2024 Central Virginia Chapter of the Society for Neuroscience

April 27, 2024

Steger Hall, Room xxx

Virginia Tech

Blacksburg, VA

Acknowledgments

VT College of Science

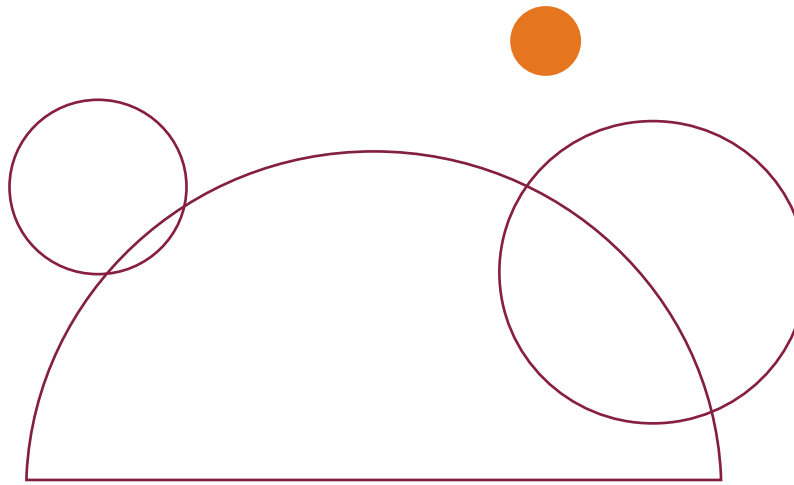
VT Office of Research and Innovation

VT Department of Biomedical Sciences and Pathobiology

VT School of Animal Sciences

VT School of Neuroscience

Fralin Life Sciences Institute

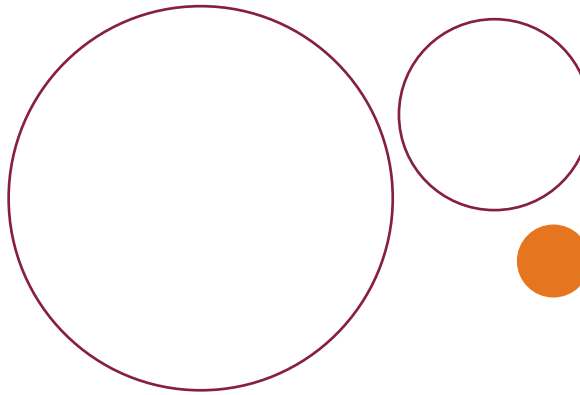


CVCSN Schedule

April 27, 2024

Steger Hall, Room

Breakfast	8:00 am – 8:45 am
Session 1	8:45 am – 10:30 am
Break	10:30 am – 11:00 am
Session 2	11:00 am – 12:30 pm
Lunch	12:30 pm – 1:30 pm
Poster Session	1:30 pm – 3:00 pm
Break	3:00 pm – 3:15 pm
Session 3	3:15 pm – 5:00 pm
Awards	5:00 pm – 5:15pm



Overview of Programs

Session 1: 8:45 am – 10:30 am

Plenary Talk

1. **Understanding aggression using the fruit fly,** Katie Schretter, Janelia Farm

12 minute talk + 3 minute question

2. **Dynamics of the 3D genome architecture in the development of the malaria mosquito nervous system**, Igor Sharakhov, Virginia Tech
3. **Perineuronal nets regulate structure and function of tripartite synapses**, Bhanu Tewari, University of Virginia
4. **A high-resolution transcriptomic atlas of cell types in the lateral geniculate nucleus**, Katelyn Stebbins, Virginia Tech
5. **Influence of the physiological state on the neural response to host and plant odors in Aedes aegypti mosquitoes,** Spruha Rami, Virginia Tech

3 minute flash talk

6. **Proprioceptive control of rapid and flexible limbs movements during Drosophila landing**, Wayne Kuo, Virginia Tech, Poster Number: 30
7. **Drosophila Larvae Cool Cell 3D Calcium Imaging Analysis using TACI**, Trisha Naidu, Virginia Tech, Poster Number: 43

Session 1: 8:45 am – 10:30 am

3 minute flash talk

8. **Miro Regulation of Neuron-Glia Interactions**, Kathleen Reuwer, University of Virginia, Poster Number: 49
9. **Investigating the potential role of adaptive immunity in dopaminergic neuron vulnerability after mild traumatic brain iniurv**. Colin Kellv. Virginia Tech. Poster Number: 24

