeptidomic dataset using serum samples of mice following 3 weeks of 4 hr/day WFS particulate (100 µg/m³) exposure was used to identify peptides with significant changes under WFS-like conditions. Significant peptides were assessed for their protein origins within the brain, contrasted against a previously generated hippocampal dataset. Putative brain-enriched serum peptides, notably Drp1, were further evaluated using immunofluorescence microscopy. Results were separately assessed across dentate gyrus (DG), hippocampal fissure (HF), CA3, CA2, CA1, and subiculum (Sub) subregions.

Mass spectrometric analysis identified 389 peptides in serum of WFS exposed mice relative to filtered air (FA), of which 160 (41.1%) were significantly responsive. 126 (78.8%) were from proteins known to be expressed in the brain, 84 of which were brain enriched in association with synaptic function and mitochondrial bioenergetics. Neurodegenerative conditions in early pathogenesis are often tied to perturbed energetics, resulting in synaptic dysfunction and apoptosis. Cend1 and Drp1 were identified as key regulators in bioenergetic dynamics of neurons. Thus, the presence of these peptides may be relevant to detecting an acute-phase response to altered brain energetics. Drp1 loss from a mitochondrial tethered state (-2.1, log₂; p<0.001) was identified in hippocampal tissue in proteomics. Immunofluorescence confirmed an overall loss in Drp1 acutely after WFS, though this response appears regionally dependent, particularly in CA1 and subiculum. Moreover, loss of Drp1 appears absent any overt loss in mitochondria density as stained by CoxIV.



CVCSN



Abstract: 6**Title: Distinct dominant and non-dominant hand fine motor impairments in early Parkinson's Disease****Taylor L¹**, Gebremariam N¹, Manning J¹, Selb C¹, Barrett MJ², Berman BD², Pidcoe P³, Krusienski D⁴, Dexheimer B¹¹Department of Occupational Therapy, Virginia Commonwealth University, Richmond, VA²Department of Neurology, Virginia Commonwealth University, Richmond, VA, USA.³Department of Physical Therapy, Virginia Commonwealth University, Richmond, VA⁴Department of Biomedical Engineering, Virginia Commonwealth University, Richmond, VA**Abstract:**

Initial Parkinson's Disease (PD) motor symptoms, including tremor, bradykinesia, and rigidity, typically begin unilaterally. Notably, individuals with initial symptoms on their non-dominant side are diagnosed sooner, relative to symptom onset, than those with initial dominant side symptoms. This on-going study examines whether early PD motor deficits differ in functional impact based on symptom laterality (dominant vs. non-dominant) and task component (trajectory control vs. stabilizing control). Participants performed a bimanual fine motor task on a touchscreen tablet. One hand moved a stylus to on-screen targets (trajectory control), while the other stabilized a second stylus over a stationary target (stabilizing control). A tension band connected the styluses to simulate real-world bimanual forces. Trajectory control was assessed via peak velocity and deviation from the linear path, while stabilizing control was measured by mean stylus displacement. Our preliminary dataset includes 15 right-handed (determined via the Edinburgh Handedness Inventory) individuals: 13 with early PD (6 male, 7 female, Hoehn & Yahr 1–2; mean age 71.8 ± 8.1 SD) and two controls (1 male, 1 female, mean age 69.0 ± 4.2 SD). PD severity was measured using the Movement Disorder Society Unified PD Rating Scale (MDS-UPDRS) Part III subsection. On the fine motor task, PD and control participants had similar trajectory control deviation and peak velocity, though dominant hands showed significantly lower deviation overall ($p=0.002$). Stabilizing control was significantly impaired in PD participants ($p=0.03$), though mean displacements were similar between hands. Correlational analysis revealed that dominant-side MDS-UPDRS scores were significantly associated with dominant hand stabilization ($r^2 = 0.61$, $p=0.002$) and near-significantly associated with trajectory control ($r^2 = 0.25$, $p=0.08$). Non-dominant side MDS-UPDRS scores correlated near-significantly with peak velocities ($r^2 = 0.29$, $p=0.059$) but not with trajectory or stabilizing control measures. These findings suggest that early PD impairs stabilizing control mechanisms, a key aspect of fine motor control. Furthermore, motor severity appears to correlate only with dominant-side deficits. Data collection is ongoing, but preliminary results indicate that left- and right-side PD symptoms differentially impact fine motor deficits.

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Abstract: 7**Title: Modeling Interstitial Cystitis/Bladder Pain Syndrome in Mice: Pain-Depressed Locomotor Effects of Cyclophosphamide****Alayna S. Palamar¹, Naomi Carter¹, Julie A. Suyama², & S. Stevens Negus¹**¹Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA, 23298²Department of Obstetrics and Gynecology, Virginia Commonwealth University, Richmond, VA, 23298**Abstract :**

Pain is a debilitating symptom commonly associated with illness, injury, and disease that can lead to diminishing effects in our personal lives and careers. Preclinical models of pain-depressed behavior are needed to aid in discovery and development of new analgesics to restore baseline behavioral function. Interstitial cystitis/bladder pain syndrome (IC/BPS) is a heterogeneous clinical syndrome characterized by bladder/pelvic pain and associated behavioral depression (Tay & Grundy, 2023). In rodent models, the chemotherapeutic drug cyclophosphamide (CYP) has been administered through intraperitoneal (IP) injection to study IC/BPS. CYP is metabolized to the bladder toxin acrolein which produces bladder inflammation and hematuria, like the effects of IC/BPS (Talar-Williams et al., 1996). In a previous study, administration of IP CYP showed reduced locomotor activity in mice (Bon et al., 2023). This study aims to develop a preclinical model of IC/BPS-related behavioral depression for use in analgesic drug development by evaluating potency, effectiveness, and time course of IP CYP in reducing mouse locomotor activity. We used IP injection of dilute lactic acid as a positive control in this study as it has been previously used in the lab to examine locomotor effects related to pain-depressed behavior. To assess locomotion, we used a 2-compartment activity chamber to measure the number of crosses and movement counts across a 15-minute session with each mouse. Subjective behavior was also recorded for grimace, hunched posture, and piloerection scores during IP CYP assessment. Injection of IP lactic acid indicated concentration-dependent effects on locomotion with relative dissipation after 80 minutes. For IP CYP, a high dose of 32 mg/ml significantly reduced movement counts in mice, but dose largely did not affect crossing behavior. Movement also significantly decreased at shorter pretreatment times with 32 mg/ml CYP, but no significant differences were found for crosses. Additionally, CYP produced both dose- and time-dependent increases in posture and grimace scores. These findings indicate signs of discomfort that may be linked to bladder inflammation and therefore pain. Further research should aim to investigate the use of different analgesics to alleviate these signs of pain-depressed behavior.



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Abstract: 8**Title: S1P-S1PR1 signaling drives layer specific cortical astrocyte morphogenesis.****Tuck C.**^{1,2}, Chandra Rao S.², Tariq Z², Gonzales J.P.^{1,2}, Marcelli P.¹, Dominguez O.^{1,2}, Singh S.²¹Biomedical Sciences Doctoral Portal Program, VCU; ²Department of Biochemistry and Molecular Biology, VCU.**Abstract:**

Development of the mammalian brain is stringently coordinated and organized, mediated by intricate physical, secretory, and spatiotemporal modes of cross-communication between nervous system cells. Astrocytes, one of the most abundant cell types in the brain, come into direct physical contact with or proximity to neighboring cells through their complex, ramifying processes, serving as an interface for intercellular communication. These interactions allow astrocytes to provide metabolic and trophic support to energetically demanding neurons, coordinate with microglia to eliminate debris and prune excess synapses during synaptogenesis and induce oligodendrocyte-dependent axonal myelination. However, the intercellular signaling events and molecular mechanisms that establish these fine astrocytic processes are not fully characterized. Recently, our lab described that neuronal contact induces expression of a sphingosine-1-phosphate (S1P) sensitive G-protein coupled receptor, sphingosine-1-phosphate receptor 1 (S1PR1), in astrocytes which in turn drives expression of synaptogenic factors and morphogenesis of astrocytes *in vitro*. In this work, we utilized an adeno-associated viral based approach to sparsely label cortical astrocytes (using membrane targeted (Ick)-GFP and GFP fill) to assess their morphological complexity in astrocyte specific S1PR1 knock-out mice. Our data exhibited region specific differences in morphological maturation of astrocytes lacking S1PR1. These findings highlight the existence of a neuron-to-astrocyte S1P-S1PR1 signaling axis necessary for the proper structural maturation of astrocytes.



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Abstract: 9**Title: Astrocyte-Oligodendrocyte Crosstalk Dependent myelination via secreted protein YKL40****Dominguez O¹, Munir S¹, Tuck C¹, Gonzales J¹, Rao S², Singh S²**¹Biomedical Sciences Doctoral Portal, VCU; ²Department of Biochemistry and Molecular Biology, VCU**Abstract:**

Chi311, otherwise known as YKL-40, is a secreted glycoprotein that is primarily expressed by astrocytes in the brain. Increased levels of YKL-40 are heavily associated with inflammatory and disease conditions throughout the body. Interestingly, high levels of YKL-40 in CSF are associated with Alzheimer's disease and multiple sclerosis progression. Despite its prevalence and use as a biomarker in these diseases, the fundamental role of YKL-40 in the developing brain remains untapped. In this study, we discovered that YKL-40 is at the center of astrocyte-oligodendrocyte crosstalk dependent myelination in the developing brain. We find that YKL-40 is expressed by astrocytes in the developing brain coinciding with OPC differentiation and myelination. Interestingly, expression of YKL-40 is specifically induced in astrocytes by the cocultures of OPCs *in vitro*, which in turn promotes OPCs' differentiation. Mechanistically, purified YKL-40 significantly induced the expression of Olig2 and MYRF (Myelin regulatory factor) transcription factors in the OPCs. Moreover, mice with YKL-40 deleted specifically in astrocytes showed delayed developmental myelination. These results identified that astrocytic YKL-40 is vital for OPC-astrocyte cross communication dependent myelination.



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Abstract: 10**Title: Astrocytes exhibit reduced complexity and morphology when Shh pathway and S1PR1 receptor is blocked****Harris, J.,¹ Thumu, S.C.R.,¹ Gonzales, J.P.,¹ Tuck, C.,¹ Munir, S.,¹ Dominguez, O.,¹ Singh, S.¹**¹Department of Biochemistry and Molecular biology, Virginia Commonwealth University, Richmond, Virginia, USA**Abstract:**

Astrocytes are the most abundant cells in the mammalian brain and are acknowledged for multiple functions which include nutritional support to neurons, maintenance of blood brain barrier integrity, neuronal circuit assembly and numerous physiological functions. Several of these manifold functions are established through their complex morphology, which is also pivotal for the bidirectional communication between neurons and astrocytes. We have previously shown that throughout the brain, astrocytes are the cells that explicitly express S1PR1 receptors and is responsible for maintaining their morphology through its interaction with S1P generated by neurons. However, the pathways that regulate this communication remain uncertain. In this work, we show the impacts of cell differentiation and intracellular interaction on astrocyte growth through the application of Sonidegib and W146 in astrocyte neuron cell cultures. For this, we isolated mixed cells from S1PR1-GFP, P2 mouse pup brains and treated either with 5uM of Sonidegib (Shh pathway inhibitor) or 200 nM of W146 (S1PR1 inhibitor) for 4 days. The cells were then subjected to immunohistochemistry analysis and imaged. Assessment of astrocyte morphology using sholl analysis showed that in astrocyte-neuron cultures exposed to these substances, a sharper decrease in branch complexity across distance was observed when compared to DMSO control. In addition, the overall morphology analyzed through the measurement of perimeter and area of the astrocytes remained significantly reduced when Shh pathway and S1PR1 receptor were blocked. Additional experiments are underway to determine the exact mechanism to substantiate these findings.



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Abstract: 11**Title: Glioblastoma secretome as a key modulator of reactive astrogliosis and glutamate dysregulation****Nalluri H.**¹, Escalante M.², Fleischel E.², Menna S.¹, Sontheimer H.²¹College and Graduate School of Arts & Sciences, University of Virginia, Charlottesville, VA 22904, USA²Department of Neuroscience, School of Medicine, University of Virginia, Charlottesville, VA 22903, USA**Abstract:**

Glioblastoma (GBM) is the most aggressive primary brain tumor, with a poor prognosis of approximately 15 months post-diagnosis. As the tumor progresses, patients often experience debilitating complications, including headaches, cognitive impairment, and tumor-associated epilepsy (TAE). A key contributor to the aggressive nature of GBM is its capacity to manipulate the central nervous system (CNS) microenvironment to promote tumor progression. Astrocytes, the predominant glial cell type in the CNS, play essential roles in neural development and homeostasis by providing metabolic and trophic support, facilitating neurovascular coupling, and regulating synaptogenesis, synaptic transmission, and plasticity. In response to various insults, astrocytes undergo a highly heterogeneous and dynamic process known as "reactivity," marked by significant transcriptomic and functional changes that depend on the nature of the initiating insult. Recent studies underscore the role of reactive astrocytes in glioma progression and TAE, with evidence showing that depleting reactive astrocytes halts tumor growth, while epileptogenesis correlates with increased system x_c^- (SXC) activity in peritumoral astrocytes. However, the underlying mechanisms remain poorly understood, largely due to the tumor's complex interactions with its microenvironment. We hypothesize that glioma-secreted factors drive both tumor progression and hyperexcitability in the tumor microenvironment (TME). Here, using indirect glioma-astrocyte cocultures, functional assays, and bulk RNA-seq, we found that GBM cells enhance astrocytic SXC activity and induce pronounced astrocyte reactivity through secreted factors. Further analysis revealed that the increase in SXC activity is driven by a protein component in the glioma secretome, leading to transporter upregulation at the plasma membrane and a corresponding increase in mRNA levels. In contrast, reactive gliosis appears to be mediated by a non-protein component, triggering widespread overexpression of A1-, A2- and pan-reactive genes, along with proliferation-associated genes. These findings highlight the complexity and heterogeneity of astrocytic responses to glioma-secreted factors. Finally, using orthotopic patient-derived xenograft tumor models and in situ hybridization (ISH), we corroborate an increase of SXC mRNA transcripts in tumor-associated astrocytes. Collectively, these findings suggest that the secretome of GBM is sufficient to disrupt astrocyte function and glutamate homeostasis in the tumor microenvironment, which may contribute to cortical hyperexcitability, excitotoxicity and tumor progression.



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Abstract: 12**Title: Exploring the role of ZFP189 mediated transcription on microglial activity in the prefrontal cortex**Carwile N^{1,2}, Truby NL¹, Hassan A^{1,3}, Ferede L¹, Lefkowitz B², Hamilton PJ¹.¹Department of Anatomy and Neurobiology, VCU²Department of Biology, VCU³Department of Pharmacology and Toxicology, VCU**Abstract:**

Rationale: Microglia are phagocytic cells which reside in the brain and spine. Once thought to be passive in the healthy brain, microglia are now recognized as key cellular mediators of neuroplasticity and behavior modulation. Previous research identified ZFP189 as a transcription factor (TF) linked to stress resilience, with its overexpression in the prefrontal cortex (PFC) protecting against social deficits. Using synthetic ZFP189 TF variants, our lab demonstrated that altering its transcriptional control impacts immune gene expression, dendritic spine morphology, and social behavior. This study aims to investigate how ZFP189-driven transcription specifically within PFC pyramidal neurons influences microglial activity and synaptic pruning. This work may provide insight into the molecular mechanisms underlying neuroplasticity and social behaviors. **Objectives:** We hypothesize ZFP189-mediated transcriptional control within the PFC controls the presentation of neuronally expressed markers that are detected by microglia and promotes microglial-driven spine pruning at manipulated neurons. **Methods:** Male and female C57BL/6J mice (8-10 wks old) from JAX Labs were used. Stereotaxic surgery was employed to virally deliver synthetic transcription factor variants to the prefrontal cortex. Three days following surgery, mice were perfused, and brains were removed and postfixed in PFA. Following postfix, whole brains were cryoprotected in a sucrose/sodium azide solution. Brains were flash frozen in isopentane over dry ice and stored at -80°C. A Leica CM1860 cryostat was used to obtain 20µm slices of the PFC for each brain. Tissues underwent immunohistochemistry to stain for IBA-1, a microglia-specific ionized calcium binding adaptor protein, and GFP, which is co-expressed with our synthetic transcription factors. Tissues were mounted and imaged using a Keyence BZ-X810 microscope. Images were analyzed using Imagej software for percent coverage and Sholl analysis. **Results:** Preliminary results from percent coverage analysis suggest both synthetic repressive and opposite activating forms of ZFP189 TF in the PFC result in reduced localization of microglia to transduced pyramidal neurons. Further Sholl analysis may provide a deeper insight into the activity of microglia in relation to ZFP189 transcriptional modulation and microglial activity in the prefrontal cortex.



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Imaging in Neuroscience

Abstract: 13

Title: Exploring the Role of Microglia in Engram Representations of Social Memories
Campbell, E.A.^{1,2}, Tatera, W.J.¹, Lee, S.¹, Sanchez, B.¹, Kraszewski, P.¹, Cope, E.C.¹

¹Department of Neuroscience, Brain Immunology and Glia Center, University of Virginia's School of Medicine

²University of Virginia Department of Psychology

Abstract:

Social memory is a necessary cognitive-behavioral process that allows organisms to recognize other members of their species and form meaningful relationships. Deficits in social memory are an especially devastating, yet poorly understood symptom of many neurodegenerative and neuropsychiatric conditions, including Alzheimer's disease, autism spectrum disorder, and major depression. To identify therapeutic targets to remedy such deficits, we must gain mechanistic insight into the neural processes and circuitry that underlie social memory. The hippocampal CA2 subregion and its excitatory projection to ventral CA1 (vCA1) are essential for the processing, storage, and retrieval of social memories. Memories are believed to be encoded in cellular ensembles termed "engrams". These neuronal populations are activated by a learning experience and, when reactivated, induce memory retrieval of that experience. Microglia, the brain's immunocompetent cells, are known to modulate neuronal activity, indicating a mechanism by which microglia-neuron interactions may shape engram representations of social memories. We observed marked social memory deficits in mice depleted of microglia via colony-stimulating factor 1 receptor inhibitor, PLX-3397. When allowed to repopulate, microglia exhibited morphological changes associated with a more "activated" state, including increased soma volume and reduced process complexity. Current work utilizes the Targeted Recombination in Active Populations (TRAP2) tamoxifen-inducible genetic system to investigate how microglia regulate neuronal activity underlying social memory. *TRAP2^{+/-};ZsGreen^{+/-}* mice were produced by crossing *TRAP2^{+/+}* mice with a *ZsGreen^{+/-}* reporter. 4-OHT injection was used to induce recombination in the TRAP2 system and permanently "tag" neurons activated during social memory encoding; combined with immunolabeling of reactivated cells expressing Fos (activation marker) during social memory retrieval, putative "engram" neurons will be identified. *TRAP2;ZsGreen* mice were depleted of microglia with PLX-3397, subjected to novel and familiar social interactions, and perfused to examine Fos with immunolabeling. ZsGreen⁺ and Fos⁺ cell counts in CA2 and vCA1 will reveal whether microglia depletion alters neuronal activity in response to a novel or familiar conspecific, respectively. ZsGreen⁺/Fos⁺ co-labeled cell counts will elucidate whether microglia modulate neuronal ensembles activated during social memory encoding and reactivated upon retrieval (i.e., social memory engrams). Future work will explore how changes in CA2 neuronal activity influence microglia-neuron interactions.