Session 2: 11:00 am – 12:30 pm

Plenary Talk

10. **Western-Style Diet Alters Neuronal Morphology in the Hippocampus and Entorhinal Cortex of Ovariectomized Female Rats with and without Estradiol**, Sarah Blythe, Washington and Lee

12 minute talk + 3 minute question

11. **A zinc finger transcription factor tunes social behaviors while controlling transposable elements and immune response in prefrontal cortex**, Natalie Truby, Virginia Commonwealth University
12. **Midnolin as an Alternative, Sex-Specific Protein Degradation Pathway Necessary for Fear Memory Formation**, Phill Gwin, Virginia Tech
13. **Chronic adolescent stress attenuates morphine-induced antinociception and central amygdala activity in adult male and female rats,** Hannah Fulenwider, Virginia Commonwealth

Session 2: 11:00 am – 12:30 pm

3 minute flash talk

14. **Diversity of mitochondrial morphology across hippocampal circuits**, Mayd Alsalman, Virginia Tech, Poster Number: 1
15. **Investigating the Distinct Roles of the Anterior and Posterior Insula in Gating of Nociceptive Stimulus using Low-Intensity Focused Ultrasound**, Gabriel Isaac, Virginia Tech, Poster Number: 20
16. **Enhancing Double-Blind Clinical Trials in Neuromodulation: A Novel Application of 3D-Printed Thermoplastics and High-Density Gel Matrices in Low-Intensity Focused Ultrasound**, Aditya Kapoor, Virginia Tech, Poster Number: 22
17. **Application of the human 3D Neuron-Glial Brain Assembloid reveals the differential role of ApoE isoforms in the pathogenesis of Alzheimer's disease**, Eliana Sherman, University of Virginia, Poster Number: 54
18. **Characterizing the Mcoln1-/- mouse as a model of early Age-related Macular Degeneration**, Jonathan Miller, James Madison University, Poster Number: 38

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#1: **Diversity of mitochondrial morphology across hippocampal circuits,** Mayd Alsalman, Virginia Tech

#2: **The Impact of Buprenorphine on Astrocyte Communication after Traumatic Brain Injury in Rats**, Rana Ansari , Virginia Commonwealth University

#3: **The Expression of Ionotropic Receptors Impacts the Sensitivity of Drosophila Larval Cool Cells under Hypertonic Conditions**, Hua Bai, Virginia Tech

#4: **Transgenerational Effects of Ethanol Exposure in Caenorhabditis elegans**, Ava Berckmueller, Amenah Holt, Aryan Bangalore, Virginia Tech

#5: **Effects of complex I deficiency and PINK1/ Parkin mediated mitophagy on neurogenesis of the subventricular zone in Ndufs4 KO mouse model of Leigh syndrome**, Sahitya Ranjan Biswas, Virginia Tech

#6: **Developmentally regulated "don't eat me" signaling in the lateral cortex of the inferior colliculus during peak pruning**, Maddie Book, James Madison University

#7: **Examination of cannabidiol oil on C. elegans behavior and cellular stress,** Claire Branscome, Virginia Tech

#8: **Investigating the role of MCU in powering circuit-specific forms of structural plasticity and social behavior**, Mikel Cawley, Virginia Tech

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#9: **Role of extracellular matrix in Alzheimer Disease-associated social memory impairment**, Lata Chaunsali, University of Virginia

#10: **Wandering with the wanderer: vagal modulation of glucose tolerance and cardiac function**, Nicholas Conley, University of Virginia

#11: **Investigating the electrophysiological features of post traumatic epilepsy in rat hippocampus**, Michael Dexheimer, Virginia Commonwealth University

#12: **Auditory and Somatosensory Multimodal Pre-Pulse Inhibition in Control and Complement Receptor 3 Knockout Mice**, Alyssa Diana, James Madison University

#13: **Starvation resistance and increased food consumption in serotonin transporter (dSert) mutants in Drosophila melanogaster**, Abigail Forrest, Eastern Mennonite University

#14: **The Effects of an Acidic Pollutant on the Nervous System of C. elegans**, Ariana Garrastegui Segarra, Virginia Tech

#15: **Altered Hippocampal Network Dynamics in a Lateral Fluid Percussion Injury model of Post-traumatic Epilepsy**, Adam Gibson, Virginia Commonwealth University

#16: **Dorsal Motor Nucleus of the Vagus Can Induce Bradycardia and Reduce Anxiety-like Behaviors**, Patricia Castro-Martinez, University of Virginia