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Abstract: 14**Title: Characterization of Paclitaxel-Induced Neuroinflammation: Temporal, Sex, And Tissue-Specific Molecular Analysis****Rakholia Y¹, Soleo L¹, Das P², Wages NA², Damaj MI¹.**¹Department of Pharmacology and Toxicology, ²Department of Biostatistics, School of Public Health, Virginia Commonwealth University, Richmond, VA 23298, USA**Abstract:**

Peripheral neuropathy is one of the most prevalent neurotoxic, dose-limiting side effects of paclitaxel, a chemotherapy agent used widely in solid cancers. The mechanism of paclitaxel-induced peripheral neuropathy (PIPN) is poorly understood, and thus there are no approved treatments currently. Notably, neuroinflammation has been described as a cardinal component in the pathogenesis of PIPN. However, animal studies of PIPN assessing neuroinflammation mediators have mostly focused on gene expression, not protein, and usually in one neuronal tissue and/or at one time point in male mice. Thus, characterization of inflammation mediators in both sexes, in different neuronal tissues, and at different timepoints is critical to understanding PIPN. Paclitaxel (8 mg/kg, i.p.) or vehicle was administered every other day for a total of four injections in C57BL/6J mice. Mechanical and cold sensitivity, nerve conductance, and 22 cytokines and chemokines levels in the dorsal root ganglia (DRG) and spinal cord were measured at different time points (7, 14, and 21 days) in both sexes. Paclitaxel induced mechanical and cold hypersensitivity and decreased nerve conduction amplitude, the latter was more pronounced in male than female mice. Multiplex cytokine analysis revealed that paclitaxel induced increase in neuroinflammation is time-, sex-, and tissue- dependent. Our findings contribute to current knowledge about neuroinflammation as an important mechanism in PIPN and thus advance efforts to identify targets for novel therapies. In addition, the results inform us about potential mechanistic sex differences that can guide precision medicine.



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Abstract: 15**Title: Evaluating P2RY12 as a Pan-Microglial Marker for Comprehensive Sampling in the Developing Multisensory Midbrain**Sophie M. Khadr¹, Sophia V. Lindauer¹, Mark L. Gabriele¹¹Department of Biology, James Madison University**Abstract:**

The inferior colliculus (IC) is a midbrain relay hub for auditory processing, with shell regions that receive and integrate a rich array of multisensory inputs. Among these, the lateral cortex of the IC (LCIC) exhibits a compartmentalized structure that emerges during a defined early postnatal window (P0–P12), wherein somatosensory and auditory afferents segregate into distinct zones—modules and matrix, respectively. Our lab has previously demonstrated that microglial cells (MGCs) play a crucial role in refining these afferent patterns through selective pruning mechanisms. Notably, distinct microglial subpopulations with unique molecular signatures appear to occupy different LCIC compartments, potentially executing specialized functions during circuit maturation. To advance our investigation into microglial heterogeneity in the LCIC, we sought a broadly expressed marker that would facilitate the comprehensive isolation of these cells. P2RY12, a purinergic G-protein coupled receptor with microglia-specific expression, emerged as a strong candidate. This study examined P2RY12 labeling in neonatal GAD67-GFP and CX3CR1-GFP mice to determine its spatial expression profile within the developing LCIC. Our findings reveal robust P2RY12 expression across all IC subdivisions, with widespread presence in the LCIC. Compared to previously tested microglial markers (e.g., Iba1, CX3CR1, CD11b, TMEM119, SIRP- α), P2RY12 demonstrated the most extensive distribution. These results support its utility in future studies leveraging P2RY12Cre lines for microglial isolation and transcriptomic profiling, enabling deeper exploration of microglial diversity in multisensory midbrain development.



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Abstract: 16**Title: Social memory dysfunction coincides with microglia-dependent remodeling of hippocampal CA2 perineuronal nets****Sanchez B*¹, Lee S*¹, Remy CM¹, Campbell EA¹, and Cope EC¹**¹Department of Neuroscience and Center for Brain Immunology and Glia (BIG), UVA

*Authors contributed equally

Abstract:

Social memory is required for adaptive social interactions, with its dysfunction being a feature of different neuropsychiatric and neurodegenerative disorders. The hippocampal CA2 region and its connections form a unique circuit critical for social memory function. One structural distinction that separates the CA2 from its neighboring subregions is its high abundance of perineuronal nets (PNNs), condensed extracellular matrix structures that ensheath particular neurons and regulate their plasticity. Previous work showed that disrupting CA2 PNNs impairs social memory, and restoring atypical CA2 PNN levels improves social memory in mouse models of social dysfunction. While this suggests that PNNs may serve as a potential therapeutic target for social memory, the specific cellular and molecular processes that regulate CA2 PNNs remain unexplored. Microglia, the brain's resident macrophages, participate in the homeostatic remodeling of PNNs and contribute to their loss in disease. In current work, we explored the role of microglia in remodeling CA2 PNNs under homeostatic conditions. First, we fed mice pexidartinib (PLX3397) to pharmacologically inhibit colony-stimulating factor 1 receptor, effectively depleting microglia. In mice lacking microglia, we observed social memory impairments such that they were unable to distinguish between novel and familiar conspecifics. Although we did not find differences across all structural components of PNNs, we observed increased intensity of *Wisteria floribunda agglutinin* (WFA), which labels the glycosaminoglycan chains attached to chondroitin sulfate proteoglycans, in the CA2 after microglial depletion. Additionally, we observed increased intensity of the chondroitin sulfate proteoglycan aggrecan, an essential PNN component. This indicates that microglial elimination causes an accumulation of some, but not all, PNN components. Using WFA, we then examined PNNs in other hippocampal regions and found little to no change in mice lacking microglia, suggesting that the CA2 may be more vulnerable to microglial-mediated PNN remodeling. Next, we investigated whether repopulation of microglia reverses CA2 PNN accumulation. After cessation of the PLX3397 diet, we found that restoration of microglial numbers coincided with a reduction of WFA+ PNN intensity in the CA2 back to control levels. Ongoing studies are exploring the turnover rate of PNNs induced by microglial depletion and repopulation in the hippocampal CA2 region.



Abstract: 17**Title: NIK and IKK2-binding protein (NIBP), a novel host protein that restricts HIV-1 reactivation in CHME5 microglial cells****Hossain RA¹, Yuan X¹, and Hu W¹**¹Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, 1101 E Marshall St. Richmond, VA 23298**Abstract:**

Though mammalian cells express restriction factors that interfere with various stages of viral infection, HIV-1 is able to circumvent and even exploit this machinery to maintain latency in HIV-1 reservoir cells. Elucidating the mechanisms behind restriction factors and the HIV-1 life cycle allows for the development of targeted therapeutics against conditions such as NeuroHIV. Our lab previously identified NIK and IKK2-binding protein (NIBP) which enhances cytokine-induced NFκB activation in neurons and cancer cells. Contrary to expectations of NFκB-dependent upregulation of HIV-1 long terminal repeat (LTR) promoter activity, NIBP overexpression significantly suppressed TNFα-induced activation of HIV-1 LTR-luciferase reporter activity in neural stem cells. NIBP deficiency in human fibroblast cells increased TNF-induced LTR-luciferase activation, which was reversed by NIBP overexpression. In the TZM-bl cell line which stably expresses LTR-luciferase reporter, adenovirus-mediated NIBP overexpression blocked HIV latency reactivation induced by TNFα or TSA. Given microglial cells are well known for HIV infection/latency and lack of NIBP expression, we hypothesize that *NIBP gene therapy may suppress HIV-1 replication/reactivation in HIV-1 permissive cells such as microglia and CD4 T cells.* Using microglia cell line CHME5, which latently expresses HIV-1 d2eGFP, we found that infection of CHME5 cells with adenovirus carrying full-length NIBP significantly suppressed HIV-1 reactivation induced by various latency-reversing agents, such as TNFα, TSA, and PMA, when compared to the control. The N-terminal deletion of NIBP abolished this suppressive effect. These findings suggest that NIBP may serve as a novel restriction factor to suppress or block HIV-1 infection/reactivation. Since NIBP is highly expressed in HIV-1 non-permissive neurons but not in permissive microglia/macrophage and T cells, NIBP gene delivery into HIV-1 permissive cells could serve as a novel gene therapy to treat HIV and NeuroHIV.



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Abstract: 18**Title: Novel CD4 targeting dual DARPin-coated lipid nanoparticle (LNP) delivers CRISPR/Cas editor to eradicate HIV proviral genome in CD4 T cells****Abdul Rasheed Baloch**¹, Subhra Mandal², Danmeng Zhang¹, Qingsheng Li² and Wenhui Hu¹¹ Department of Anatomy and Neurobiology, VCU School of Medicine, Richmond, VA, USA² Nebraska Center for Virology, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE, USA.**Abstract:**

Antiretroviral therapy (ART) has revolutionized HIV-1 treatment, transforming a once-fatal disease into a manageable chronic infection. However, even brief ART interruption (ATI) triggers rapid viral rebound from hidden reservoirs in cells and tissues. To achieve a true “sterile” cure, novel strategies are urgently needed to eradicate or permanently silence HIV-1 proviruses. CRISPR/Cas9-mediated editing has shown promise in excising HIV-1 proviruses *in vitro*, *ex vivo*, and *in vivo*, bringing us closer to a potential cure. However, a critical challenge remains in precisely, efficiently, and safely delivering editors to HIV latent reservoirs like CD4 T cells. In this study, we designed a synthetic lipid nanoparticle (LNP) to deliver spCas9 mRNA and HIV-targeted duplex sgRNAs specifically to CD4 T cells *in vivo* using human CD4-specific Designed Ankyrin Repeat Protein (DARPin). We selected dual DARPins (-55 and -57) to enhance CD4 receptor binding. Our study indicates that CD4-specific DARPin-coated LNPs carrying SpCas9 mRNA and LTR+GagD sgRNAs achieved selective uptake by CD4 receptor-expressing monocytes and CD4 T cells in PBMCs isolated from a healthy donor, while showing minimal accumulation in non-target cells (CD8 T cells and B cells). *In vitro* HIV proviral DNA excision studies on latent J-Lat and D10 cells demonstrated that the sgRNA-guided spCas9 CD4-LNP system could effectively induce HIV proviral excision both in basal latent states and following reactivation. The *in vivo* study in a humanized mouse model confirmed bioaccumulation of CD4-targeted LNPs and effective delivery of sgRNAs and EGFP-Cas9 mRNA across various HIV-prone anatomical compartments and cellular reservoir sites. In conclusion, these findings demonstrate that CD4-targeted dual DARPins can effectively direct duplex sgRNA/spCas9 editors via novel LNPs to CD4 T cells both *in vitro* and *in vivo*, leading to efficient eradication of the HIV proviral genome. Our proof-of-concept study underscores the therapeutic potential of this highly specific CD4-targeting LNP system for delivering CRISPR/Cas editors to eliminate latent cellular reservoirs for a HIV cure.



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Abstract: 19**Title: AAV bar-coded library screening identified microglia-targeting and BBB-crossing AAV-BM serotypes****Danmeng Zhang¹, Xinxu Yuan¹, and Wenhui Hu¹**¹Department of Anatomy and Neurobiology, Virginia Commonwealth University, School of Medicine, 1101 E Marshall St. Richmond, VA 23298**Abstract:**

In the past years, extensive efforts using various types of AAV library screening have identified an increasing number of blood brain barrier (BBB)-crossing serotypes (AAV-B) that can efficiently transduce neurons and astrocytes. However, efficient transduction of microglia remains a challenge. To address this, we developed a novel dual-lock AAV transfer vector, which consists of a microglia-specific promoter (HexB or CD68) and a miRNA target sequence (4x miR9T) to enable increased transgene expression specifically in microglia. Using this optimized vector, we encapsulated four bar-coded AAV transfer vectors into existing, publicly available AAV-B serotypes (28) to construct a bar-coded (bc) AAV-B library for screening serotypes that can efficiently transduce microglia. Using primary microglia cultured from LoxP-STOP-LoxP-tdTomato (LSL-tdT) mice (Ai14), we identified eight AAV-B serotypes that can transduce microglia at the efficiency comparable to R2e-MAC. To ascertain the potential of these 8 AAV-B serotypes (AAV1/2, AAV-DJ8, AAV6TM, AAV9, AAVrh.10, AAV-HSC16, AAV-Pal2, and AAV-MDV1A) in transducing microglia in vivo, we performed intracerebral injection (IC) of a mixed AAV-B formulation (1.34e10 GC/ μ l each) in adult LSL-tdT mice (Male, 3-month-old, 2 μ l/site, 4 sites). At 1 week after injection, tdT+ microglia were observed at the injection sites, suggesting some of these 8 AAV-B serotypes can transduce microglia in vivo. Microglial transduction was further validated through intravenous (IV) individual AAV-B injections (28) and confocal colocalization analysis with anti-IBA and anti-GFAP antibodies. The most effective AAV-Bs to transduce microglia in vivo include AAV1/2, AAV-DJ8, AAV-9p31, AAVRh10, AAV-Rh39, AAV-Pal2, AAV6-TM and AAV-HSC16. In conclusion, our novel AAV transfer vector with a dual-lock system enabled previously uncharacterized AAV-B serotypes to cross the BBB and selectively transduce microglia. These serotypes show promise for microglia-targeted gene therapy, though transduction efficiency varies by serotype and dosage.



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Abstract: 20**Title: Microglial Ramification and Landscape Rearrangement in Lipopolysaccharide Induced Respiratory Infection****Lawani O** ^{1,3,4,5}, Doceti M^{2,3,5}, Duffy A ^{3,4,5}, Eyo U ^{3,4,5}¹Program in Fundamental Neuroscience²Department of Microbiology, Immunology, and Cancer³Center for Brain, Immunology, and Glia⁴Department of Neuroscience⁵University of Virginia**Abstract:**

Microglia are brain-resident immune cells that serve a central role in regulating neuroinflammatory processes in cerebral pathology. One of their most notable features is the extensive morphological plasticity by which they perform surveillance of the central nervous system in both physiology and pathology. While microglial dynamics have been well documented in physiology, microglial behavior in infection, particularly respiratory infection, remain poorly understood. Here, we show that intratracheal lipopolysaccharide (IT-LPS) injection, used to simulate respiratory infection, induces increased microglial emergence, clearance, and migration in wildtype mice, a process that can be broadly described as microglial rearrangement. Further, we show that IT-LPS treated mice exhibit a higher density of CD68+ microglia, despite both experimental and control groups having the same total number of microglia, indicating a more phagocytic microglial phenotype. Fascinatingly, Csf1r^{FIRE} (Fire KO) mice—a genetically modified model in which microglia fail to develop—do not exhibit sickness behavior (i.e., significant weight loss), highlighting microglia's potential role in immune response. Based on this data, we hypothesize that microglia play a necessary role in producing immune response in respiratory infection. Given that microglia respond to respiratory infection of lipopolysaccharide (LPS) by modulating their behavior, it remains of interest to determine whether microglia modulate the structure and function of neighboring structures, particularly cerebrovasculature in respiratory infection. By studying these interactions, we hope to uncover novel relationships between microglia and vasculature relevant for brain function in various pathologies.



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Abstract: 21**Title: Anti-inflammatory and Neuroprotective Effects of Intramuscular Verapamil following Organophosphate DFP-induced Status Epilepticus in Rats**Y. Paudel¹, E. Hawkins¹, R. Blair¹, and L. Deshpande¹.¹Virginia Commonwealth University, Richmond, VA.**Abstract:**

Background and Purpose: Organophosphate (OP) compounds are chemical threat agents. Exposure to these compounds at high doses triggers a hypercholinergic response that could lead to status epilepticus (SE) and even death. Despite the treatment with standard-of-care therapy, OP-SE survival is associated with long-term neurological morbidities, including recurrent seizures and memory deficits. Neuronal injury and sustained neuroinflammation have been reported post-OP induced SE. Therapeutic strategies targeting cell death and persistent inflammation have produced beneficial outcomes in rat models of OP-SE. Verapamil (VPM) is a calcium-channel blocker that is used clinically for blood pressure and angina management. It has been reported to exert neuroprotective and anti-inflammatory effects in animal models of CNS injuries reflecting its re-purposing potential. Herein, we investigated the safety and toxicity profile of VPM therapy and compared its pharmacokinetic (PK) profile when administered via intramuscular (I.M.) and oral route. Further, we evaluated the neuroprotective and anti-inflammatory effects of VPM following DFP-SE. **Methods:** Rats (SD; male and female; 250-300g) were injected with DFP (4 mg/kg, S.C.). One minute later, atropine sulfate (0.5 mg/kg, I.M.) and 2-PAM (25 mg/kg, I.M.) were injected. Rats immediately developed SE, which was then controlled with midazolam (1.78 mg/kg, I.M.) at 1-h post SE onset. Rats were then treated with VPM (10 mg/kg, I.M.) b.i.d. for 3 days. Mortality was assessed daily. On the fourth day, rats were perfused, and brains were post-fixed for sectioning. Neuronal injury was assessed using the FJC staining. Activation of microglia and astrocytes were detected with immunohistochemical analysis using their respective markers IBA1 and GFAP. Quadriceps muscles were also dissected and analyzed for injection site pathology using H&E staining. Control and OP-SE rats were treated once with VPM orally or I.M. and blood and brain were collected at various time points ranging from 0 min to 24 h. Blood plasma and brain homogenates were analyzed for VPM levels using LC-MS/MS. PK parameters were compared between these two routes of administration using non-compartment analyses. **Results:** DFP exposure produced a rapid onset of SE. VPM (10 mg/kg, I.M.) combined with atropine, 2-PAM, and midazolam therapy was well-tolerated, and no significant increase in mortality was noted compared to the rats treated with the standard-of-care alone. Muscle safety assessed in the quadriceps using a standard 4-point inflammation scale showed mild to moderate inflammation following VPM therapy. However, the pathology inflammation scores were not significantly different from saline-treated controls (SAL: 1.38 ± 0.74 , VPM: 2.0 ± 0.76 , n= 8/group, t-test, *p



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Abstract: 22**Title: A potential beneficial role of astrocyte secreted protein YKL40 in cuprizone induced de/remyelination model****Munir S¹, Dominguez OA¹, Gonzales JP¹, Tuck C¹, Rao S¹ and Singh SK¹**¹Department of Biochemistry, Virginia Commonwealth University, Richmond, VA**Abstract:**

Multiple sclerosis (MS) is a neurodegenerative disease affecting approximately 2.9 million people worldwide, characterized by myelin loss and cognitive decline. Among others, neuroinflammation, reactive-astrocytes and -microglia are hallmarks of MS and may contribute to the de/remyelination. Interestingly, cerebrospinal fluid levels of YKL40 are elevated in individuals with multiple sclerosis, yet its role during de/remyelination is unclear. Previously, our lab identified YKL40 as an astrocyte-secreted factor whose expression is increased during developing brain and in neuroinflammation. Moreover, recently, our lab discovered that astrocytic YKL40 is crucial for developmental myelination in the mouse brain. However, its role in de/myelination remains unclear. To investigate this, we generated a tamoxifen-inducible astrocyte-specific YKL-40 knockout mice and subjected them to a well-established cuprizone-induced de/remyelination model. Brain tissue was analyzed using qPCR and immunofluorescence to assess de/remyelination associated markers. Our preliminary results show that astrocytic YKL-40 loss impacts neuroinflammation, impairs oligodendrocyte precursor cell (OPC) maturation, and affects oligodendrocyte survival, leading to overall worsened demyelination. These findings indicate a protective role for YKL-40 in de/remyelination and suggest it as a potential therapeutic target for MS and other demyelinating diseases.