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#17: **Biochemical Characterization of the Retina-specific Transcription Factor CRX,** Aparna Gupta, Ali Carl, James Madison University

#18: **Acetaminophen and ibuprofen do not have a direct effect on nerve activities in the central nervous system in crickets**, Henry Holley, Virginia Tech

#19: **Responses of different Drosophila species to temperature changes**, Ainul Huda, Virginia Tech

#20: **Investigating the Distinct Roles of the Anterior and Posterior Insula in Gating of Nociceptive Stimulus using Low-Intensity Focused Ultrasound,** Gabriel Isaac, Virginia Tech

#21: **Inducible knockout of autism gene Itgb3 and MORF3 labeling of excitatory pyramidal neurons during early postnatal development of the cerebral cortex**, Anna Kalinowski, James Madison University

#22: **Enhancing Double-Blind Clinical Trials in Neuromodulation: A Novel Application of 3D-Printed Thermoplastics and High-Density Gel Matrices in Low-Intensity Focused Ultrasound**, Aditya Kapoor, Virginia Tech

#23: **The Effect of unc-53 on C. elegans Apoptosis,** Evan Kauffman, Eastern Mennonite University

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#24: **Investigating the potential role of adaptive immunity in dopaminergic neuron vulnerability after mild traumatic brain injury,** Colin Kelly, Virginia Tech

#25: **Input-specific organization of NMDA receptors in the thalamus,** Rabeya Khondaker, Virginia Tech

#26: **The Role of Brain Stem 5HT1A Receptors in the Protective Hypothermic Response to Hypoxic Stress,** Catherine Kirkhorn, James Madison University

#27: **Vasoactive intestinal peptide-expressing interneurons are impaired in SCN8A Epileptic Encephalopathy,** Shrinidhi Kittur, University of Virginia

#28: **Hippocampal perineuronal net plasticity is associated with astrocyte dysregulation,** Piotr Kraszewski, University of Virginia

#29: **Multiple glial subtypes interact and compensate for the loss of nearby glial function,** Kevin Krause, University of Virginia

#30: **Proprioceptive control of rapid and flexible limbs movements during Drosophila landing,** Wayne Kuo, Virginia Tech

#31: **The nociceptive withdrawal response in intact, unanesthetized rats exhibits strong dependence on initial posture but weak dependence on stimulus location**, Claire Larson, James Madison University

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#32: **Microglial depletion impairs social memory function and diminishes hypothalamic afferents to the hippocampal CA2 region**, Sabrina Lee, University of Virginia

#33: **Navigating the Navs: Applying RNA-seq Tools to Understand Differential Isoform Expression of UNC-53 in C. elegans**, Meredith Lehman, John Jantzen, Eastern Mennonite University

#34: **Interactions between positive allosteric modulation of muscarinic acetylcholine receptors and orexin receptor antagonism in cognitive flexibility in rats (Rattus norvegicus),** Mutian Li, William & Mary

#35: **Regional astrocyte changes following diffuse traumatic brain injury and buprenorphine administration in rats**, Radina Lilova, Virginia Commonwealth University

#36: **Exploring P2RY12 as a marker for widespread harvesting of microglia in the early postnatal multisensory midbrain**, Sophia Lindauer, James Madison University

#37: **The Relationship Between Purkinje Cells and Nuclei Cells in Mouse Models for Cerebellar Movement Disorders**, Alyssa Lyon, Virginia Tech

#38: **Characterizing the Mcoln1-/- mouse as a model of early Age-related Macular Degeneration**, Jonathan Miller, James Madison University

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#39: **The Role of Brain Stem 5HT1A and GABAA Receptors in the Thermoregulatory Response to Handling Stress,** Halle Miller, James Madison University

#40: **The Effect of Diet-Induced Obesity on Cognitive Performance in Rodent Model**, Haley Minnery, Washington and Lee University

#41: **Perineuronal net plasticity in age-related cognitive function**, Jacqueline Moats, University of Virgnia

#42: **Characterization of Metachromatic Leukodystrophy Disease Model**, Emily Moran, Virginia Commonwealth University

#43: **Drosophila Larvae Cool Cell 3D Calcium Imaging Analysis using TACI,** Trisha Naidu, Virginia Tech

#44: **Neuromelanin Contrast Ratio's Inverse Relationship with Locus Coeruleus Volume and Its Impact on Attentional Ability**, Joshua Neal, Virginia Tech

#45: **A Novel Feeding-Activated Arcuate Neuron Subtype That Controls Body Weight, But Not Feeding,** Roberta Onoharigho, University of Virginia

#46: **mTBI in higher order animal models: Assessing changes in Yucatan Minipig sociability following diffuse central fluid percussion injury**, Mark Pavlichenko, Virginia Commonwealth University

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#47: **Spatiotemporal multi-omics analysis of the hippocampal-amygdala circuit during contextual fear memory consolidation**, Xiguang Xu, Virginia Tech

#48: **Investigation of Low-Intensity Focused Ultrasound (LIFU) for Neuromodulation of Anxiety**, Arthi Prabhakar, Virginia Tech Carilion School of Medicine

#49: **Miro Regulation of Neuron-Glia Interactions**, Kathleen Reuwer, University of Virginia

#50: **Mediodorsal Thalamus Projections to the Prefrontal Cortex and Their Roles in Cue Detection**, Kelly Runyon, Virginia Tech

#51: **Synaptic transmission alterations in a mouse model of Szt2 loss-of-function**, Mona Safari, Virginia Tech

#52: **Microglial-mediated perineuronal net accumulation in the hippocampal CA2 region coincides with social memory impairments**, Brenda Sanchez, University of Virginia

#53: **Loss-of-function in the Drosophila serotonin transporter (dSert) gene changes sleep and activity and decreases life span**, Marciella Shallomita, Eastern Mennonite University

#54: **Application of the human 3D Neuron-Glial Brain Assembloid reveals the differential role of ApoE isoforms in the pathogenesis of Alzheimer's disease,** Eliana Sherman, University of Virginia

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#55: **Heterozygous expression of a Kcnt1 gain-of-function variant has differential effects on SST- and PV-expressing cortical GABAergic neurons**, Amy Shore, Virginia Tech

#56: **Inhaled Wildfire Smoke Particulate Drives Aberrant Proteinopathy-related Changes in the Adult Mouse Brain**, Mohammad Siddiqi, Virginia Commonwealth University

#57: **Mapping neural connectivity of strain-sensing proprioceptive sensory neurons in the leg of Drosophila melanogaster**, Himadri Sunam, Virginia Tech

#58: **Uncovering the molecular logic of dendritic RNA localization to synapses**, Renesa Tarannum, Virginia Tech

#59: **Assessing The Effect of Caffeine on Associative Butanone Learning in C. elegans**, Caleb Townsend, Virginia Tech

#60: **Investigating the Efficacy of a Targeted Therapy on Reducing Seizures in a Mouse Model of Epilepsy,** Jessica Urbanczyk, Virginia Tech Carilion School of Medicine

#61: **Defining Molecular Mechanisms Underlying Thermoreceptors IR21a and IR68a**, Thomas Vaden, Virginia Tech

#62: **The Role of Brain Stem GABA-A Receptors in the Protective Hypothermic Response to Hypoxic Stress,** Carissa Vergeres, James Madison University

Poster Session: 1:30 pm – 3:00 pm

Poster Session 1 (Odd Numbers): 1:30 pm – 2:15 pm

Poster Session 2 (Even Numbers): 2:15 pm – 3:00 pm

#63: **Ultrasonic Modulation of Insular Subregions Reveals Distinct Roles in the Gating of Nociceptive Stimulus,** Annie Walls, Virginia Tech

#64: **Developing a New Animal Model of AQP4 Autoimmunity,** Leon Zheng, Julie McVoy, Yoichiro Abe, Masato Yasui, Unsong Oh, Virginia Commonwealth University & Keio University

#65: **Neuroscience PhD Program at Virginia Tech**, Alicia Pickrell, Virginia Tech

Session 3: 3:15 – 5:00 pm

Plenary Talk

19. **Molecular Mechanisms of Autophagy in Substance Use Disorder: Uncovering a Novel Cocaine Receptor**, Maged Harraz, University of Maryland School of Medicine

12 minute talk + 3 minute question

20. **Low-intensity focused ultrasound to the human insular cortex differentially modulates the heartbeat-evoked potential: a proof-of-concept study,** Andrew Strohman, Virginia Tech
21. **Mechanisms of Ethanol Anxiolysis: The Role of Ninein Deletion on Ethanol and Anxiety-like Behaviors,** Emma