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Imaging in Neuroscience

Abstract: 23

Title: Validating a MS Progression Risk Model using Advanced MRI
Pearsall RQ¹, Spears TA², Fletcher T², Chen SD³, Oh U¹, Goldman MD¹.

¹Neurology, Virginia Commonwealth University, Richmond, VA; ²Electrical and Computer Engineering, University of Virginia, Charlottesville, VA; ³Biostatistics, Virginia Commonwealth University, Richmond, VA.

Abstract:

Background: Progression occurs throughout the MS disease course and across all phenotypes, including the relapsing phase of the disease. Progression is insidious and often identified only in retrospect at which point there are no effective treatments to reverse acquired disability. We currently lack a validated risk-stratification tool for MS progression. We have developed a novel outcome, the 6- minute walk gait speed trajectory (6MW^{GST}), that captures between- and within walk performance simultaneously and have demonstrated that the 6MW^{GST} can be used to stratify patients with high or low risk of progression. For this study, we validated our 6MW^{GST} progression risk model approach using advanced MRI measurements of myelin and axonal integrity utilizing diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI). **Methods:** Using our published growth mixture model (GMM) approach, MS participants were classified as high or low risk progressors (HRP or LRP, respectively). HC, HRP, and LRP were compared on MRI outcomes. MR Images were obtained on a Philips Ingenia 3.0 Tesla MRI Scanner. **Results:** A total of 38 MS and 5 HC completed 6MW and MRI and were available for analysis. Our GMM approach identified 9 out of 38 as HRP. Five participants had problems with MRI analysis, one participant was removed for inconsistent classification, and one LRP participant was removed as an outlier in MRI measures (>5 standard deviations from mean). This left 33 MS (24 LRP, 9 HRP) and 10 HC. We computed average MRI measures (NODDI intracellular volume fraction, NODDI isotropic compartment, and DTI fractional anisotropy) in normal appearing white matter (NAWM) voxels and averages in lesion voxels for each participant. We found significant differences between HRP and LRP groups in average NODDI ISO (free water component) in NAWM ($p = 0.035$). In lesion tissue, we found HRP to have significantly lower FA than LRP ($p = 0.020$) and moderately lower NODDI ICVF ($p = 0.169$). **Conclusions:** Our study identified differences on several MRI metrics between our HRP and LRP groups. The pattern of MRI outcomes in the HRP group is consistently in the direction of reduced white matter integrity.



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Imaging in Neuroscience

Abstract: 24**Title: Investigating the Role of the Endogenous Peptide Nociceptin in Progressive Multiple Sclerosis.****Baker J**^{1,2}, Poore M¹, Bigbee J², Oh U³, Sato-Bigbee C¹.¹Departments of Cellular, Molecular & Genetic Medicine,²Departments of Anatomy & Neurobiology and³Departments of Neurology. Virginia Commonwealth University School of Medicine, Virginia, USA.**Abstract:**

We have previously found that the endogenous peptide Nociceptin blocks the premature differentiation of oligodendrocytes into mature myelin-making cells, a mechanism that prevents untimely precocious myelination in the developing brain. Consistent with this function, Nociceptin levels are developmentally regulated, sharply decreasing with the initiation and progression of myelination. We now found that, when compared with age-matched controls and relapsing-remitting multiple sclerosis (RR-MS) patients, Nociceptin expression is highly elevated in the cerebrospinal fluid from patients with the most severe “progressive” forms of multiple sclerosis (PMS). This posed the question of whether Nociceptin plays a role in the arrested oligodendrocyte maturation observed in PMS, thus obstructing remyelination. To test this possibility, we developed a model of PMS by inducing experimental autoimmune encephalomyelitis (EAE) in older mice (8-9-month-old), at an age equivalent to the one correlating with increased risk of transition of RR-MS into PMS. Our findings showed that, like in humans with PMS, older EAE animals develop persistent and highly debilitating clinical symptoms. These animals also exhibit an increase in brain activated microglia and reactive astrocytes, accompanied by elevated Nociceptin levels. Preliminary studies indicated that treatment of these mice with an antagonist of the Nociceptin receptor elicits a regression of clinical scoring that correlates with lower levels of reactive astrocytes and increased myelination. These findings support the possibility of using Nociceptin as a biomarker of PMS and target of pharmacological treatments for this devastating disease. Supported by NMSS grant RG-2206-39621.



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Imaging in Neuroscience

Abstract: 25

Title: The Impact of Early Life Stress on Patient Reported Outcomes in Multiple Sclerosis
Pearsall RQ¹, Goldman MD¹, Chen SD², Neigh G³, Oh U¹.

¹Neurology, Virginia Commonwealth University, Richmond, VA;

²Biostatistics, Virginia Commonwealth University, Richmond, VA;

³Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA

Abstract:

Background: Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) affecting an estimated one million Americans. There is significant variability in the disease course and symptom burden among persons with MS (pwMS) that may be related to - environmental factors. Prior studies have implicated early life stress (ELS) as a contributor to the risk of developing autoimmune disorders including MS. Less is known, however, about the relationship between ELS and MS following the diagnosis. We hypothesized that the history of ELS would be associated with worsened disease course and symptom burden in pwMS. **Methods:** In a single-center, cross-sectional study, pwMS were invited to complete a composite of surveys assessing ELS and patient reported outcomes (PRO). ELS was assessed using two validated surveys: Adverse Childhood Experiences (ACE) and Childhood Trauma Questionnaire (CTQ). MS-relevant PRO included patient determined disease steps (PDDS), MS Performance Scales, MS Impact Scale (MSIS-29) and MS Quality of Life (MSQOL-54). Multiple linear regression models were used to assess the associations between ELS and outcomes while adjusting for potential confounding variables. **Results:** A total of 133 pwMS completed the study. Sixty percent of participants had a history of ELS. ELS was associated with greater likelihood of comorbid depression and anxiety. ELS was significantly associated with worse scores in several domains of the MS Performance Scale including Fatigue ($p = 0.009$), Pain ($p = 0.027$), and Tremor/Loss of Coordination ($p = 0.023$). ELS was associated with significantly worse scores on the MSIS-29 and the MSQOL-54 ($p < 0.001$). Both the physical and the psychological/mental components of MSIS-29 and MSQOL-54 were significantly worse among pwMS with a history of ELS. The relationship between the severity of ELS and worse outcomes on MSIS-29 and MSQOL-54 remained significant after adjusting for potential confounding variables. **Conclusion:** In pwMS, a history of ELS is associated with significantly worse MS symptom burden and quality of life. Increased exposure to ELS was associated with worsened PRO scores, with higher ELS scores demonstrating worsened outcomes.



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Imaging in Neuroscience

Abstract: 26**Title: Sarm1-Dependent Programming of Schwann Cells Following Nerve Injury**Lee J¹, Stepanova E^{1,2}, Deppmann C^{1,2}.¹Program in Fundamental Neuroscience, UVA²Department of Biology, UVA**Abstract:**

Schwann cells (SCs), a type of glial cell in the peripheral nervous system (PNS), have been known to play a key role in the support and regeneration of PNS neurons following axonal injury. Current knowledge in the field suggests that in response to injury, Schwann cells transition directly from healthy "myelinating" and "non-myelinating" states to a "Repair" phenotype that guides injured axons through regeneration. However, recent data suggests the presence of additional distinct states SCs can acquire preceding the formation of Repair SCs. This project investigates the role of Sarm1, a toll-like receptor adaptor protein traditionally studied in the context of axon-autonomous degeneration, in mediating the transition of SCs from a protective, oxidative phosphorylation-dependent state we term "Protection Associated Schwann cells" (PASCs) to the known pro-regenerative, glycolytic repair state. In recent data obtained with single-nucleus RNA sequencing, we observe an up-regulation of genes associated with mitochondrial respiration and oxidative phosphorylation in Sarm1-knockout (Sarm1KO) SCs following injury. This is in contrast to the down-regulation of many of these same genes in wild-type (WT) SCs following injury. We propose that Sarm1KO SCs are gated in the PASC state, while WT SCs with normal Sarm1 expression continue towards the Repair SC phenotype. In co-cultures of WT dorsal root ganglia neurons with either WT or Sarm1KO SCs, we observed a significantly lower percentage of degeneration at 8-hours post-injury in WT axons cultured with Sarm1KO SCs compared to WT axons cultured with WT SCs. Using the Seahorse XFe96 Analyzer as an assessment of oxidative phosphorylation, we also found that Sarm1KO SCs display a higher oxygen consumption rate compared to WT SCs following injury. Current and future directions of this project include investigating the role of Sarm1 as an NADase to program metabolic state and developing alternative cell death assays to confirm the preference of Sarm1KO SCs for oxidative phosphorylation over glycolysis. From this project, we hope to elucidate a previously unknown role of Sarm1 in Schwann cell metabolic programming, laying the foundation for future work on potential targets for neurodegenerative diseases and injuries.



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Imaging in Neuroscience

Abstract: 27**Investigating the role of IL-12 signaling on OPC maturation and differentiation**

Stephanie Moy^{1,2}, Andrea Merchak^{1,2,3}, Alison Curtis², **Jakenzie Fletcher-Thrower**², Farooq Khan², & Alban Gaultier^{1,2}

¹University of Virginia Neuroscience Graduate Program, Department of Neuroscience, Charlottesville, VA, ²University of Virginia Department of Neuroscience, Charlottesville, VA,

³University of Florida, Department of Neuroscience, Gainesville, FL

Abstract:

Multiple sclerosis (MS) is a multifaceted, chronic disorder of the central nervous system in which the myelin sheath is destroyed due to an immune-mediated response. Despite decades of work, MS has no cure and remains a debilitating disease with an enormous economic and social cost. Oligodendrocyte precursor cells (OPCs) are a glial subtype with the ability to differentiate into myelinating oligodendrocytes and replace the loss of myelin. The pathways that stimulate or prevent OPC differentiation toward remyelination and maintenance of myelin throughout life are an emerging area of research in the development of therapeutics to combat MS and other demyelinating conditions. Interleukin-12 (IL-12) is a cytokine that is the hallmark inducer of type 1 immune signaling, primarily produced by antigen-presenting cells in the periphery. Mice deficient in IL-12 signaling, *Il12a*^{-/-} and *Il12rb2*^{-/-} strains, are highly susceptible to experimental autoimmune encephalomyelitis (EAE), a well-accepted animal model of MS, suggesting a protective role of IL-12 signaling. IL-12 receptor mRNA transcripts have been shown to be expressed by cells of the central nervous system including oligodendrocytes, but the precise role of IL-12 signaling during remyelination has not been established. To test the direct effect of IL-12 signaling on OPC differentiation, we incubated primary OPCs from wildtype or *Il12rb2*^{-/-} mice and found that *Il12rb2*^{-/-} cultures do not differentiate compared to cultures from C57Bl/6J mice. We confirmed that *Il12rb2*^{-/-} cultures can mature into OPCs by PDGFRa⁺OLIG2⁺ immunofluorescent staining. We took down brains from 2-week, 10-week, and 6-month C57Bl/6J and *Il12rb2*^{-/-} mice and stained for markers of OPCs and oligodendrocytes, finding no differences between groups in cell counts in the corpus callosum or cortex. Taken together, these results suggest that the absence of IL-12 signaling has no effect on the maturation of cultured cells from neural progenitors to OPCs but inhibits differentiation of OPCs into myelinating oligodendrocytes *in vitro*. Investigating IL-12 signaling on oligo lineage cells may elucidate new pathways to promote OPC differentiation during demyelination.



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Imaging in Neuroscience

Abstract: 28**Title: The Role of GLT-1 in Oligodendrocyte Myelination: Unraveling Mechanisms of Myelin Repair in Development and Disease**

Jazmín Soto-Verdugo¹, Edna Suárez-Pozos¹, Elizabeth J. Thomason¹, Fatemah S. Afshari¹, Paul A. Rosenberg², Jeffrey L. Dupree¹ & Babette Fuss¹

¹Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, Richmond, Virginia, USA.

²Department of Neurology and the F.M. Kirby Neurobiology Center, Boston Children's Hospital, Boston, MA, USA.

Abstract:

The central nervous system (CNS) myelin sheath, formed by oligodendrocytes (OLGs), is essential for rapid signal conduction and axonal integrity. Recent studies reveal that CNS myelination is a dynamic process influenced by neuronal activity and extracellular cues, with glutamate playing a key role. The sodium-dependent glutamate transporter GLT-1 (i.e., EAAT2/SLC1A2) is crucial for maintaining glutamate homeostasis and preventing excitotoxicity. While GLT-1's role in astrocytes is well-established, its function in maturing OLGs during CNS myelination remains poorly understood. Our previous research has identified GLT-1 as a key regulator of OLG maturation, with evidence suggesting that its activation leads to a glutamate-triggered signaling cascade that promotes OLG maturation independent of the regulation of myelin gene expression. These findings are of particular relevance in the context of Multiple Sclerosis (MS), a progressive inflammatory demyelinating disease where impaired myelin repair drives disease progression. In MS, there is good evidence that glutamate signaling in the context of remyelination is compromised, limiting the efficiency of myelin repair. Here, we show that the deletion of GLT-1 in maturing OLGs during the process of remyelination attenuates myelin repair and is associated with the appearance of pathological structural myelin characteristics. On a more mechanistic level, our data point toward a role for GLT-1 activation in regulating mitochondrial energy metabolism and protein trafficking in maturing OLGs. Overall, this study suggests new mechanisms that may regulate myelin repair in the CNS and highlights potential therapeutic targets for enhancing remyelination in MS and other demyelinating disorders.



CVCSN



Imaging in Neuroscience

Abstract: 29**Title: TRIM28-mediated transposable element stability in the prefrontal cortex is implicated in social behavior changes**Smith C¹, Kim RK¹, Cui X¹, Hamilton PJ¹¹Department of Anatomy and Neurobiology, VCU**Abstract:**

Engaging in social behavior is essential for group-based organisms, but chronic stress experience can lead to changes in social functioning that constitute a serious quality of life issue. Transposable elements (TEs) are mobile DNA segments that are increasingly appreciated as highly dynamic in response to experience and are implicated in the evolution of transcriptional networks via cis regulation of genes. TE transcription is largely controlled by the KZFP family of transcription factors along with the repressive cofactor, TRIM28. Here, we employed synthetic TRIM28 constructs to exert differential control over TE transcription and observed the social behavioral and transcriptional consequences. TRIM28^{WT} mimics the endogenous protein and recruits repressive chromatin machinery; TRIM28^{VPR} utilizes a VP64-p65-Rta (VPR) domain in place of the wild-type repressive domains and recruits chromatin activating proteins; and TRIM28^{NFD} contains a KRAB-binding domain but no functional domain. After delivering a synthetic TRIM28 protein packaged in a herpes simplex virus (HSV) or an HSV-GFP control to the medial prefrontal cortex of mice, we tested social behavior in the Three-Chamber Social Interaction Test, Five Trial Social Memory Test, and Social Dominance Tube Test. We found that inverting TRIM28's natural repression with TRIM28^{VPR} or introducing the transcriptionally inert TRIM28^{NFD} causes nuanced deficits in social cognition, while overexpressing TRIM28^{WT} did not impact behavior in these stress-naïve mice. These behavioral changes were mirrored by a de-repression of TEs and dysregulation of immune-related genes, particularly interferons. To further characterize TE-related social deficits, we sought to determine whether stabilizing TEs with TRIM28^{WT} promotes resilient social behavior in response to chronic stress. We employed chronic social stress paradigms before introducing the HSV-TRIM28 variants or HSV-GFP control and measured the behavioral response in mice in the Social Interaction Test, Elevated Plus Maze, and Three-Chamber Social Interaction Test. While introducing TRIM28^{WT} following stress did not reverse social behavior deficits, future experiments will test if TRIM28^{WT} can be introduced prior to stress to prevent these behavioral changes.



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Abstract: 30**Title: Delayed neurodevelopmental milestones in conditional *Itgb3* KO mice****Vinson EC^{1*}**, Denzler CJ^{1*}, Kalinowski AR^{1*}, Jeweler KA¹, Rusak NG¹, Scoffield LK¹, Di Pietro AR¹, Golden CK¹, Benedict JMA¹, Vidal GS¹¹Department of Biology, JMU, *Co-first**Abstract:**

Integrin $\beta 3$ (*Itgb3*) mutations are associated with autism spectrum disorder. *Itgb3* is required in forebrain excitatory neurons and astrocytes for normal social and grooming behaviors in adult mice. Recent evidence from our lab shows that postnatal *Itgb3* expression is highest in cortical layer 5 and that it is developmentally regulated. Specifically, layer 5 *Itgb3* expression is strongest in the motor and somatosensory cortex between P0-P21, peaking around P7-P14. The early developmental regulation of *Itgb3* in the motor and somatosensory cortex led us to hypothesize that *Itgb3* is required for the normal development of early somatomotor behaviors. We tested conditional *Itgb3* KO mice (floxed *Itgb3* line crossed with the *Emx1-Cre* driver line) and control mice (floxed *Itgb3* line) every day from P4-P21, utilizing a battery of sensorimotor and other behavioral tests. Both mouse lines were confirmed to have the same C57BL6/J genetic background via SNP-based genetic monitoring assays. Experimenters were blinded to the genotype of the mice. Individual mice within litters were identified by microtattoo. Behaviors were quantified and analyzed via statistical testing that accounted for litter-to-litter variations. Almost all behaviors tested were validated in controls, and the onset of most sensorimotor behaviors occurred between P7-P15. Conditional *Itgb3* KO were highly delayed in the onset of only certain behaviors that require a high degree of somatomotor integration, such as body and air righting. Other developmental milestones, such as eye opening and auditory startle reflex, were not delayed in conditional *Itgb3* KO. We conclude that forebrain *Itgb3* is required for the normal development of highly integrated somatomotor behaviors.