Session 3: 3:15 pm – 5:00 pm

12 minute talk + 3 minute question

22. **Human perception of clusters in Gaussian-distributed fields of dots,** Anthony Cate, Roanoke College
23. **Effects of aging on memory brain state dynamics,** Isabelle Moore, University of Virginia

Abstracts for speakers

1. **Understanding aggression using the fruit fly,** Katie Schretter, Janelia Farm

   Whether deciding to pursue a mate or attack a competitor, social interactions are critical for survival. Aggressive behaviors are governed by social decision-making that must continuously assess the risk of injury and potential reward to guide approach, engagement, continuation, and disengagement. However, little is known about the neuronal circuit mechanisms underlying these complex aspects of aggression. The fruit fly, Drosophila melanogaster, constitutes a powerful model for the mechanistic dissection of such cognitive processes due to its genetic accessibility, complete brain-wide connectome, and sophisticated behaviors. Recently, we uncovered a cell type underlying persistent female aggression (Schretter et al., 2020; Chiu,...,Schretter, 2023). Through mapping this complete female aggression circuit, we found these neurons exert a large part of their behavioral effects through gating visual processing. Interestingly, male courtship pursuit uses many of the same circuit motifs suggesting common mechanisms for continuing social behaviors. As persistent aggression risks injury or death, mechanisms for conversely shutting down or disengaging are equally critical for survival. Further circuit and quantitative behavioral analysis uncovered a novel neuronal subset downregulating aggression in females and males. Identification of these sex-specific and shared mechanisms reveals general principles for circuits controlling social behaviors and lays the foundation for a mechanistic understanding of social decision-making.

2. **Dynamics of the 3D genome architecture in the development of the malaria mosquito nervous system**, Igor Sharakhov, Virginia Tech

   Functional interactions between regulatory elements and gene promoters play a role in regulating gene transcription during cellular differentiation and response to external stimuli. However, the 3D aspect of gene regulation in nervous system development has not been investigated in detail, especially in insects that transmit human diseases. It has been demonstrated that the 3D chromatin structure in malaria mosquitoes shares several characteristics of chromatin organization previously reported in Drosophila. However, the Anopheles mosquito also reveals novel concepts of 3D genome organization. For example, it shows the existence of heterochromatic B-compartments that are spatially separated from euchromatic B-compartments. Additionally, highly evolutionarily conserved giant multimegabase-long chromatin loops are present (Lukyanchikova et al

2022; doi: 10.1038/s41467-022-29599-5). Here, we examined the dynamic aspects of 3D genome architecture during mosquito individual development, with a focus on the sensory systems, including the antennae. The antennae are responsible for mosquito olfaction and play 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, antennas, 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 across developmental stages of the mosquito. However, we have identified long-range chromatin interactions, particularly on the 3R arm, 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 contained the majority of giant chromatin loops, while fewer loops were found in the antennas and even fewer in the maxillary palps. Interestingly, genes located at the loop anchors have roles in cell-cell signaling, sensory perception, neuron differentiation, signal transduction, and response to stimulus. The observed developmental loop dynamics often correlate 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.

3. **Perineuronal nets regulate structure and function of tripartite synapses**, Bhanu Tewari, University of Virginia

   Perineuronal nets (PNNs) are condensed deposits of the extracellular matrix that predominantly surround fast-spiking PV neurons. The lattice-like assembly of PNNs is composed of chondroitin sulfate proteoglycans, hyaluronan, and glycoproteins. PNNs stabilize synapses and the PNN maturation in the developing brain terminates the critical period of plasticity. In the present study, we find that the axosomatic synapses in the PNN holes contain astrocytic leaflets that express all homeostatic proteins. Therefore, PNN holes contain tripartite synapses. A lack or disruption of PNNs increases the astrocytic coverage in developing as well as in adult brains, however, synapses are prone to alter only in the developing brain. PNN

disruption also retards astrocytic uptake of glutamate and potassium released on synaptic activity. Our study provides evidence of a physiochemical interaction between astrocytes and PNNs at synapses. Further, our study suggests that PNN disruption in several brain diseases may cause abnormal brain activity due to glial dysfunction.