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Imaging in Neuroscience

Abstract: 31**Title: GABA_A Receptors in the Hypothermic Response to Hypoxia- Exploring the Etiology of Sudden Infant Death Syndrome (SIDS)****Kirkhorn C¹**, Boucher N¹, Breland J¹, Miller H¹, Reyes S¹, Teves L¹, Vergeres C¹, Brown J¹¹Department of Biology, James Madison University**Abstract:**

1 in 10,000 births suffer from sudden infant death syndrome (SIDS). Altered neuron development in the brainstem may prevent protective responses to exogenous stress, especially hypoxia, and thereby contribute to SIDS. Specifically, the nucleus of the raphe pallidus (NRP) in the brainstem has serotonin and GABA receptors on sympathetic nervous system (SNS) premotor neurons which coordinate protective cardiovascular responses to stress (Brown, et al., 2011). Do they also control the hypothermic response to hypoxic stress (HH)? When oxygen levels decrease a mammal's core temperature (T_c) and selected ambient temperature (ST_a) will decrease as a protective response to lower metabolism and decrease oxygen consumption for survival. Do these SNS premotor neurons in the NRP mediate the HH response? It is hypothesized that microinjection of GABA_A receptor agonists & antagonists will alter SNS premotor neuron activity at the NRP and therefore alter the HH response. A surgically implanted cannula targeting the NRP allows microinjection of drugs while an abdominal thermoprobe allows core temperature (T_c) recording. After recovery from surgery, rats are housed in a thermocline which quantifies selected ambient temperature (ST_a). Exposure to 60min hypoxic (6%) stress causes protective reductions in both T_c (-2.7±0.3se) and ST_a (-7.6±0.9se) with control (ACSF) injection while muscimol (30mM) (GABA_A agonist) injections exacerbates these responses (T_c:-5.7±0.8°C, ST_a:-8.6±1.3se). Saturated (~100mM) bicuculine mildly attenuated this response (T_c:-1.4±0.4°C, ST_a:-0.3±4.7). 30mM bicuculine had minimal effects. These data suggest GABA_A receptors at the sympathetic premotor neurons in the NRP at least partially mediate the HH response. Altered development of this brainstem locale and/or these receptors, may contribute to SIDS. Determining the mechanics of the NRP in the HH response is essential to the etiology of SIDS and may help prevent its occurrence.



CVCSN



Imaging in Neuroscience

Abstract: 32**Title: Impact of Gestational Ozone Exposure on the Fetal Environment as a Risk Factor in Neurodevelopmental Outcomes.****Bajpai R¹, Brent S¹, Hamm A¹, Garcia M², Campen MJ², Ottens AK¹.**¹Department of Anatomy & Neurobiology, Virginia Commonwealth University, Richmond, VA²Department of Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM**Abstract:**

Ozone (O₃) is a critical component of air pollution with demonstrated impacts on human health, including increased risk for premature birth and lower birth term weight. O₃ exposure has also been associated with a greater risk for developmental delays and reduced cognitive performance in children. Thus, studies here sought to understand the impact of maternal ozone exposure on fetal neurodevelopment. The placenta is generally recognized as a barrier to exogenous maternal stressors; however, we hypothesized early maternal O₃ would, shortly after implantation, cause vascular abnormality in the placenta, perturbing the fetal environment. Pregnant Sprague Dawley Rats were exposed to 0.3 ppm O₃ (consistent with global urban areas) or filtered air (FA). Dams received either an O₃ or FA 4-h exposure at gestation day GD10 or GD20 (to control for an acute phase response). At term (GD21), amniotic fluid (AF) and placental tissues were collected and snap-frozen. Unbiased AF discovery proteomics first identified relevant perturbation to the fetal environment. Overall, GD10 O₃ exposure had a greater, sustained impact on the fetal environment: a more vulnerable period in early placental/fetal development than GD20. Follow-up studies then investigated the origin of amniotic proteomic perturbation. Increased OPN throughout placental lamina explained an increase in AF-based OPN, which recruits immune cells under inflammatory conditions, affirmed by O₃-increased pro-inflammatory CD80 M1 macrophage staining. Separately, we confirmed O₃ exposure had no impact on placental integrin A5B3, discounting altered implantation. Further, e-cadherin and connective tissue growth factor are associated with abnormal tissue remodeling in preeclampsia; both were sourced from the GD10 placenta. PECAM-1 staining illustrated increased vessel wall thickening with decidual bias, consistent with maternal hypertension and impaired perfusion. However, factors like galectin-1 increased in the AF with GD10 O₃ exposure but could not be traced directly to an increase in placental tissue, perhaps indicating increased shedding to reduce inflammation in the fetal environment. Results substantiate that maternal O₃, particularly at GD10, has a significant impact on placental function comprising the fetal environment. Future research will investigate how perturbation may impact fetal neurodevelopment as it may be connected with worsened cognitive and behavioral outcomes in children.



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Imaging in Neuroscience

Abstract: 33**Title: Competitive Synapse Formation in the Cerebellum**

Kristen M Crane^{1,2,4*}, Alyssa M Lyon^{1,3,4*}, Annie E Walls^{1,4}, Jack Fernandez^{1,4}, Meike E van der Heijden^{1,2,3,4}

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⁴Virginia Tech Polytechnic Institute

Abstract:

The climbing fiber synapses in the cerebellum are the strongest synapses in the brain. This synapse is known to play a vital role in developing motor control and due to its strength, it must be tightly regulated. During development multiple climbing fibers compete with one another for synaptic connections and ultimately one climbing fiber connects to each Purkinje neuron. This “winning” climbing fiber must be carefully selected, which is thought to happen by selecting for the climbing fiber with the strongest connection to the Purkinje neuron. This belief stems from the neuroscience principle “cells that fire together wire together”. We assessed this hypothesis by leveraging an experimental mouse model in which a subset of climbing fibers are silenced. Because the silenced climbing fibers can no longer fire together with the Purkinje neurons, we expect that they would not wire with the Purkinje neurons. We assessed experimental mice and their control littermates during development and visualized this climbing fiber – Purkinje neuron synapse. Our results revealed that silenced climbing fibers unexpectedly became “winners” in the cerebellar cortex. This finding challenges our current understanding and suggests that additional mechanisms may govern the climbing fiber–Purkinje neuron synapse formation. Our experiments will ultimately provide a key understanding of competitive synapse formation in the cerebellum during development.



CVCSN



Imaging in Neuroscience

Abstract: 34**Title: Investigating the role of cortex glia in controlling neural stem cell behavior****Sagar Kasar**¹, Jaeda Coutinho-Budd², Sarah Siegrist¹¹Department of Biology, University of Virginia²Department of Neuroscience, University of Virginia**Abstract:**

Neural stem cells (NSCs) are specialized cells within the brain that have the ability to self-renew and generate new neurons and glia during a process called neurogenesis. NSCs produce an immense diversity of molecularly and functionally distinct neuron types for functional neural circuitry. Impaired NSC behaviors like proliferation, differentiation or survival can lead to various neurodevelopmental disorders and cognitive decline. In the developing central nervous system (CNS) of *Drosophila*, the NSCs (called neuroblasts) reside within molecularly and physically defined microenvironments called niches. Neurogenic niches regulate neuroblast behavior by coordinating intrinsic neuroblast genetic programs with extrinsic cues, local and systemic. Cortex glia, a glial subtype, ensheath neuroblasts and their newborn progeny, providing both structural and signaling support. During early developmental stages, cortex glia promote neuroblast reactivation from developmental quiescence. Later, cortex glia also establish an extensive membrane network in the developing CNS, but it remains unclear whether and how cortex glia control neuroblast behavior during larval neurogenesis. We aim to understand the role of cortex glia in the neurogenic niche where they form honeycomb-like cell compartments around proliferating neuroblasts and extend their membranes to accommodate newborn progeny within locally contained microenvironments. We use the Gal4/UAS system to genetically ablate cortex glia and assay neuroblast survival and proliferation. We find that the temporal loss of cortex glia causes neuroblast death that leads to significant reductions in neuroblast numbers and smaller sized brains. We also observed significant structural changes in cortex glial morphology with the formation of glial globules. Currently, we are working to further understand how cortex glia supports neuroblast survival and growth at both the molecular and cellular level. Results from this project will provide new insight into understanding how neurogenesis is regulated at the niche level.



CVCSN



Imaging in Neuroscience

Abstract: 35**Title: Neglect and Stress Dysregulation: The Effect of Parental Separation on Prairie Vole Social Behavior and Stress Response**Meagher SA¹, Connelly JJ¹, Perkeybile AM¹¹Psychology Department UVA**Abstract:**

Neglect and early life stress are common worldwide and are associated with a myriad of mental and physical health issues associated with stress dysregulation. The double-hit model theorizes that extreme stress early in life, the first "hit," results in a disruption to the hypothalamus pituitary-adrenal axis, causing the individual to react more negatively to stress later in life, the second "hit." One paradigm commonly used to study early life stress in rodents is maternal separation; however, this does not account for the biparental system which we typically find in human behavior. Prairie voles are better suited for studying human social behavior as they are both biparental and socially monogamous. This study aims to examine the effect of deficient care in the form of parental separation on prairie vole stress regulation and social behavior. To create the effect of neglect, experimental litters had dividers placed in their cages separating the pups from their parents for three hours per day for the first two weeks of life. To assess the downstream impact of parental separation as a stressor, we utilized two common stress associated behavioral tests, alloparenting and forced swim tests, as well as assaying corticosterone levels in plasma. We hypothesized that animals in parental separation condition would be less alloparental and spend more time struggling in the forced swim tests. We also hypothesized that the increased struggling would be accompanied by higher corticosterone levels. Decreased social behavior, in the form of alloparenting, and increased stress, in the form of forced swim behavior and corticosterone levels, would support the overall hypothesis that early life stress leads to negative outcomes in terms of stress regulation and social wellbeing.



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Imaging in Neuroscience

Abstract: 36**Title: The Role of Brain Stem 5HT1a Receptors in the Protective Hypothermic Response to Hypoxic Stress – Exploring the Etiology of Sudden Infant Death Syndrome (SIDS)**

Carissa Vergeres, Catie Kirkhorn, Halle Miller, John Breland, Noah Boucher, Sophia Reyes, Luana Teves, Justin Brown PhD

James Madison University

Abstract:

Abnormal brainstem development of serotonin neurotransmission is thought to increase an infant's vulnerability to exogenous stressors and contribute to sudden infant death syndrome (SIDS) (1). The nucleus raphe pallidus (NRP), an area rich in serotonin receptors (5HT1a) that mediates cardiovascular responses to stress (1, 2), may also coordinate protective thermoregulatory responses to hypoxic stress. Since 5HT1a receptors are altered in SIDS babies (3), it is hypothesized that activating these inhibitory receptors at the NRP in rats with 8-OH-DPAT, SNS premotor neurons that facilitate heat production would be inhibited thereby exacerbating the regulated hypothermic response to hypoxic stress (HH) versus control (ACSF). Conversely, injections of the 5HT1a receptor antagonist (WAY-100635: WAY) should attenuate this response. A surgically implanted cannula targeting the NRP allows microinjection of drugs while an abdominal thermoprobe allows core temperature (Tc) recording. After recovery from surgery, rats are housed in a thermocline which quantifies selected ambient temperature (STa). Exposure to 60min hypoxic (6%) stress causes protective reductions in both Tc (-2.7 ± 0.3 se) and STa (-7.6 ± 0.9 se) with control (ACSF) injection while 8-OH-DPAT (30mM) injections exacerbates these responses (Tc: -3.9 ± 0.6 , STa: -7.3 ± 1.5). 30mM or saturated (~ 100 mM) WAY injection did not significantly affect the thermoregulatory responses. It was expected that WAY would block the HH response. Perhaps the short half-life of WAY (~ 20 min) limits its effectiveness. Alternatively, redundant pathways that cause the protective HH response. The DPAT injection data suggest that the 5HT1a receptor is involved in mediating the protective HH response. Altered 5HT1a receptors at the NRP could contribute to SIDS. Determining the neural paths that coordinate the HH response is essential to the etiology of SIDS and may help prevent its occurrence.



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Imaging in Neuroscience

Abstract: 37

Title: Single-cell atlas of human neonatal CSF reveals extracellular vesicle neuroimmune mechanisms of hydrocephalus

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Abstract:

Intraventricular hemorrhage, the most common neurological complication of prematurity, causes post-hemorrhagic hydrocephalus (PHH) with high morbidity and mortality. Recent work in animal models has implicated inflammation-dependent dysregulation of cerebrospinal fluid (CSF) homeostasis in PHH, but the cellular and molecular mechanisms and relevance for humans remain unknown. Here, we performed the first integrated, multi-omics analysis of human neonatal CSF at single-cell resolution in patients with or without PHH. Our results revealed striking enrichment in PHH of activated, peripherally-derived T cells and myeloid cells in the CSF and choroid plexus (ChP). PHH CSF contained pro-inflammatory extracellular vesicles (EVs) derived from exhausted immune cells and damaged ventricular zone neural precursors. Purified EVs from PHH CSF were sufficient to elicit NF- κ B-dependent T-cell cytokine production. These data suggest that EV-mediated peripheral immune cell recruitment and activation propagate the hemorrhage-induced ChP-CSF immune-secretory response in PHH. The data suggest immunomodulation as a non-neurosurgical treatment for human PHH.



Abstract: 38**Title: Phenotypic impact of an epigenetic mutation in the oxytocin receptor gene****Duck, O. A.¹, Connelly, J. J.¹, Perkeybile A. M.¹, Page, E. A.¹**¹ University of Virginia Psychology Department**Abstract:**

Oxytocin is a key mammalian neuropeptide that modulates behavior across the lifespan and primarily exerts its effects through the oxytocin receptor. Expression of the gene encoding the oxytocin receptor (Oxtr) is epigenetically regulated, notably by DNA methylation (DNAm) at a specific CpG site within its promoter, -934. In both human and model systems, DNAm at this site varies across individuals, correlates with levels of Oxtr expression, and is sensitive to early life experience. This mark is also associated with human disorders characterized by social deficits, including autism spectrum disorder. In the biparental, socially monogamous prairie vole (*Microtus ochrogaster*), DNAm at -934 is correlated with the level of parental care received early in life, such that more care is associated with lower methylation and higher gene expression in the nucleus accumbens. Typically, animals that express higher levels of Oxtr display reduced anxiety and high-care parental behaviors later in life. Given DNAm at adjacent CpG sites are similarly correlated, a genetic knockout of -934 is required to precisely isolate the regulatory role of this CpG site on Oxtr expression and resulting phenotypic effects. Using CRISPR-Cas9, we generated prairie voles that lack CpG site -934 and show that they subsequently express Oxtr at lower levels than their wildtype littermates. We hypothesize that reduced Oxtr expression early in life will lead to a disruption in species-typical phenotypes. Here, we analyzed ultrasonic vocalization, an early emergent social behavior in the prairie vole pup. We hypothesize that -934 is a key regulator of Oxtr expression and therefore animals with a mutated -934 site will vocalize differentially when compared to the wildtype. This work will be the first to show that disruption of a single CpG site in Oxtr can disrupt outward displays of social behavior.



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Imaging in Neuroscience

Abstract: 39**Title: What's so special about dads? Establishing the importance of quality over quantity of care in offspring development**Klau SC¹, Hinton TD^{1,2}, Connelly JJ^{1,2}¹University of Virginia, Department of Psychology²Program in Fundamental Neuroscience**Abstract:**

Over two thirds of children in the US are raised in traditional two parent homes where both mothers and fathers play an integral role in caring for their children. Variation in this care structure leads to differences in children's social and biological development. We can use the prairie vole (*Microtus ochrogaster*), a rodent species that forms social bonds and parents in pairs like humans, as a model to study human parenting. Vole offspring raised by single mothers show delays in species typical pair bond behavior. To understand the impact of father specific quality of care, we compared the effects of early life care on pair bonding under four parenting conditions, biparental care, maternal only, maternal and older sister, and maternal and older brother, and found that the quantity of care received by pups raised in the single mother group is significantly lower than groups that contain two caregivers. Typical female, but not male pair bond formation was rescued by the presence of a second parent, suggesting a sex specific impact of paternal care on the development of the male brain. We aim to uncover molecular differences that may drive these behavioral results. Oxytocin plays a critical role in pair bond formation, and this peptide exerts its effects through the oxytocin receptor (OXTR). In voles decreased early life biparental care leads to increased DNA methylation of the oxytocin receptor gene (*Oxtrm*) and decreased *Oxtr* expression in a region of the brain critical for pair bond formation, the nucleus accumbens. The work presented here posits that there is a specific quality of paternal care early in life that is important for male neurodevelopment and later adult social behavior. We hypothesize that males raised without a father will show increased *Oxtrm* compared to biparental rearing, as opposed to females who will only display this increase in the single parent group. Elucidating these results will shed light on the sex specific neurobiological implications of variation in parental structure and perhaps help us to gain a better understanding of adult bonding behavior.



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Imaging in Neuroscience

Abstract: 40**Title: Rapamycin as a Potential Therapy for Post-Hemorrhagic Hydrocephalus: Targeting Myeloid mTOR Signaling**

Marie Michenkova¹, Maria Garcia-Bonilla¹, Emre Kiziltug², Rajiv Swarup¹, Kirill Shumilov¹, James P. McAllister II¹, Kristopher T. Kahle³⁻⁶, David D. Limbrick Jr¹.

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Abstract:

Introduction: Intraventricular hemorrhage (IVH) and post-hemorrhagic hydrocephalus (PHH) are major neurological complications in preterm infants, with IVH affecting roughly 35% of those born prematurely. Of these, 25–30% develop PHH, a condition associated with significant morbidity and mortality. In PHH, myeloid cells infiltrate the cerebrospinal fluid (CSF) and invade critical brain regions such as the ventricular and subventricular zones (VZ/SVZ), integral to neural development. mTOR signaling regulates neurogenesis in these areas by controlling neural stem cell proliferation and differentiation, and its dysregulation is associated with neuroinflammation and developmental disorders. Targeting mTOR signaling could modulate immune responses and mitigate PHH progression. Rapamycin, an FDA-approved mTOR inhibitor known for its immunomodulatory and neuroprotective properties, emerges as a promising therapeutic candidate. **Objectives:** To evaluate the therapeutic potential of rapamycin in reducing myeloid cell infiltration, modulating neuroinflammation, and mitigating PHH progression in a neonatal mouse model, guided by insights from human PHH CSF profiles. **Methods:** Human neonatal CSF with PHH was analyzed using scRNAseq and flow cytometry. Samples were stained with cell viability dye (ZombieNIR) and immune cell markers (e.g., CD14, C1QC, F4/80, MHCII), and analyzed on a CytexAurora Analyzer. scRNAseq libraries were prepared with 10X Genomics Chromium, sequenced on an Illumina NovaSeq S4, and analyzed with Seurat. In a mouse model of neonatal PHH induced by bilateral intraventricular injection of 5uL of lysed syngeneic blood, rapamycin (5 mg/kg pre-induction, 1 mg/kg every other day) was administered intraperitoneally. Periventricular myeloid cell infiltration was assessed via flow cytometry, and ventricular volumes were quantified using T2 MRI. **Results:** Flow cytometry detected enrichment of myeloid cell subpopulations (e.g., CD14+ monocytes, Q1QC+ macrophages) in PHH CSF. ScRNAseq data showed enrichment of DEGs involved in the mTOR signaling pathway, including genes coding for MAPK and mTOR activators (LAMTOR1–2, 4–5, p -value <0.0001) in PHH myeloid cells. Rapamycin treatment significantly reduced macrophage and monocyte infiltration in the periventricular regions and decreased median ventricular volumes in treated mice (1.439 mm³; IQR 1.146, 1.980) compared to controls (2.039 mm³; IQR 0.1365, 2.408). **Conclusion:** Rapamycin reduces infiltrating myeloid cells in experimental PHH, supporting further clinical investigation of its therapeutic potential in neonates.



Abstract: 41**Title: Extracellular Vesicles from an In Vitro Model of Preterm Intraventricular Hemorrhage Drive Neuroinflammation in Neonatal Mice****Vohra HZ¹**, Michenkova M¹, Limbrick O², Lane A², Isaacs A³, McAllister JP¹, Limbrick DD¹, Garcia-Bonilla M¹¹Department of Neurosurgery, Virginia Commonwealth University School of Medicine, Richmond, VA, USA,²Department of Neurosurgery, Washington University in St Louis, MO, USA,³Department of Neurosurgery, Nationwide Children's Hospital, Ohio State University, Columbus, OH, USA**Abstract:**

Introduction: Post-hemorrhagic hydrocephalus (PHH) is a debilitating complication of neonatal intraventricular hemorrhage (IVH), occurring in 40-50% of preterm neonates with severe IVH. PHH is driven by disrupted cerebrospinal fluid (CSF) dynamics, neuroinflammation, ventricular zone (VZ) disruption, and extracellular matrix remodeling. We previously identified increased extracellular vesicles (EVs) in the CSF of infants with IVH and PHH, originating from immune and neural stem cells. This study examines whether VZ-derived EVs contribute to the pathophysiology of IVH/PHH by promoting neuroinflammation. We hypothesized that blood-exposed VZ-EVs activate immune cell subpopulations, contributing to periventricular inflammation. **Methods:** Primary neural stem cells from the VZ of postnatal day 4 (P4) C57BL/6 mice were cultured and exposed to 25 μ L whole blood for 24 hours, with PBS-treated cultures as controls. Following a 24-hour washout period, conditioned media was collected for EV isolation. EVs were characterized using nanoparticle tracking analysis (NTA), flow cytometry, and proteomics. P4 mice underwent bilateral intracerebroventricular injections of 4 μ L EVs (2.25×10^9 particles/mL). On post-injection day 7, MRI assessed ventricular volume, while quantitative histological analyses evaluated ventricular wall integrity, choroid plexus function, and inflammatory markers. **Results:** NTA revealed a higher concentration of blood-exposed VZ-EVs than control-EVs ($p=0.0465$), with a greater abundance of smaller EVs (50-150 nm) [exo]. Flow cytometry confirmed EV sorting by size and PKH67 membrane labeling. Proteomic analysis identified enrichment of extracellular matrix remodeling proteins (Tnn, Serpina1a, Plxdc2, Loxl3) and inflammatory mediators (S100a9, Gpx1, Apom, Trem2). Differentially expressed proteins ($p<0.05$, $\text{Log}_2\text{FC} >1$) were linked to cytokine regulation (Apoa1, Serpina1a), immune cell differentiation (Tfrc), and TGF- β signaling (Dnm2, Usp15). Despite no significant difference in ventricular volume on MRI, histological analysis showed increased GFAP⁺ astrocytes and Iba1⁺ microglia and macrophages ($p<0.04$) in the periventricular white matter of blood-exposed VZ-EV-injected mice. No differences were observed in choroid plexus transport protein expression (SPAK, NKCC1) or VZ disruption (GFAP, β IV-tubulin). **Conclusion:** Blood exposure *in vitro* induces VZ-derived EVs with proinflammatory cargo. When administered *in vivo*, these EVs drive periventricular inflammation, suggesting a role in the pathophysiology IVH and PHH. Targeting EV-mediated immune activation may offer a novel therapeutic strategy for treating IVH and preventing PHH.



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Imaging in Neuroscience

Abstract: 42**Identifying native NMDA receptor diversity in the mouse thalamus****Pozo-Aranda AE^{1,4}, Swanger SA^{2,3,4}, Gilmore BG²**

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Abstract:

N-methyl-D-aspartate receptors (NMDARs) are ionotropic glutamate receptors that are crucial for neuronal communication including synapses. They're also important for maintenance, development, and plasticity. NMDARs are exceptionally diverse tetrameric complexes with the assembly being comprised of two GluN1 and two GluN2 subunits (GluN2A – 2D) or GluN3 subunits (GluN3A or 3B) that allow for variation in biophysical, pharmacological, and signaling properties. NMDARs subtypes with distinct subunit composition contribute and lead to distinct brain functions. Disappointingly, we have very limited knowledge of native NMDAR subunit composition and how they're coupled with other proteins, limiting our ability to target specific NMDAR populations. We study NMDAR organization and synapse diversity in the thalamus due to its diverse glutamatergic input and gene expression. The objective of this project is to investigate the mechanisms that lead to synaptic diversity by identifying the specificity of NMDAR subunit assembly. We hypothesize that GluN2 subunits assemble preferentially in tetramers and have subunit specific interactions with other macromolecular complexes that lead to distinct subcellular organization in the brain. To determine the composition of NMDAR tetramers, immunoprecipitation was performed on solubilized mouse thalamus to isolate crude synaptoneurosomes containing pre- and postsynaptic compartments, followed by western blotting. Our preliminary results have qualitatively shown varying prevalence of NMDAR assemblies including a novel NMDAR complex including the GluN2B and GluN2C subunits. Outcomes of this project will guide treatment strategies in ongoing studies with mouse disease models and the development of sub-type selective NMDAR modulators.



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Imaging in Neuroscience

Abstract: 43**Title: Oral Administration of Fluoxetine/L-DOPA Disrupts Behavioral Self-Control in Siamese Fighting Fish (*Betta splendens*)**

Joshua Weber, Kate Watson, Ethan Hoffman, Jamie Martin, Micaela Flores-Vaccari, Rachel Glather, Emily Person, Jacob Wilcox, Zachary Giles, Rommel Pagkalinawan, Claire Tarallo, Grayce Cookenour, Emily Person, Trent Kirchoff, & Andrew Velkey (PI)¹.

Abstract:

Prior research in our lab indicates that male *Betta splendens* are capable of behavioral self-control on a discrete-choice instrumental-response task for food reward, where they consistently preferred a larger-later (LL) reward over a smaller-sooner (SS) reward. Subsequent research revealed that oral delivery of the dopamine precursor L-DOPA to male *B. splendens* significantly reduced self-control, resulting in a greater preference for the impulsive SS alternative. Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, have been shown in earlier research on people and non-human animals to help reduce impulsivity and aggressiveness. In the present study, male *B. splendens* are given oral fluoxetine as an attempt to restore behavioral self-control compromised by L-DOPA. Subjects were randomly assigned to one of two groups: one group received fluoxetine alone (F) while the other group received fluoxetine plus L-DOPA (FD). One week before the trials started and continuing throughout the study period, subjects in both groups received a single oral dosage of fluoxetine (2 mg/kg) at 9 am. Before each daily trial, participants in the FD group also received an oral dosage of L-DOPA (60 mg/kg). Using a submerged T-maze, subjects were given the option to choose between an SS reward (one food pellet supplied immediately) and an LL reward (three pellets delivered after a 15-second delay) during three daily instrumental-choice trials. More than half of the individuals in both groups were removed from the research due to aberrant movements/responding. There was a discrepancy in attrition; the FD group had almost twice as many excluded participants as the F group. While outcomes in the F group were inconsistent, with twice as many participants stabilizing on the SS option as the LL option, all remaining subjects in the FD group stabilized on the SS option. According to the FD group's stability rates, it does not seem that the tested dosage of fluoxetine can restore behavioral self-control disrupted by L-DOPA. Moreover, in this paradigm, fluoxetine-only dosage appears to also interfere with behavioral self-control.

Keyword (Complete): SSRI; L-DOPA ; SELF-CONTROL ; BETTA SPLENDENS

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Abstract: 44**Title: Characterization of novel SNPs within Oxtr and their impact on Oxtr-H mRNA expression in the prairie vole****Leone KD¹, Page EA^{1,2}, Connelly JJ^{1,2}.**¹University of Virginia, Department of Psychology²University of Virginia, Program in Fundamental Neuroscience**Abstract:**

Oxytocin is a nonapeptide that regulates many physiological processes, including complex social behaviors and reproduction, through its interaction with the oxytocin receptor. Previous research has shown that oxytocin receptor gene (OXTR) expression is correlated with several single nucleotide polymorphisms (SNPs) within its third intron. The expression of Oxtr in prairie voles, a translational animal model used to study oxytocin-dependent processes, is similarly associated with SNPs within this region. Our lab has previously identified a novel alternative transcript Oxtr-H originating from the third intron with unknown biological function. The expression of this transcript appears to be partially associated with genotype at the SNP K LW2. We seek to identify a SNP that better explains the differential expression of Oxtr-H. We hypothesized that a SNP within the promoter of Oxtr-H may be responsible for the differential expression of this transcript. RNA expression data of Oxtr-H and Oxtr-B, the main transcript, was collected for all samples in the brain and the uterus. Sanger sequencing was conducted to identify novel polymorphisms within a 1540 bp region containing the putative Oxtr-H promoter. Genotype was subsequently investigated at four novel polymorphisms KDL1 (C/G), KDL2 (-/C), KDL3 (C/A), and KDL4 (T/C). No significant association was found between Oxtr-H expression and genotype at KDL1, KDL2, or KDL3. Interestingly, the novel polymorphism KDL4 may be associated with Oxtr-H and displays linkage disequilibrium with K LW2. This finding provides support for the identification of causative SNPs and associated differences found in Oxtr expression, which has significant downstream effects on complex social behaviors.



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Imaging in Neuroscience

Abstract: 45

Title: Chromatin loops in the sensory organs of the malaria mosquito *Anopheles coluzzii*
Sharakhov I¹, Lukyanchikova V¹, Brusentsov I¹

Department of Entomology, Virginia Tech, Blacksburg, VA

Abstract:

Interactions between regulatory elements and gene promoters regulate transcription during cellular differentiation and response to external stimuli. However, the 3D aspect of gene regulation in the nervous system of mosquitoes has not been investigated. Here, we examined the dynamic aspects of 3D genome architecture during mosquito individual development, with a focus on the sensory system that plays a crucial role in host-seeking, foraging, oviposition, and mating behaviors. We performed genome-wide chromatin conformation capture (Hi-C) on embryonic, larval, and adult stages of mosquito development, as well as on body parts of adult females and males, including heads, antennae, proboscises, maxillary palps, thoraxes, and gonads of *Anopheles coluzzii*. In parallel, we conducted RNA-seq to understand the transcriptional changes that occur during the development. Comparison of Hi-C maps obtained from adult and embryonic tissues revealed the presence of several autosomal and X-chromosomal giant loops. We also identified long-range chromatin interactions that occur at specific stages or in certain body parts during mosquito development. Some giant loops are specific to the soma, as they are absent in ovaries or testes but present in the thoraxes and heads of adult mosquitoes. Additionally, heads have stronger contacts, as well as additional giant loops that are absent in thoraxes, suggesting their possible function in the nervous system. The eyes/brain samples as well as the antennae and the maxillary palps contained the majority of giant chromatin loops. Interestingly, genes located at the loop anchors have roles in cell-cell signaling, sensory perception, neuron differentiation, signal transduction, and response to stimulus. We also identified a network of smaller head-specific loops (120-2,000 kb) in the intercalary heterochromatin that contains genes encoding for neural-cadherin at their anchors. The observed developmental loop dynamics correlates with transcriptional changes of genes located in the loop anchors. The dynamic nature of the chromatin interactions in different organs suggests their functional significance for the development and function of the nervous system in malaria mosquitoes.



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Abstract: 46**Title: Zebrafish (*Danio rerio*) Visually Detect Conspecific Reactions to Different Doses of the Synthetic Alarm Substance, Hypoxanthine-3 N-Oxide (C₅H₄N₄O₂)****Ethan Hoffman**¹, Rommel Pagkalinawan¹, Kaitlyn Kinslow², Jamie Martin², Haley Dewitt², Sara Chaari¹, Joy Kanapala¹, Jack Medlin¹, Grady Fleming¹, Ellie Barry³, & Andrew Velkey² (PI)¹Cellular, Molecular, Physiological Biology²Neuroscience 3: Kinesiology**Abstract:**

As an anti-predation behavior, shoaling enhances survival among prey species by reducing individual predation risk through mechanisms like the dilution effect and collective vigilance. Zebrafish (*Danio rerio*) — a highly social and genetically tractable species — are valuable for studying these behaviors. The present study examined zebrafish's social preferences in a 3-chamber open-tank free-swim task, assessing whether visual cues alone could be used to distinguish between an undisturbed shoal and an alarmed shoal exposed to different doses of the synthetic alarm substance H₃NO. During single subject sessions, subjects were exposed to shoals (n = 4, 2 males & 2 females) in flanking tanks. One flanking tank housed an alarmed shoal exposed to either a 1.5 nM or a 5 nM concentration of H₃NO while the other flanking tank housed an undisturbed shoal. The movement of each individual subject in the center tank was digitally recorded and subsequent movement analysis performed with EthoVision XT16. Analysis of the subjects' movements exposed to shoals in the 1.5 nM condition reveal that subjects spent more time near the undisturbed shoal, were more active in the periphery zones, and had reduced velocity in their swim speed near the unalarmed shoal. Males also demonstrated more freezing responses than females. These results indicate that zebrafish can visually detect differences in conspecifics' responding to an alarm substance. Movement analyses of subjects responding to shoals in the 5 nM condition are pending. This research expands the understanding of zebrafish's social dynamics and provides a robust framework for future exploration of the neural mechanisms underlying social behavior and threat assessment in zebrafish.



CVCSN



Imaging in Neuroscience

Abstract: 47**Title: Characterizing the signaling pathway for intrinsic photo responses in the vertebrate iris****McCarthy C¹, Tobin D¹, and Walker MT¹**¹Biology Department, James Madison University**Abstract:**

All light detection for visual function in vertebrate animals such as humans occurs in the eye. The iris is a muscular tissue in the anterior of the eye which controls the amount of light entering the eye. The size of the iris aperture (i.e. pupil) is controlled by neurological signals initiated in the retina and transmitted to the brain, which signals the iris muscle to constrict during pupillary light reflex. The iris helps to improve visual sensitivity and acuity, it can also protect the retina against light-dependent stress and photoreceptor degeneration. Non-primate vertebrates have an additional intrinsic pathway to drive light-dependent iris constriction. This additional pathway requires intrinsic iridial photoreceptors (IIPs) that initiate light responses. The mechanism by which IIPs communicate with the iridial sphincter muscle cells is still unknown. Some research studies suggest that gap junctions are required for intercellular signaling in the intrinsic signaling pathway. We hypothesize that gap junctions are required for cell-to-cell communication in the intrinsic iridial signaling. We have identified a set of putative gap junction proteins (connexin) expressed in mouse and rat irises. To test vertebrate irises for the expression and distribution of connexin proteins we used quantitative PCR and immunohistochemical staining. Our results show that connexin expression is evolutionarily conserved in vertebrate irises and is the signaling conduit for the light-driven signaling pathway in the iris.



CVCSN



Abstract: 48**Title: Exploring the Function of Alternative Oxytocin Receptor Transcripts****Bruns LR^{1,2}, Page EA^{1,2}, Connelly JJ^{1,2}**¹Department of Psychology, University of Virginia, Charlottesville, VA²Program in Fundamental Neuroscience, University of Virginia, Charlottesville, VA**Abstract:**

Redundancy forms the biological basis for nearly all living organisms and is reflected in countless phenomena from karyotypes to intracellular signaling cascades. Alternative transcripts serve as a prime example of how redundancy can promote survival for a cell, adaptability, and introduce individual variability across individuals. The rodent oxytocin receptor (Oxtr), a rhodopsin-like G protein-coupled receptor, encodes at least six alternative transcripts where two of interest show concurrent activity upon exogenous oxytocin stimulation. Little is known about these putative isoforms, including if they are expressed in humans. We investigated the functional relevance of two alternative transcripts in vitro, denoted OxtrB, a seven transmembrane receptor, and OxtrH, a single transmembrane receptor. Chinese Hamster Ovary (CHO) cells were transiently transfected to express isoforms and subsequently stimulated with exogenous oxytocin. Cells were then imaged to analyze patterns of localization in conjunction with flow cytometry to observe fluctuations in intracellular calcium release resulting from ligand binding. Preliminary data suggests that OxtrB and OxtrH differentially localize within distinct cellular compartments and co-localize at specific oxytocin concentrations. While further exploration is needed to understand the mechanisms and interactions through which the protein products of these alternative transcripts function, clear phenotypic differences in their behavior within the cell exist that suggest novel functional significance. Our study aims to form a more comprehensive understanding of oxytocin-oxytocin receptor signaling while shining light on a novel mode of GPCR regulation.



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Abstract: 49**Title: Antibody transport vehicle (ATV)-mediated lentivirus-like particle delivery of spCas9-RNP/sgRNAs to cross blood-brain barrier for STOP eradication in LSL-tdT reporter mice**Reid L², Jaijyan D¹, and Hu W¹¹ Department of Anatomy and Neurobiology, Virginia Commonwealth University, School of Medicine, 1101 E Marshall St. Richmond, VA 23298²Biomedical Sciences Doctoral Portal**Abstract:**

The Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-associated enzyme-Cas9 system is a revolutionary gene-editing tool with the potential to cure a range of central nervous system (CNS) disease and injuries. This powerful technology enables precise corrections of harmful point mutations, the disruption of genes responsible for CNS diseases, and the excision of HIV proviral DNA from host cells. The lentivirus-like particles (LVLPs) have been extensively studied for efficient delivery of CRISPR/Cas ribonucleoprotein (RNP) and mRNA in peripheral systems for “hit-and-run” editing therapy. However, there have been no reports demonstrating the capability of LVLPs to cross the blood-brain barrier (BBB). In this study, we developed an innovative BBB-crossing LVLP delivery of spCas9 RNP via receptor-mediated transcytosis (RMT). We incorporated a transferrin receptor (TfR)-specific antibody transport vehicle (ATV) into the glycoprotein VSV-G to facilitate the delivery of LVLPs across the BBB. Using LV-CMV-EGFP transfer reporter, we validated the capability of ATV-LVLP to cross BBB and transduce neural cells both in vitro and in vivo. Then, we prepared ATV-LVLP carrying spCas9 RNP and sgRNA targeting STOP in LoxP-STOP-Lox-tdTomato (LSL-tdT) mice (Ai14). Intracerebral injection (IC) induced extensive tdT expression in endothelial cells, neurons, astrocytes, and microglia. Intravenous injection (IV) induced tdT expression in brain, and peripheral organs such as spleen, lung, liver. The editing of endogenous LSL was validated by PCR fragmental deletion genotyping, showing 80% editing efficiency in IC brain and 50% in IV brain, as well as 60% in spleen after IV injection. These findings underscore the potential of our innovative ATV-LVLP delivery system to enhance the efficacy of gene editing therapies for neurological conditions, paving the way for future advancements in targeted treatment strategies.