4. **A high-resolution transcriptomic atlas of cell types in the lateral geniculate nucleus**, Katelyn Stebbins, Virginia Tech

   Axons from over 40 subtypes of retinal ganglion cells convey visual information from the outside world to distinct brain regions. However, in many retinorecipient nuclei, there is a lack of understanding of the target cells and circuitry underlying these important connections. The lack of a comprehensive catalog of transcriptomically defined cells in the visual system has hampered our ability to study visual circuits associated with these brain regions and gain a clear understanding of how they contribute to visual behaviors. In this study, we use state-of-the-art single-cell sequencing and proteomics to identify the first-ever comprehensive list of cells in the ventral lateral geniculate nucleus (vLGN), the third largest retinorecipient region in the rodent brain. Additionally, we employed the same technique to study cell types in the neighboring dLGN which has been thoroughly characterized in terms of cytoarchitecture and neuronal morphology, but for which transcriptomic identity has not been identified for these cell types. Systematic analysis of the neuronal, non-neuronal, and immature neuronal cell types across LGN reveals nearly 20 potential subtypes of vLGN neurons, including a previously undetected population of GABAergic cells in a hidden layer of the vLGNe the first-ever characterization of excitatory neurons in vLGN. The results also suggest the identity of unique features of cell type organization in dLGN; in particular, the identity of 15 potential subtypes of dLGN neurons including multiple inhibitory neuron subtypes. This comprehensive transcriptomic atlas of the mouse LGN establishes a benchmark reference atlas and a foundational resource for deep and integrative investigations of cell type and circuit function, development, and evolution of an essential nucleus of the mammalian visual system. These datasets also provide potentially generalizable principles in how sensory information is organized in the brain.

5. **Influence of the physiological state on the neural response to host and plant odors in Aedes aegypti mosquitoes,** Spruha Rami, Virginia Tech

   Each year, several hundred thousand people worldwide are affected by diseases spread by Aedes aegypti mosquitoes, such as chikungunya, dengue, and Zika. Female mosquitoes are vectors for these viruses when they bite hosts to acquire the blood meal required for developing

eggs. The host-seeking behavior of female mosquitoes is influenced by several extrinsic and intrinsic factors. Among them, the quality of the larval growing conditions will produce adult mosquitoes of a range of body sizes. A previous study in the lab showed that while larger mosquitoes readily host-seek, smaller mosquitoes prefer seeking plant scent, likely because they need a nectar meal to build energy reserves before investing in reproduction. In addition, the response to carbon dioxide (CO2) was found to be mating status-dependent, but independent of body size. However, the neural processes behind these state-dependent effects remain to be determined. As a first step toward closing this knowledge gap, we analyzed the olfactory integration of CO2, and plant and host scent by performing electrophysiological recordings of the mosquito antennal lobes (i.e., the primary olfactory centers in the mosquito brain). Specifically, we stimulated female Ae. aegypti with CO2 in the presence or absence of artificial host and plant odor blends. Our results revealed bi-directional interactions between CO2 and plant and host chemicals, which modulate each other's representation in the mosquito perceptive space. The results from this study expand our understanding of olfactory integration in mosquitoes and, more specifically, shed light on the neural mechanisms driving state-dependent effects on olfactory behavior. Results will be discussed in the context of their implication for the transmission of mosquito-borne diseases.

6. **Proprioceptive control of rapid and flexible limbs movements during Drosophila landing**, Wayne Kuo, Virginia Tech, Poster Number: 30

   Proprioception, the sense of self-movement and body position, is vital for highly coordinated movement. However, the neural mechanisms underlying proprioceptive control of behavior remain poorly understood. Here, we developed a novel behavioral assay using the fruit fly, Drosophila melanogaster, to determine how proprioceptive feedback coordinates limb movements. We constructed a high-resolution 3D kinematics analysis paradigm to track the leg joints, wings, and body of tethered, flying flies. We will use this assay to dissect how the central nervous system integrates leg proprioceptive information to coordinate a behavioral transition from flight to a stable landing. We induced landing behavior by elevating a platform to contact the flying fly's legs, mimicking the contact that happens during landing. Flies were more likely to land when the distal tibia-tarsus (TTa) joint was contacted compared to the proximal coxa-trochanter-femur (CTF) joints. Additionally, stimulation at TTa joint led to landings with lower latency compared to stimulation at CTF joint. We further examined how behavioral state modulates landing by comparing the behavior of starved and fed flies. We found that starvation increased flies' likelihood of landing in response to contact of the CTF joint.

Overall, our study reveals that flies exhibit distinct landing responses in response to contact of different leg joints, suggesting that limb mechanosensory cues can influence landing coordination, but may do so in different ways. This assay will enable novel insights into how proprioceptive feedback rapidly coordinates ethologically important behaviors.