CVCSN



Imaging in Neuroscience

Abstract: 50**Exin21 boosts transgene expression for AAV gene therapy**Yuekun Li¹, Abdul Sattar Baloch¹, Jian Xu¹, Dabbu Jaijyan¹, and Wenhui Hu¹¹Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, 1101 E Marshall St. Richmond, VA 23298**Abstract:**

AAV gene therapy holds immense promise for treating a wide range of diseases, particularly genetic and infectious diseases. Boosting transgene expression is crucial for the success of AAV gene therapy, as enhanced expression can improve efficiency, reduce required dosages, and potentially minimize side effects. By optimizing transgene expression, we can maximize therapeutic benefits and broaden the applicability of AAV gene therapy. Recently, we discovered a novel Exin21 cis-regulatory motif that enhances the protein expression and secretion of various antibodies, mRNA vaccines, and recombinant proteins (Zhu et al., 2023). While initial attempts to increase packaging efficiency by inserting Exin21 into the Cap protein of the AAV capsid were unsuccessful, we explored its potential to enhance transgene expression when inserted into the AAV transfer vector. Using our established dual gd-Luciferase/EGFP reporter system (Zhu et al., 2023), we cloned Exin21 into the AAV transfer vector using the NEBuilder HiFi DNA Assembly cloning kit. The final AAV transfer vectors were validated by whole plasmid sequencing. Transient transfection studies in HEK293T cells showed a 7-11-fold increase in gd-Luciferase activity in the culture media with Exin21 inclusion. After packaging into AAV2 or AAV-PhP.eB capsids, crude AAV virus delivery induced a 6-15-fold increase in gd-Luciferase activity due to Exin21 addition. Confocal imaging of EGFP expression further validated the boosting activity of Exin21. These data suggest that the addition of Exin21 in the AAV transfer vector can significantly increase transgene production in cultured cells. The in vivo boosting efficacy of AAV transfer vectors with Exin21 remains under study.



CVCSN



Imaging in Neuroscience

Abstract: 51**Title: Novel Modified Adeno-Associated Virus-Like Particle Enables Packaging and Delivery of Both Linear and Circular mRNA**Jian Xu¹, Dabbu Jaijyan¹, and Wenhui Hu¹¹Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, 1101 E Marshall St. Richmond, VA 23298**Abstract :**

Adeno-associated viruses (AAV) are a most promising tool for DNA delivery in gene therapy. AAV is well known for its ability to deliver cDNA for long-term expression. However, this characteristic is not ideal for CRISPR/Cas-mediated gene knockout, which requires a "hit and run" strategy to induce gene editing. A recent study has demonstrated the feasibility of AAV-like particles (**AVLP**) to deliver linear mRNA for transgenes through aptamer-binding proteins (ABP) such as MS2-coupling protein (MCP). The circular RNAs (cRNAs) form covalently closed loop structures that provide exceptional stability, protecting them from exonuclease degradation. Incorporating cRNA into AVLP will not only increase mRNA stability during transduction but also enhance mRNA stability during AVLP packaging, potentially increasing AVLP gene delivery efficiency. Here, we have modified the AVLP packaging system to accommodate both linear mRNA and cirRNA. Using nanoLuciferase reporter assay, we found that MCP fusion at the N-terminal Rep of our newly-discovered AAV-R2e-Mac capsid (AAV-MCP-R2e-MAC) can effectively deliver both cRNA and linear mRNA. ReTo investigate whether AVLP can deliver spCas9 mRNA and sgRNA for gene editing, we packaged AAV-MCP-R2e-MAC with AVLP transfer vectors TP1328 (MS2-mediated sgRNA targeting LoxP sites) and TP1363 (MS2-mediated spCas9), both of which lack the standard AAV ITR. The resulting crude AVLP virus was used to treat neural stem cells from LoxP-STOP-LoxP-tdTomato reporter mice (Ai14). The presence of tdTomato cells indicates successful delivery of spCas9 mRNA (>4.2 kb) and sgRNA for functional LoxP editing. These findings suggest that MCP-mediated AVLP can deliver not only linear and circular mRNAs but also relatively large cargo of spCas9 mRNA for "hit-and-run" gene editing purposes.



CVCSN



Imaging in Neuroscience

Abstract: 52**Title: Evaluation of Low Intensity Focused Ultrasound to the Dorsal Anterior Cingulate Cortex for Effects on Pain Intensity and Unpleasantness**

Aditya Kapoor

Abstract:

Chronic pain is a major public health challenge, driving high economic costs and reduced quality of life. The dorsal anterior cingulate cortex (dACC), is crucial for pain perception and autonomic regulation making it a promising target for pain modulation. However, its deep location precludes access with common noninvasive techniques such as transcranial magnetic stimulation (TMS). Low intensity focused ultrasound (LIFU) offers a novel, noninvasive approach using acoustic energy to alter neural activity with high spatial resolution and deep focal lengths. N = 5 healthy volunteers completed a cold pressor task during continuous electroencephalography (EEG), electrocardiography (ECG), electrodermal (EDR), and respiratory rate (RR) measurements. Following a brief anticipation period, participants immersed their right foot in ice water (20°C) for one minute, rating their pain on a 0–10 numerical scale (0 = no pain, 10 = worst pain imaginable) at 10-second intervals. Emotional discomfort was measured at baseline, immediately after foot removal (60 seconds), and at 2 minutes post-removal using the Wong-Baker scale. The task was performed twice before and twice after either LIFU or sham administration. The LIFU condition showed a greater reduction in pain ratings than sham at time points 20–40 seconds, with mean differences of -0.8 ± 0.2 to -0.9 ± 0.4 points relative to sham. LIFU did not affect peak pain at 60 seconds. At foot immersion, the ACC condition showed a larger initial increase in emotional discomfort than sham (0.4 ± 0.1 vs. 0.1 ± 0.1). By 60 s, ACC returned to baseline (0.0 ± 0.16) while sham remained elevated (0.5 ± 0.5), and at 120 s, ACC stayed at baseline versus sham (0.2 ± 0.34). Preliminary findings suggest that a single-session LIFU intervention targeting the dACC modestly reduced both pain and emotional discomfort compared to sham. These results support the feasibility and tolerability of dACC LIFU, indicating promise as a therapeutic intervention. However, longer treatment durations and fully powered studies are needed to confirm its efficacy in reducing pain intensity and unpleasantness.



CVCSN



Abstract: 53**Title: Visualizing Dopamine Dynamics In-Vivo****Michaela McCormack**¹, Savanna Hinson¹, Aaditya Deshpande², Jill Venton², Jay Hirsh¹University of Virginia, ¹Department of Biology,
²Department of Chemistry**Abstract:**

The Hirsh Lab studies neurological dopamine mechanisms within a *Drosophila melanogaster* model. Dopamine biosensors, which are fluorescently tagged D2-like receptors, spatially and temporally track dopamine in vivo. Sangston et al. (2019) observed that heat shifts in flies with dTrpA1 driven in dopamine neurons (DANs) induced a rapid spike followed by a decay in locomotor activity (Figure 1). The activity spike results from dopamine expression, but the cause for the rapid activity decay during the heat shift remains unclear. A GRAB-DA biosensor and a perfusion method are being used to answer this question. After repeated proof of concept trials meant to confirm that the GRAB-DA works, picospritzing confirmed its utility. Ongoing studies are being completed to continue to refine the method of using dissected brains in perfusion studies.



CVCSN



Imaging in Neuroscience

Abstract: 54**Title: Association of host-seeking behavior with chromosomal inversions in the mosquito *Aedes aegypti*****Maria Sharakhova**^{1,2}, Jiangtao Liang¹, Andrey Yurchenko¹, Varvara Lukyanchikova¹, Ilya Brusentsov², Noah Rose³, Zhijian Tu⁴, Carolyn McBride³¹Department of Entomology and Fralin Life Science Institute, Virginia Polytechnic and State University, Blacksburg, USA;²Laboratory of Cell Differentiation, the Federal Research Center, Institute of Cytology and Genetics, Novosibirsk, Russia;³Department of Ecology and Evolutionary Biology, Princeton University, Princeton, USA;⁴Department of Biochemistry and Fralin Life Science Institute, Virginia Polytechnic and State University, Blacksburg, USA**Abstract:**

The impact of chromosomal inversions on the evolution of diverse organisms has been demonstrated in previous studies. However, they have not been characterized in the genome of the arboviral vector *Aedes aegypti* due to the lack of readable polytene chromosomes. The *Ae. aegypti* mosquito consists of two subspecies *Ae. aegypti aegypti* (*Aaa*) and *Ae. aegypti formosus* (*Aaf*) that exhibit notable differences in their adaptations to human and blood feeding preferences. While *Aaf* has an opportunistic host choice and its feeding behavior is likely dependent on host availability, *Aaa* is strongly anthropophilic. Using a genome-based approach, this study identified 20 chromosomal inversions in the *Ae. aegypti* subspecies from around the world and showed that inversions are strongly associated with the geographic origin of the strains and the *Ae. aegypti* subspecies. Inversions were more common in African *Aaf* strains than in non-African *Aaa* strains. To assess whether the inversions could potentially affect traits important for disease transmission, we attempted to correlate the locations of the chromosomal inversions with the locations of clusters of chemoreceptor genes involved in smell and taste. We found significant overlap of the 1qF inversion, which is endemic to West Africa, with clusters of odorant receptor genes located near to the telomere on 1q. As shown before, this region overlaps with the highest peaks of SNP differences between strains in West Africa and includes a gene, *Or4*, previously implicated in mosquito preference for human odors. In addition, the overlapping 3pB/3pD inversions and the 3pC inversion located in the middle of the 3p arm overlapped with the clusters of gustatory, and ionotropic receptor genes, respectively. Our discovery of a large pool of structural variation in the *Ae. aegypti* genome represents a groundbreaking step forward in our understanding of the genome organization and function of this important disease vector.



CVCSN



Imaging in Neuroscience

Abstract: 55**Nicotinic Acetylcholine Receptor Antagonism via Dihydro- β -erythroidine in Conditioned Place Preference****Baldwin, A¹, League, S², Fernandez, G²**

¹ Department of Neuroscience, Christopher Newport University; ² Department of Psychology, Christopher Newport University

Abstract:

Adolescence is considered to be a critical period for neural development associated with higher-order cognition—often characterized by risk taking and reward seeking behaviors resulting from uneven maturation across brain structures (Larsen & Luna, 2018; Spear, 2015). While areas such as the mesolimbic dopamine (reward) pathway are fully mature at the start of early adolescence, other structures comprising the mesocortical pathway (cognition) continue to undergo key developmental changes (Spear, 2000). Exposure to nicotine during adolescence has been linked to increased susceptibility to developing substance use disorders (Smith et al., 2015). These behavioral disruptions have been extensively correlated with changes in cholinergic signaling pathways found in the developing mesocortical pathway (Counotte et al., 2012; Dwyer et al., 2009; Ehlinger et al., 2016; Slotkin et al., 2007). This project pharmacologically manipulates the cholinergic system and examines its effect on nicotine-context association observed in conditioned place preference (CPP). Sprague Dawley rats were injected subcutaneously with twelve doses of 0.4 mg/kg nicotine (equivalent to one pack of cigarettes, or one e-cigarette cartridge) or saline during adolescence (post-natal day [PND] 28-49). Animals were tested as adults using a biased, single trial, nicotine CPP paradigm in which a single 0.4 mg/kg nicotine dose was associated with a non-preferred context. Each conditioning session also included a co-administered, subcutaneous injection of 5 mg/kg Dihydro- β -erythroidine (DH β E; an α -4 nAChR antagonist) or vehicle (dimethylsulfoxide [DMSO]). We replicated prior research which found DH β E attenuated CPP in adult cohorts. Interestingly, animals pretreated with adolescent nicotine and co-administered DH β E did form single trial, nicotine CPP compared to all other groups. These findings suggest that adolescent nicotine exposure leads to a long-term disruption in cholinergic system functioning.



CVCSN



Imaging in Neuroscience

Abstract: 56**Title: Site and outcomes of lynx1 allosteric modulation of $\alpha 3\beta 4$ -nicotinic receptors with relevance to nicotine use disorder****Kneisley DL¹, Oh H¹, Cao Y², Im W², Miwa JM², Whiteaker P¹**¹Department of Pharmacology & Toxicology, Virginia Commonwealth University School of Medicine²Department of Biological Sciences, Lehigh University**Abstract:**

Smoking, maintained by nicotine-seeking behaviors, is the leading cause of preventable death worldwide. Despite most users' desire to cease nicotine usage, and the availability of multiple cessation therapies, cessation success rates remain low. In the brain, nicotine acts on nicotinic acetylcholine receptors (nAChR). The $\alpha 3\beta 4$ -nAChR subtype suppresses somatic nicotine withdrawal signaling by enhancing activity of GABAergic interneurons of the interpeduncular nucleus. The prototoxin lynx1 is highly expressed in these interneurons, and lynx1 allosterically diminishes $\alpha 3\beta 4$ -nAChR response, suggesting that lynx1 may play a role in modulating nicotine withdrawal signaling. This points toward lynx1 as a potential modulator of a critical withdrawal circuit and a target for future nicotine cessation therapies. Lynx1 notably exhibits different potency depending on $\alpha 3\beta 4$ -nAChR subunit ratio. In order to define the interactions through which lynx1 exerts its effects and understand its mechanism of action, molecular dynamics simulations were used to identify residues of $\alpha 3\beta 4$ -nAChR where lynx1 may interact. We hypothesized that modifying key lynx1 binding residues suggested by the simulations would result in mutant $\alpha 3\beta 4$ -nAChR that are less sensitive to lynx1's allosteric modulation. Two-electrode voltage-clamp electrophysiology (TEVC) and cell-attached single-channel electrophysiology were used to assess the effects of mutating the putative lynx1 allosteric binding site residues (at the $\alpha 3/\alpha 3$ subunit interface) on macroscopic receptor function. As hypothesized, some of these mutations at the suspected binding site decreased the sensitivity of the receptor to lynx1's effects, supporting the hypothesis that lynx1 effects are mediated by the site indicated by molecular dynamics. Surprisingly, other mutants increased sensitivity of the receptor to lynx1, and all mutants also diminished ACh-induced function in the absence of lynx1. Some of these residues at the $\alpha 3/\alpha 3$ interface are conserved with those that are important for agonist binding at orthosteric sites, located at $\beta 4/\alpha 3$ interfaces, possibly revealing a previously-unknown ACh binding site at the $\alpha 3/\alpha 3$ interface. The results of these experiments will elucidate the mechanisms by which lynx1 interacts to alter receptor function. Understanding the lynx1/receptor interaction under the described project may provide a new target for future smoking cessation therapies by alleviating somatic withdrawal, making it easier for individuals to stop using nicotine products.



CVCSN



Imaging in Neuroscience

Abstract: 57**Title: Effects of behavioral economic variables on methamphetamine-vs-food choice in Sprague-Dawley and Long-Evans rats****Baldwin A¹ and Banks M¹**¹Department of Pharmacology and Toxicology, Virginia Commonwealth University.**Abstract:**

Methamphetamine is the second leading cause of drug-related overdose deaths, and no FDA-approved pharmacotherapies exist for methamphetamine use disorder (MUD). This highlights the need for preclinical research to uncover the basic biological mechanisms of methamphetamine reinforcement. The present study aimed to determine the economic substitutability between methamphetamine and liquid food (Ensure®) using a methamphetamine-vs-food choice procedure in rats by manipulating reinforcer magnitude and response requirement ("cost"). Sprague Dawley (SD) (n = 6M/6F) and Long Evans (LE) (n = 6-7M/6F) rats were implanted with IV catheters and trained on daily 2-hour behavioral sessions. The terminal choice procedure consisted of five 20-minute response components where methamphetamine doses (0, 0.01, 0.032, 0.1, and 0.32 mg/kg/infusion) were available as an alternative to liquid food. In each component, subjects could make up to 10 choices of either liquid food or methamphetamine, both requiring a fixed ratio (FR) of 5 lever presses. Once responding stabilized, the magnitude of the liquid food reinforcer was manipulated weekly by changing the concentration of Ensure® in water (0, 10, 32, and 100%). Subsequently, the response requirement for methamphetamine was manipulated weekly using FR values of 1, 5, 25, and 125. Methamphetamine maintained a dose-dependent increase in choice for both SD and LE male and female rats. Increasing Ensure® concentrations caused rightward shifts in methamphetamine choice dose-effect functions in both strains (SD $p = 0.020$, LE $p = 0.005$), with methamphetamine choice highest when the alternative was water and lowest with 100% Ensure®. Both SD ($p = 0.059$) and LE ($p = 0.028$) rats were equally sensitive to methamphetamine FR cost manipulations. These findings provide an empirical foundation to study the pharmacology and neurobiology of methamphetamine reinforcement in a drug choice context. This project was made possible through funding from R01DA055825 and IMSD T32GM148403.



CVCSN



Imaging in Neuroscience

Abstract: 58**Title: Dose-Dependent Effects of Synthetic Alarm Substance (C₅H₄N₄O₂) on Established Zebrafish**

Shoals Kinslow K¹, Hoffman E², Bowers M², Surisetty B², Martin J¹, Kanapala J², Caterbone R², Dwyer M³, Kirchoff T², Williams S⁴, Fleming G², Dewitt H¹, Barry E², Chaari S², Medlin J², Velkey A¹.

¹Neuroscience Program, CNU

²Department of Molecular Biology & Chemistry, CNU

³Department of Organismal and Environmental Biology, CNU

⁴Department of Psychology, CNU

Abstract:

Zebrafish (*Danio rerio*) are a valuable model for studying social behavior due to their highly gregarious nature, which includes shoaling. Shoaling occurs when members of a group of fish congregate loosely, with limited coordination of movement among the members. When a predator attacks a shoal member, the injured fish releases an epithelial alarm substance which serves as a threat signal to other shoal members. Previous studies indicate that exposure to alarm substances disrupts shoaling and elicits anti-predatory responses, including freezing, bottom-dwelling, and a lower intermember distance within the shoal. The present study explored the responses of small mixed-sex shoals (2 males & 2 females each) exposed to varying concentrations of asynthetic alarm-substance as part of a Open-Tank Free-Swim Test (OTFST). Adult wild-type zebrafish were used in control and experimental shoals in 5L tanks flanking either side of the tank holding a single subject for a related study. The experimental shoals were exposed to either a 1.5nM or 5nM concentration of the synthetic alarm substance; the control shoal remained unperturbed. Digital video recordings were obtained during the OTFST and subsequently analyzed using EthoVision XT 15.0. While shoals demonstrated variability in intraindividual movement and shoal density, there are differences between shoals exposed to different doses. Compared to intact shoals, and shoals exposed to 1.5nM, the shoals exposed to 5nM spent substantially less time in the upper half of the tank, a behavioral marker of anxiety in fish. Further analyses are pending. Understanding these responses in regard to fear contagion has implications regarding anxiety, fear responses, and potentially research on neurodevelopmental disorders of social development.



CVCSN



Imaging in Neuroscience

Abstract: 59**Title: The Impact of Mef2C on Alcohol Induced Memory Deficiency in Adolescents**
Connor J Wright¹ and Dr. Wolstenholme¹¹VCU Pharmacology**Abstract:**

It is known that adolescent consumption of alcohol has many detriments to the physical and psychological functioning of those who partake in it. Despite this, over 1 in 4 individuals between the ages of 12-20 in the United States report that they have consumed alcohol in the past year. This reality indicates the necessity of researching adolescent alcohol consumption. Mef2C is a protein associated with many crucial functions of brain development such as neuronal migration, activity-dependent cell survival, neuronal differentiation, axon guidance and pruning, dendritic formation and remodeling, as well as synaptic development and neuronal excitability. Our goal is to investigate the effect this gene and its associated protein have on the severity of these known detriments caused by drinking. Adult mice, males and females, underwent stereotaxic injection to knockout the Mef2C gene (pENN.AAV8.hSyn.HI.eGFP-Cre.WPRE.SV40 (titer $\geq 1 \times 10^{13}$ vg/mL)), or a control virus, pAAV8-hSyn-EGFP (titer $\geq 7 \times 10^{12}$ vg/mL). For two days prior to testing, mice were habituated to the behavior room for one hour and then given 0.9% saline injections (0.2 mL i.p.) before being placed in the locomotor boxes for 15 minutes to allow for habituation to the chamber (light intensity = 5). On testing day, the mice were again habituated to the behavior room for one hour, then placed in the same chambers for a 15-minute habituation (Phase 1). The mouse is then removed, injected with either 0.9% saline or ethanol (2.0 g/kg 10% w/v in 0.9% saline) and then immediately returned to the chamber for NOR (novel object recognition) testing. The results indicate that the Mef2C knockout mice which received injections of ethanol had significant deficiencies in their ability to recognize the objects they had previously been exposed to compared to the control group. This indicates that when exposed to alcohol as adolescents, the memory of mice without Mef2C is more severely impacted compared to the mice which had intact Mef2C code in their genome. Further investigation is needed to examine exactly how the absence of Mef2C affects neuronal development and arrangement when there is alcohol exposure during adolescence.



CVCSN



Imaging in Neuroscience

Abstract: 60**Title: Investigating the Stability of Transposable Elements within the Nucleus Accumbens as a Causal Mechanism Required for the Progression of Substance Use Disorders****Hassan A**^{1,2}, Picone JA², Carwile N², Tramonte BJ¹, Hamilton PJ²¹Department of Pharmacology and Toxicology²Department of Anatomy and Neurobiology
Virginia Commonwealth University**Abstract:**

Repeated use of addictive substances causes long-lasting changes in the function of the brain's reward circuitry, including the neurons of the nucleus accumbens (NAc). Specifically, cocaine use induces adaptations within NAc cells persisting beyond acute use, implicating long-term molecular modifications in gene regulatory networks and chromatin landscape. Despite growing prevalence of cocaine misuse and increasing overdose fatalities, no FDA-approved pharmacotherapies currently exist for cocaine use disorder (CUD). Transposable elements (TEs) are mobile DNA sequences that comprise about half of the human genome. Emerging evidence suggests that TEs are transcriptionally repressed by KRAB zinc finger proteins (KZFPs), the largest class of transcription factors. This research investigates the role of TE expression and their KZFP transcriptional controllers in the NAc as potential molecular mediators of CUDs. We use virally delivered synthetic variants of TRIM28, a repressive co-factor for KZFPs, to the NAc to dysregulate the function of KZFPs in preclinical SUD models. We hypothesize that KZFP-mediated transcriptional repression of NAc TEs enables the progression of CUDs and that activating NAc TEs through TRIM28 variants exacerbates its pathogenesis. We performed a dose-response experiment using male C57BL/6J mice implanted with jugular vein catheter to operate intravenous self-administration (IVSA) of cocaine. Mice were tested with four randomized doses of cocaine for four consecutive sessions on a fixed-ratio five schedule of reinforcement for three hours daily. Following completion of our initial baseline dose response, mice undergo intra-NAc stereotaxic delivery of our synthetic TRIM28 variants and repeat the same paradigm to directly investigate the effects of KZFP dysregulation on cocaine reinforcement. Additionally, male C57BL/6J mice were also used for cocaine conditioned place preference (CPP) to investigate rewarding effects upon KZFP dysregulation. Preliminary data reveals that KZFP dysregulation using synthetic variants of TRIM28 does not significantly alter CPP behaviors. However, dysregulating the function of NAc KZFPs decreases the IVSA responses for cocaine at intermediate doses, hinting towards a downward shift in the reinforcing effects of cocaine self-administration. These findings point to the possibility that the KZFP-mediated transcriptional control of NAc TEs contributes to the molecular processes that govern drug taking behaviors.



CVCSN

**Imaging in Neuroscience**

Abstract: 61**Title: Investigation of Neuronal Activation via Gq DREADDs to Mitigate Ethanol-Induced Memory Deficits in Adolescence****Rodriguez, S¹, Morgan, A², Wolstenholme, JT, PhD^{1,2}**¹Department of Pharmacology and Toxicology, VCU²VCU-Alcohol Research Center, VCU**Abstract:**

Adolescent binge ethanol exposure has been linked to delays in oligodendrocyte (OL) differentiation, suggesting that ethanol disrupts myelination and contributes to cognitive deficits in adulthood. Neuronal activation, the process by which neurons send electrical signals to other neurons, plays a crucial role in oligodendrocyte precursor cell (OPC) differentiation and cognitive learning. However, ethanol exposure interferes with this process, impairing OPC differentiation. To address ethanol-induced deficits, a pilot study using chemogenetic Gq DREADD activation of prefrontal cortex (PFC) neurons was conducted to assess whether activity-dependent myelination could mitigate cognitive impairments and increase markers of myelination. A virus containing a Gq DREADD was injected into the medial PFC of nine C57BL/6J mice, which then underwent binge ethanol exposure from postnatal days 29–42. Three weeks after viral delivery, the Gq DREADD was activated using DCZ or vehicle 30 minutes before behavioral tasks. The experimental groups were as follows: Group 1 received vehicle (saline) for 15 days, Group 2 received DCZ for 15 days, and Group 3 received DCZ for 8 days. To evaluate memory deficits, novel object recognition testing was conducted. There were no significant differences between the DCZ-treated groups and the vehicle. However, a statistically significant difference was observed between the DCZ-8 and DCZ-15 groups, suggesting possible dose dependent differences. Following behavioral assessments, brain tissue was collected for immunohistochemistry. Dual staining for mCherry and myelin basic protein (MBP) was performed to confirm viral delivery and assess myelination. Following image quantification using ImageJ/FIJI, we observed no significant differences between the vehicle group and the DCZ-treated groups. However, there was a trend toward increased MBP immunoreactivity in the DCZ-8 and DCZ-15 groups compared to the vehicle group. Future studies will focus on further investigating the epigenetic mechanisms by which adolescent binge ethanol alter OL differentiation. This will involve using snCUT&Tag to identify differential regulation of H3K9me3 in OPCs and OLs in the PFC.



CVCSN



Imaging in Neuroscience

Abstract: 62**Title: Role of DAGL β in Locomotion, Pain Sensitivity, and Ethanol Metabolism in Alcohol-Induced Peripheral Neuropathy****Mohiuddin A¹**, Moncayo L¹, Chiang A¹, Dahman AR¹, Siddiqi AS¹, Cruz M¹, Adu-gyamfi F¹, Patel P¹, Akbar Z¹, Khan A¹, Adusumalli S¹, Patel T¹, Rauf A¹, Damaj MI¹¹Department of Pharmacology & Toxicology, Virginia Commonwealth University,
Richmond, VA 23284**Abstract:**

Diacylglycerol lipase beta (DAGL β) is an enzyme involved in synthesizing the endocannabinoid 2-arachidonoylglycerol (2-AG), which regulates neural signaling, pain, and metabolism. The role of DAGL β in locomotor activity, pain sensitivity, and ethanol metabolism remains unclear, particularly in alcohol-induced peripheral neuropathy (AIPN). This study investigates the effects of DAGL β knockout (KO) on these processes. Male and female DAGL β KO and wild-type (WT) mice were assigned to a control (0% EtOH) or experimental (5% EtOH) diet for four weeks following a one-week baseline on the control diet. Pain sensitivity was assessed weekly via mechanical (paw withdrawal threshold) and cold hypersensitivity (paw withdrawal latency) tests. Locomotor activity was measured using beam breaks. The first behavioral tests occurred after the baseline diet before dietary reassignment. Additionally, WT mice were administered a DAGL β inhibitor before receiving an acute ethanol dose via oral gavage. Blood samples were collected at 30, 60, 120, and 240 minutes to measure ethanol and acetaldehyde levels. Both male and female DAGL β KO mice on the 5% EtOH diet exhibited increased paw withdrawal thresholds and decreased withdrawal times compared to WT mice, indicating greater pain sensitivity in WT mice. Mechanical hypersensitivity was more pronounced in KO males, whereas female KO mice displayed significantly longer withdrawal times compared to their WT counterparts. Cold hypersensitivity also increased with ethanol exposure, and pain sensitivity worsened over time in WT mice. Despite differences in pain sensitivity, locomotor activity remained unchanged between KO and WT mice under both dietary conditions. Blood analysis following ethanol gavage revealed no significant differences in ethanol absorption. However, DAGL β KO mice exhibited a trend toward faster ethanol-to-acetaldehyde conversion, particularly at the four-hour mark. These findings suggest DAGL β influences pain sensitivity and ethanol metabolism in AIPN. The increased hypersensitivity in WT mice implicates DAGL β in pain modulation under chronic alcohol exposure. Additionally, the altered ethanol metabolism in KO mice suggests DAGL β plays a role in regulating this pathway. These insights may contribute to understanding endocannabinoid-based interventions for alcohol-induced pain and metabolism.



CVCSN



Imaging in Neuroscience

Abstract: 63**Title: Nucks1 Overexpression in mPFC decreases Ethanol Drinking in Male Mice****Morgan A¹**, Wright CJ¹, Miles MF^{1,2}, Wolstenholme JT^{1,2}¹Alcohol Research Center, VCU²Department of Pharmacology and Toxicology, VCU**Abstract:**

Newer pharmacotherapies are needed for the treatment of alcohol use disorder. The goal of the VCU-Alcohol Research Center is to use cross-species studies to identify genetic risk for alcohol misuse with the aim of identifying targetable therapeutic strategies. To identify potential candidate genes involved in the escalation of ethanol drinking, the Diversity Outbred mice underwent a 2-bottle choice intermittent ethanol drinking paradigm for five weeks. Nuclear casein kinase and cyclin dependent kinase substrate 1, *NUCKS1*, a gene highly expressed in the mouse cortex and hippocampus, was identified within a highly suggestive quantitative trait loci (QTL) for ethanol preference during the last week of drinking. Human genetic association studies in the GWAS & Sequencing Consortium of Alcohol and Nicotine use database (GSCAN) also found *NUCKS1* was associated with alcohol consumption. Here, we overexpressed *Nucks1* in the frontal cortex of C57BL/6j males and females to investigate its role in ethanol consumption and acute ethanol behaviors. *NUCKS1* overexpression and accurate localization was validated by immunohistochemistry. *NUCKS1* overexpression in the PFC decreased in ethanol intake at 24 hours and ethanol preference at 2 and 24 hours in males, but not females. Open field test, novelty suppressed feeding, loss of righting reflex, saccharin and quinine testing was also completed. *NUCKS1* impacted behavior in the novelty suppressed feeding task in a region-dependent manner. Overall, these data suggest that *NUCKS1* may be involved in the escalating effects of ethanol consumption.



CVCSN



Imaging in Neuroscience

Abstract: 64**Title: Exploring the effect of psilocybin on gene expression of nicotinic receptors and plasticity-related genes in mice****Klein S¹**, Buzzi B¹, Palamar A¹, Kalck E¹, Groener Y¹, Buttar B¹, Gonzalez-Maeso J², Damaj MI¹¹Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA23298.²Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA 23298.**Abstract:**

Tobacco use is one of the leading causes of preventable deaths worldwide, and with e-cigarette usage increasing among young adults, nicotine dependence remains a critical public health issue. The classical psychedelic psilocybin has shown promise as a potential smoking cessation treatment in an initial clinical study (Johnson et al. 2014). Preliminary studies done within our lab have shown that psilocybin (1 mg/kg) post-acutely (24 hours after) reduces nicotine withdrawal signs in mice. Human and animal studies suggest that the therapeutic effect of psychedelics may be due to an increase in neuroplasticity (Ly et al. 2021), In this study, we investigated the molecular mechanisms of how psilocybin impacts neuroplasticity-related genes in brain regions that mediate nicotine withdrawal. For that, we injected male and female mice with psilocybin (1 mg/kg i.p.) and 24 hours later, we dissected 3 important brain regions, the prefrontal cortex (PFC), medial habenula (MHb) and interpeduncular nucleus (IPN), previously shown to play a role in nicotine withdrawal, and investigated gene expression of nicotinic receptor subunits (B2, B4, a5) and neuroplasticity-related genes (synaptophysin, synaptotagmin1, BDNF) using quantitative polymerase chain reaction (qPCR). We found that psilocybin increased neuroplasticity-related genes in the MHb and IPN in mice, two regions that are activated during nicotine withdrawal. However, we found no significant changes in gene expression of nicotinic receptor subunits in any brain regions tested. Future studies will investigate the changes in these genes in nicotine-dependent animals and explore additional doses of psilocybin.

This research was supported by the National Institute on Drug Abuse of the National Institutes of Health under award numbers P30DA033934 to MID, T32DA007027 to BB, and a small pilot grant from Virginia Youth Tobacco Projects (VYTP).



CVCSN



Imaging in Neuroscience

Abstract: 65**Title: The Effect of Psilocybin on Nicotine Vaping in a Novel Oronasal-Restricted Vapor Self-Administration Mouse Model****Buttar B¹**, Buzzi B¹, Oros A¹, Klein S¹, Groener Y¹, Kalck E¹, Gonzalez-Maeso J², Damaj MI¹¹Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298.²Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA 23298.**Abstract:**

Nicotine is one of the leading causes of preventable deaths across the world. Meanwhile, accessibility and use of electronic nicotine delivery systems has increased. Although many long-term risks of vaping remain relatively unknown, novel pharmacological interventions can improve the currently limited scope and efficacy of cessation treatments. Psilocybin, a classical psychedelic, has been suggested to be effective as a smoking cessation tool in a small clinical study at Johns Hopkins University (Johnson et al., 2014). However, psilocybin has not been thoroughly examined as a treatment for nicotine vaping, especially among young adults. This study uses a recently published novel, oronasal-restricted, nicotine vapor self-administration mouse model (Akinola et al., 2025) to investigate the effect of the psychedelic drug, psilocybin, on nicotine intake behavior in young adult female mice. Additionally, a separate cohort was examined to investigate if psilocybin alters acute nicotinic behaviors, including body temperature, analgesia, and locomotor activity. After training and operantly conditioning female mice to self-administer nicotine, psilocybin was acutely administered (1 and 3 mg/kg, i.p.). The results show that psilocybin reduces nicotine intake, and not nicotine-free e-liquid, 24 hours after administration, and for up to 6 days post psilocybin injection. In addition, psilocybin did not alter any acute effects of nicotine when administered 24 hours prior. In conclusion, psilocybin may be a potential treatment option for smoking cessation of e-cigarettes. Future research is needed to examine possible sex differences and to investigate the neurobiological mechanisms of psilocybin on regions of the brain activated by nicotine.

Funding: This research was supported by the National Institute on Drug Abuse of the National Institutes of Health under award numbers P30DA033934 to MID, T32DA007027 to BB, and a small pilot grant from Virginia Youth Tobacco Projects (VYTP).



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Imaging in Neuroscience

Abstract: 66**Title: Respiratory Depressant Effects of Fentanyl and Xylazine and Their Reversal in Mice****Lewis MR¹, Walentiny DM¹**¹Department of Pharmacology & Toxicology, Virginia Commonwealth University School of Medicine.**Abstract:**

Background: Opioid misuse remains a critical public health crisis in the U.S. with approximately 75,000 overdose deaths attributed to fentanyl or related synthetic opioids occurring in 2023. Opioid overdose results from hypoventilation mediated by μ -opioid receptor (MOR) activation. Recently, illicitly manufactured fentanyl has been increasingly adulterated with xylazine, an alpha-2 adrenergic receptor (α 2AR) agonist used in veterinary medicine. This drug combination, often referred to as “tranq dope”, can cause severe complications, including skin wounds and abscesses, polydrug dependence, and hypoventilation that may be more resistant to naloxone reversal, thus increasing overdose risk. In 2023, xylazine was found in 30% of fentanyl powder seizures, prompting concerns over its role in the overdose crisis. This study examined fentanyl- and xylazine-induced respiratory depression in mice and evaluated reversal effectiveness of the MOR antagonist naloxone and the α 2AR antagonist yohimbine. **Methods:** Respiration was measured in adult male C57Bl/6J mice via whole-body plethysmography. Dose-response curves were established for fentanyl (0.01-10 mg/kg) and xylazine (0.3-10 mg/kg). A dose of 0.3 mg/kg of each agonist produced an approximate 50% reduction in baseline minute volume (MVb), and was utilized in subsequent reversal tests with naloxone (0.1-10 mg/kg) and yohimbine (1-10 mg/kg). **Results:** Fentanyl reduced MVb by decreasing respiratory frequency (Freq) while increasing tidal volume (TVb), and these effects were reversed by naloxone. Yohimbine briefly worsened fentanyl-depressed MVb. Xylazine dose-dependently reduced MVb by lowering both TVb and Freq which was reversed by yohimbine but not naloxone. The fentanyl and xylazine combination affected respiration similarly to fentanyl alone (i.e., increasing TVb and decreasing Freq) and was reversed by naloxone but not yohimbine. However, combining naloxone and yohimbine led to a faster reversal of MVb than naloxone alone. **Conclusions:** Respiratory depressant effects of fentanyl and xylazine are primarily mediated via MOR and α 2AR, respectively. Naloxone reversed respiratory depression caused by fentanyl and xylazine coadministration, supporting its continued use as a first-line overdose treatment. The combination of naloxone with an α 2AR antagonist may offer faster or more effective overdose reversal, though additional studies performed under a broader range of experimental conditions are needed to further test this hypothesis.