7. **Drosophila Larvae Cool Cell 3D Calcium Imaging Analysis using TACI**, Trisha Naidu, Virginia Tech, Poster Number: 43

   Drosophila melanogaster, commonly known as the fruit fly, have cells capable of responding to environmental temperature fluctuations. Specifically under cooler conditions, three dorsal organ cool cells (DOCCs) have been found to help the fly larvae sense suboptimal temperature conditions, with two classified as type-A and one as type-B .However, imaging these cells in live larvae presents challenges due to the difficulty in controlling organismal movements. To address this issue, our lab developed a new ImageJ plugin called TrackMate Analysis of Calcium Imaging (TACI) which enhances the accuracy of neuron tracking while accommodating motion in all directions. TACI takes into consideration not only the x and y axes but also the z-axis, enabling identification of the maximum fluorescence value across all the z-positions a neuron appears in and uses it to represent the neuron's intensity at the corresponding time point. By using TACI which factors in z-planes, I have been able to efficiently analyze the DOCCs in fly larvae even when there are neurons that are overlapping in the lateral direction. This efficient method of analysis has allowed for a clear visualization of the Drosophila larvae cool cell temperature responses.

8. **Miro Regulation of Neuron-Glia Interactions**, Kathleen Reuwer, University of Virginia, Poster Number: 49

   Glia support many aspects of neuronal cell function, including supplying metabolic support for neurons to keep up with their immense energy demand. Miro, a mitochondrial rho GTPase, is a mitochondrial outer membrane protein known for connecting mitochondria to motor proteins to aid in the transport along cellular architecture. Not surprisingly, mutations in Miro result in disrupted mitochondrial motility with mitochondria becoming sequestered in the neuronal soma, as well as perturbations to mitochondrial function, and have been implicated in neurodegenerative diseases such as Parkinson’s and Alzheimer’s pathology. Using Drosophila melanogaster, we investigate how Miro dysfunction affects the interaction between glia and other cells in the central nervous system (CNS), particularly focusing on cortex glia, a glial subtype that support neuronal cell bodies and neural stem cells (neuroblasts). Interestingly,

global Miro knockout results in a unique degradation in these cortex glia in the thoracic region of the ventral nerve cord (VNC) of late stage larvae, a region that undergoes a second wave of neurogenesis at this time. Whether the loss of cortex glia and the onset of neuronal expansion in these mutants are linked remains to be explored. This unique loss of cortex glia provides a tool to investigate the role of glia in the metabolic support of neuronal cell bodies and neuroblast function. Employing cell-specific Miro RNAi knockdown and rescue constructs, we aim to uncover how cortex glia interact with surrounding cells such as neurons, neuroblasts, and other glial subtypes to adapt in the presence of mitochondrial dysfunction. Using structure-function analysis to rescue the global Miro knockout with human disease mutations lacking specific functional domains and molecular interaction sequences, we aim to delve deeply into the mechanistic role of Miro in these processes, and discover potential targets for restoring disrupted cell-cell interactions and neuronal degeneration associated with Miro loss in disease.

9. **Investigating the potential role of adaptive immunity in dopaminergic neuron vulnerability after mild traumatic brain injury**, Colin Kelly, Virginia Tech, Poster Number: 24

    Parkinson's disease (PD) is the most common motor deteriorating neurodegenerative disease, resulting in 90,000 diagnoses each year in the U.S. alone and over 10 million current diagnoses worldwide. Traumatic brain injury (TBI) is a known risk factor for PD development later in life, with chronic neuroinflammatory processes drawing growing interest. A brain injury may trigger activation of neuroinflammatory signaling pathways that are similarly activated in PD patients to increase susceptibility to neurodegenerative pathologies, though the exact mechanisms by which TBI increases this risk remain unknown and understudied. Previous studies have demonstrated that specific subtypes of T cells can mediate inflammation and neurodegeneration in preclinical models of PD in response to α-synuclein, the protein implicated in PD pathology. In addition, human PD patients display the T-cell antigen presenting molecule MHC-I in dopaminergic (DA) neurons, which are susceptible to degeneration. T cells release cytokines and interferons, like interferon gamma (IFN-y), which can maintain a pro-inflammatory feedback loop and trigger MHC-I expression, leading to DA neuron death. Our transcriptome analysis of DA neurons from the
    striatum of mice revealed upregulation of genes related to neuroinflammation, peripheral immune signaling, and IFN-y, and dysregulation of genes known to play a role in PD, 90 days post-mild TBI (mTBI). Additionally, we also see significant DA neuron cell death in the substantia nigra 90 days post-injury, with resulting alterations in

neurotransmitter concentrations in the striatum of these brains. Future directions of this study will determine how mTBI impacts neurodegenerative properties in a combinational mTBI + preclinical PD model, and if T-cell dependent signaling pathways are directly responsible for the sensitivity of DA neurons after injury.