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Imaging in Neuroscience

Abstract: 67**Title: Exploration into psilocybin's effects on perineuronal nets in the cortex and hippocampus of C57BL/6J mice.****Zylko AL¹**, Pulugujju B¹, Todupunoori S¹, Saraswat M¹, Gonzalez-Maeso J² and Lasek AW¹¹Department of Pharmacology and Toxicology, Virginia Commonwealth University²Department of Physiology and Biophysics, Virginia Commonwealth University.**Abstract:**

In recent years, the psychedelic compound psilocybin has emerged as a promising therapeutic for a wide variety of diseases, including major depressive disorder, anxiety disorders, and substance use disorders. While underlying causes of these diseases vary, psilocybin's therapeutic efficacy for each may be related to its ability to induce neuroplasticity across the brain. Perineuronal nets are extracellular matrix structures that are critical for regulating neuronal outgrowth and formation of new synapses. This project examined the effects of systemic administration of psilocybin on perineuronal nets and the parvalbumin-expressing interneurons they surround in the anterior insular and cingulate cortices, and the CA1 region of the dorsal and ventral hippocampus. We used the plant lectin WFA to visualize perineuronal nets and immunohistochemistry with antibodies to parvalbumin and c-fos on mouse brain sections to quantify the number of perineuronal nets, parvalbumin neurons, and c-fos positive cells, as well as the intensity of perineuronal net staining. Mice were treated with a single intraperitoneal injection of 1 mg/kg psilocybin or saline, and tissue was collected 2.5 or 24 hours after injection. Results uncover brain region, time, and sex-specific effects of psilocybin. 24 hours after psilocybin treatment, we observed a decrease in the number of perineuronal nets in the psilocybin-treated group in the cingulate cortex, as well as a sex difference showing females had a higher number of parvalbumin neurons regardless of treatment. At 2.5 hours, no changes were observed in the anterior cingulate cortex or dorsal hippocampus, however there was a decrease in the intensity of perineuronal nets in the insular cortex and ventral hippocampus of psilocybin-treated female mice. We also observed an increase in c-fos positive cells after psilocybin in both males and females only in the insular cortex at this time point. The decrease in WFA staining intensity indicates a decreased density of perineuronal nets, which can "reopen" windows of plasticity, and alter firing of the parvalbumin neurons they surround. These data warrant further study into the effects of psilocybin on perineuronal nets, and the implications the results can have into the therapeutic mechanisms of psilocybin.



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Abstract: 68**Title: Characterization of the ipRGC's Role in Non-Image-Forming Pathways in Glaucoma**Wenjin Xu¹, Shichu Chang¹, Mingna Liu¹, Ignacio Provencio^{1,2,3}, Xiaorong Liu^{1,2,3,4}¹Department of Biology, University of Virginia, Charlottesville, Virginia, USA²Department of Ophthalmology, University of Virginia, Charlottesville, Virginia, USA³Program in Fundamental Neuroscience, University of Virginia, Charlottesville, Virginia, USA⁴Department of Psychology, University of Virginia, Charlottesville, Virginia, USA**Abstract:**

We investigate the mechanism through which intrinsically photosensitive retinal ganglion cells (ipRGCs), crucial mediators of light's influence on sleep, cognition, and mood regulation, function within the non-image-forming pathways in the context of glaucoma. We have used two behavioral paradigms, the open-field test (OFT) and elevated plus maze (EPM), to evaluate the anxiety level in *Angiopoietin1* conditional knockout mice (A1 cKO), a model of open-angle glaucoma. To examine the ipRGC circuits, we crossed *Opn4*^{Cre-ERT2/Cre-ERT2} mice (Jackson Laboratory, 035926) with *R26*^{Syp-tdT/+} mice (Jackson Laboratory, 012570) to accurately report the projections of ipRGCs in adult brain. At 3 months old, the progeny receive an *i.p.* injection of tamoxifen on five consecutive days to induce ipRGC-specific expression of the synaptophysin-tdTomato fusion protein. Two weeks post-injection, brains and retinas were prepared for immunohistochemistry and confocal imaging. We also injected a viral tracer into brain targets of interest to analyze target-specific ipRGC subtype projections. In addition, cholera toxin subunit B (CTB) was injected into the eye 5 days prior to sacrifice. The long-term damage by ocular hypertension in A1 cKO mice results in a reduced duration spent "in center" during OFT and "in open arms" in the EPM when compared to WT control, indicating a trend toward anxiety-like behavior. Sparse yet discernible tdTomato signals were detected in key regions associated with sleep promotion, including the ventrolateral preoptic nucleus, as well as mood-regulating centers such as the lateral habenula. CTB anterograde tracing and anti-PSD-95 immunostaining in these specific regions support their identity as ipRGC terminals. We observed anxiety-like behaviors in the glaucoma mouse model and characterized ipRGC projections in the adult mouse brain with genetic tools. Our findings suggest potential neuronal circuitry underlying glaucomatous insult to the non-image-forming system. Our subsequent investigations will delve into the analysis of how these target-specific ipRGCs degenerate as glaucoma progresses, and we will elucidate the impact of their loss on behaviors.



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Abstract: 69**Title: Assessing TBI-responsive changes of the clinically relevant proteins MafA and PURA in brain tissue following CCI injury****Wang J¹, Platfoot KE¹, Ottens AK¹.**¹ Department of Anatomy and Neurobiology, VCU.**Abstract:**

Traumatic brain injury (TBI) often results in long-term disability and affects millions globally. Blood-based biomarkers have been sought for TBI as a lower-cost, pathology-driven diagnostic that has the potential for repeated use in patient management. Recent work from the Ottens lab identified peptide fragments from MafA and Pur-alpha (PURA) in the serum of TBI patients that correlate with important clinical metrics of patient status across time. The distribution of their parent proteins, and colocalization with additional relevant markers, were assessed using immunohistochemical staining of brain tissue collected from rats injured using a controlled cortical impact (CCI) model of TBI to gain a better understanding of their role in TBI pathobiology. The role of MAFA within the glial scar was investigated by assessing expression in mature, reactive astrocytes using GFAP, scar-forming astrocytes using β -catenin, and proliferating cells using Ki67. MAFA signal was reduced in the tissue immediately adjacent to the lesion coincident to reduced GFAP signaling. Additionally, there was no colocalization with β -catenin or Ki67, suggesting changes in MAFA are specific to astrocyte functional subtypes and unrelated to scar formation. Disruption of mRNA trafficking by PURA was assessed via colocalization with CDK5R1 and MAP2 and colocalization between the PURA substrate α -catenin and the dendritic spine marker ACTR3. Near the site of impact, PURA colocalization with both CDK5R1 and MAP2 was significantly reduced, suggesting impaired activation of Kif5 by CDK5R1 is contributing the limited distribution of PURA along dendrites. Colocalization increased further from the site of injury. A similar distribution was observed with α -catenin and ACTR3 indicating an inability for PURA substrates to reach distal ends of the dendrite and further supporting the hypothesis that PURA trafficking is disrupted following TBI. Taken together, these results indicate MafA and PURA are relevant to the pathobiology of TBI.



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Abstract: 70

Title: Profiling cerebrospinal fluid metabolites in traumatic brain injury and stroke
Harge C¹, Carlson AP¹

¹Department of Neurosurgery, UVA

Abstract:

Neurological disorders like traumatic brain injury (TBI) and stroke are the leading causes of deaths worldwide. They can affect patient's cognitive function, motor skills and sensory perception. At a clinical level, TBI and stroke can be managed by maintaining adequate oxygenation, cerebral perfusion pressure, cerebrovascular pressure as well as analysis of cerebrospinal fluid (CSF). TBI and stroke can disrupt blood-brain barrier causing leakage of substances from blood into CSF, altering its composition. Injured brain tissue can also release specific molecules into the CSF. Analysis of such molecules in TBI and stroke patients can help determine CSF signatures in patient recovery. Recent data science advances provide tools to analyze complex data from CSF samples to identify potential biomarkers. We analyzed metabolite profiles present in CSF after TBI and Subarachnoid Hemorrhage (SAH) stroke. We characterized patterns in CSF metabolites and physiological indicators in TBI and SAH patients with poor and good recovery outcomes. We are further utilizing detailed data obtained through CSF mass spectrometry to identify specific molecules that are significantly altered following TBI/SAH. Combining CSF data with other neuromonitoring assessments may be used to build robust predictive models for TBI/SAH prognosis.



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Abstract: 71**Title: Endogenous peptides detected in the serum of TBI patients correlate with clinical metrics to inform on patient status.****Platfoot KE¹, Roy AK², Valadka AB³, Ottens AK¹**¹ Department of Anatomy and Neurobiology, VCU² Department of Neurosurgery, VCU³ Department of Neurological Surgery, UT Southwest**Abstract:**

Traumatic brain injury (TBI) is a leading cause of death and disability worldwide. Despite medical advances improving patient survival, many are left with lifelong cognitive and functional disabilities ultimately impacting their quality of life. Blood-based biomarkers would be particularly advantageous in TBI as they are faster and more accessible than imaging, such as CT or MRI, and allow for repeated measures over time without concerns of radiation exposure or extreme cost. Currently, FDA approved blood-based biomarkers GFAP and UCH-L1 are used to aid clinicians in the diagnosis of relatively mild injuries, but markers capable of informing on patient status and underlying pathobiology are still sorely needed. This study used mass spectrometry to identify and quantify endogenous peptides detected in the serum of TBI patients admitted to the NSICU at VCU Health. Correlation of these peptides with important clinical metrics across time was assessed using generalized linear mixed models (GLMM). Peptides from the following proteins were found to strongly correlate with varying combinations of GCS, age, blood pressure, CO₂, and blood glucose: MafA (AIC=47.15), Pur- α (AIC=47.36), PTPRZ1 (AIC=52.22), HAPLN4 (AIC=53.84), and TRIM33(AIC=54.94). By comparison, GFAP and UCH-L1 correlated significantly worse with AIC values of 110.2 and 111.5, respectively, though this was to be expected given their diagnostic, not observational, purpose. These results lay the foundation for the development of a clinical assay with the potential to provide insight into the impetus of changes in patient status. Subsequent studies will reverse-translate this work to a rodent model of TBI to better understand pathobiological relevance of these peptides to TBI patient status.



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Abstract: 72**Title: Single-cell RNA sequencing reveals the impact of TBI and Notch1 conditional knock down on functional changes of hippocampal adult-born neurons.****Jakob C. Green**¹, Xufeng Qu², Nicole Weston¹, Jinze Liu², Dong Sun^{1*}¹ Department of Anatomy and Neurobiology, Virginia Commonwealth University, Richmond, VA² Department of Biostatistics, Virginia Commonwealth University, Richmond, VA.**Abstract:**

Traumatic Brain Injury (TBI) is a debilitating condition associated with pathological changes in the brain. Following TBI there is an enhanced generation of new neurons from neural stem cells (NSCs) in the neurogenic regions of the brain, i.e. the dentate gyrus (DG) of the hippocampus and the subventricular zone (SVZ). Studies have demonstrated that these injury-induced new neurons participate post-injury brain repair, however, the reaction of distinct pools of NSCs in response to TBI, the molecular control of injury-induced activation of these cells, the influence of injury severity on their maturation stages and functioning in post-TBI neural circuit reorganization are largely unknown. Utilizing transgenic mice with tamoxifen-induced GFP expression and Notch1 knock-out in nestin+ NSCs, this study examined the impact of TBI and Notch1, a master regulator of stem cells, on the functional changes of newly generated neurons in the hippocampus at the chronic stage after TBI using single-cell RNA sequencing (RNAseq). To do this, we used the tamoxifen (TAM) inducible Nestin-CreER^{T2}/ R26R-EYFP, and a conditional Notch1 knockout (Notch1 cKO) generated from a Notch1 Flox, nestin-CreER, and R26R-EYFP mouse lines. Animals were treated with tamoxifen for six days followed by a lateral fluid percussive injury (LFPI). Hippocampal tissue was harvested 8 weeks later and cells isolated for FACs. EYFP positive cells were isolated and prepared for RNAseq using the 10x Genomics Chromium platform. Cell populations were identified using an automated cell-type identification tool "scType." We found TBI and Notch1 cKO induced changes in NSC progenies and expression profiles of genes regulating functions of mitochondria, cell cycle, senescence, synaptogenesis, DNA/RNA processing, inflammatory, and various signaling and metabolic pathways. Supported by VCU CCTR grant.



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Abstract: 73**Title: Electrophysiological Investigation of Ionotropic and Non-Ionotropic Signaling in NMDA Receptors with Chimeric GluN2 Subunits****Diborah Gutema**, Dr. Greta Ann Herin and Dr. Theodore DumasCollaborative Undergraduate Neuroscience Lab
Physiological and Behavioral Neuroscience in Juveniles Lab
Neuroscience Department, College of Science, George Mason University**Abstract:**

NMDA receptors (NMDARs) play a critical role in synaptic plasticity and cognitive function by mediating both ionotropic and non-ionotropic signaling. The GluN2A and GluN2B subunits of NMDARs exhibit distinct functional properties, with a developmental shift from GluN2B to GluN2A influencing synaptic maturation. While previous research has explored the physiological and behavioral consequences of this transition, the precise contributions of ionotropic and non-ionotropic mechanisms remain unclear. This study aims to dissect these signaling pathways by utilizing chimeric GluN2 subunits engineered to separate ionotropic and non-ionotropic functions. Chimeric constructs are currently being subcloned into *Xenopus laevis*-compatible vectors for functional expression in oocytes. Once subcloning is complete, the constructs will be injected into *Xenopus laevis* oocytes, followed by two-electrode voltage clamp (TEVC) recordings to measure receptor responses to glutamate and glycine. Statistical analyses using two-way ANOVA will be conducted to evaluate differences across experimental groups, with the goal of identifying distinct electrophysiological profiles between chimeric and native GluN2A- and GluN2B-containing NMDARs. Findings from this research will advance our understanding of NMDAR-mediated plasticity and may inform therapeutic strategies for neurological disorders linked to NMDAR dysfunction.



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Abstract: 74**Title: The Impact of MEF2C deletion on Memory Deficits and Ethanol Sensitivity in Mice****Wright, C¹**, Morgan, A², Betancourt-Toscano, I², Miles, MF^{1,2}Wolstenholme, JT, PhD^{1,2}¹Department of Pharmacology and Toxicology, VCU²VCU-Alcohol Research Center, VCU**Abstract:**

Cross-species studies in the VCU-Alcohol Research Center have identified MEF2, as a candidate gene that may impact initial sensitivity to ethanol, influencing a potential risk for alcohol misuse. MEF2C, myocyte enhancer factor 2C, is a protein coding gene that plays a role in neuronal survival and neural pruning as well as cell differentiation, survival, and apoptosis. In the VCU-ARC, MEF2C has been found to influence the self-rating effects of alcohol in humans and altered MEF2 expression impacted ethanol sedation in *Drosophila*. Our goal is to investigate the effects of knocking down MEF2C protein expression in the prefrontal cortex to assess its role in ethanol sensitivity and memory. Adult male and female mice underwent stereotaxic injection to knockdown the *Mef2C* gene (pENN.AAV8.hSyn.HI.eGFP-Cre.WPRE.SV40 (titer $\geq 1 \times 10^{13}$ vg/mL)), or a control virus, pAAV8-hSyn-EGFP (titer $\geq 7 \times 10^{12}$ vg/mL) in the prefrontal cortex (PFC) of mice expressing a floxed *Mef2C* gene. Three weeks following surgeries to allow for MEF2C knockdown, mice were tested for potential deficits in short-term memory using the novel object recognition (NOR) task and ethanol sensitivity using the loss of righting reflex task. MEF2C knockdown in the PFC shows a trend towards recognition deficits in both males and females. Ethanol sensitivity to a sedating ethanol dose, however, did not differ between MEF2C knockdowns and controls. This suggests that MEF2C knock down may alter learning and memory, but may not play a strong role in ethanol sedation.



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Abstract: 75**Title: A New Animal Model of AQP4 Autoimmunity Zheng L¹, McVoy J¹, Mufti F¹, Abe Y², Yasui M², Oh U¹**¹Virginia Commonwealth University School of Medicine, Richmond, VA, USA²Keio University School of Medicine, Tokyo, Japan**Abstract:**

Background: Preclinical research in neuromyelitis optica spectrum disorder associated with aquaporin 4 antibody (AQP4 NMOSD) is limited by the lack of a model that recapitulates the autoimmunity and the pathology of AQP4 NMOSD in the same animal. Central immune tolerance prevents the development of autoimmunity against AQP4 in wild type mice, and AQP4 null mice, though susceptible to AQP4 autoimmunity, do not exhibit the pathology of AQP4-directed autoimmunity due to lack of target antigen. Thus, the existing models of AQP4 NMOSD require adoptive transfer, which confounds interpretation of studies evaluating therapeutic effect, particularly those targeting anti-AQP4 T cells. Objectives: To develop a new animal model of AQP4 NMOSD, a novel transgenic mouse strain containing a loxP flanked LacZ sequence inserted before the start codon in exon 0 of the *Aqp4* gene (AQP4.LacZ) was tested. Methods: AQP4 expression was assessed by polymerase chain reaction (PCR), immunoblotting and immunohistochemistry in various tissues and primary astrocyte cultures from AQP4.LacZ mice and following adeno-associated virus (AAV)-mediated Cre recombinase transduction. AQP4 function was assessed by detecting intracellular calcein fluorescence quenching as a measure of cell volume change in response to hypotonic challenge in cultured astrocytes. AQP4.LacZ animals were imaged using Magnetic Resonance Imaging (MRI) to examine the brain morphology. Results: Quantitative reverse transcriptase (qRT)-PCR, immunoblotting and immunohistochemistry confirmed the lack of *Aqp4* transcript and protein in the AQP4.LacZ mouse. The time to peak swelling and regulatory volume decrease were significantly blunted in response to hypotonic challenge in primary cultured astrocytes from AQP4.LacZ mice compared to wild type, confirming a functional loss of AQP4. AAV-mediated transduction of Cre recombinase partially restored AQP4 expression and function with notable increase in the M23 transcript variant. Conclusion: The AQP4.LacZ mouse is a AQP4 null strain amenable to restoration of AQP4 expression in the presence of Cre recombinase. Future work will test if selectively restoring AQP4 expression in astrocytes in the AQP4.LacZ mouse following immunization will result in the development of AQP4-directed autoimmunity and pathology in the same animal.



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Awards

Awards will be presented at the end of the conference.

Stay tuned for the winners.

Closing Remarks

Dear CVCSN 2025 Attendees,

As we wrap up the annual Central Virginia Chapter of the Society for Neuroscience Conference, we want to acknowledge the incredible journey we have shared today. Our time together has not only focused on the latest advancements in neuroscience but has also fostered inspiring conversations that propel our mission forward. Thank you to our speakers for their insights, our sponsors for their support, and the organizing committee for their hard work. Your contributions have made this event a success. Let's take the knowledge and inspiration gained here to make a meaningful impact in our communities and for those affected by neurological disorders. We encourage you to stay connected and continue collaborating. Thank you for being a part of this year's conference. Safe travels, and we look forward to seeing you at our next gathering!

Sincerely,

The CVCSN 2025 Organizing Committee



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Venue Information

Conference Venue: Delta Hotel, Richmond Downtown,
555 E Canal St, Richmond, VA 23219

Parking: On-site parking will be available on a limited basis
(\$5 for a day)

Paid off-site parking is available at City Parking Lot #61.

Check this link for more nearby parking options.

https://en.parkopedia.com/parking/locations/555_east_canal_street_richmond_virginia_23219_united_states_00abdq8vtc95p2ei79/?country=us&arriving=202503290830&leaving=202503291730#google_vignette

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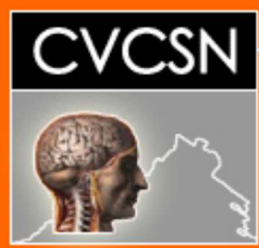


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Meeting adjourned



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