10. **Western-Style Diet Alters Neuronal Morphology in the Hippocampus and Entorhinal Cortex of Ovariectomized Female Rats with and without Estradiol**, Sarah Blythe, Washington and Lee

    As rates of obesity and dementias are rising, research suggests an association between diet-induced obesity and cognitive impairments; however, past studies have primarily utilized male subjects. Due to estrogens' effects on behavior and neuronal structure, it is crucial to explore the effect of obesogenic diets in the presence and absence of estrogens. This is of particular interest considering that all women will enter menopause, which has been associated with an increased risk of developing Alzheimer's disease. Additionally, over 10.6 million women utilize birth control pills, which provide constant, low-doses of estrogens and eliminate natural cycling. While prior studies have utilized gonadally intact females, we compare the effect of a Western-style Diet (WSD) on memory task performance and neuronal morphology in female Sprague-Dawley rats (n = 38) with and without administration of a constant low-dose estradiol pellet. All females were ovariectomized (OVX) and half were implanted with a slow-release 17 β-estradiol pellet (OVX+E). After ten weeks of diet exposure, spatial and episodic memory were assessed using the Morris Water Maze (MWM) and Novel-Object Recognition (NOR) tasks, respectively. Brains were removed at termination and tissue was stained using the Golgi-Cox method. Neurons in the hippocampus and entorhinal cortex (EC) were imaged and digitally reconstructed. Results demonstrated higher body adiposity and TNF-α levels in WSD-fed animals, and lower cholesterol levels in females with estradiol. WSD OVX+E females showed reduced dendritic arborization in a Sholl analysis of the entorhinal cortex, raising questions about estradiol's neuroprotective actions. WSD OVX females demonstrated reduced dendritic complexity in the CA1 hippocampus. These results demonstrate the importance of further investigating the role of estrogens in obesity-related impairments.

11. **A zinc finger transcription factor tunes social behaviors while controlling transposable elements and immune response in prefrontal cortex**, Natalie Truby, Virginia Commonwealth University

    Social behaviors are central to the health of society and the individual and are disrupted in a number of psychiatric illnesses. However, the neurobiological origins of complex social behaviors are incompletely

understood. The Zfp189 gene product is a KRAB zinc finger transcription factor whose expression and function in the rodent prefrontal cortex (PFC) was previously determined to be protective against stress-induced social deficits. To interrogate the function and gene targets of ZFP189, we reprogrammed the endogenous ZFP189WT by replacing the repressive KRAB domain with an enhanced transcriptional activation domain (VP64-p65-Rta (ZFP189VPR)) or by removing the functional moiety entirely (ZFP189NFD). Upon packaging these ZFP189 variant constructs in viral vectors and delivering to mouse PFC, we interrogated the transcriptional and behavioral adaptations mediated by these synthetic ZFP189 transcription factors. We observed that dysregulation of ZFP189-mediated transcription in this brain area, achieved by delivery of synthetic ZFP189VPR, precipitates social behavioral deficits in terms of social interaction, motivation, and the cognition necessary for the maintenance of social hierarchy, without other observable behavioral deficits. By performing RNA sequencing in virally manipulated prefrontal cortex tissues, we discovered that ZFP189 transcription factors of opposing regulatory function have opposite influence on the expression of genetic transposable elements as well as genes that participate in immune functions. Collectively, this work indicates that ZFP189 function in the prefrontal cortex coordinates transcriptional neuroadaptations necessary for social behaviors by directly binding transposable element-rich regions of DNA to regulate immune-related genes. Given the evidence for a co-evolution of social behavior and the brain immune response, we posit that ZFP189 may have evolved to augment brain transposon-associated immune function as a way of enhancing an animal's capacity for functioning in social groups.

12. **Midnolin as an Alternative, Sex-Specific Protein Degradation Pathway Necessary for Fear Memory Formation**, Phill Gwin, Virginia Tech

    Approximately 6% of the US population suffers from PTSD, with its genetic and molecular mechanisms largely unknown, limiting treatment efficacy. Women are 2-3 times more likely to be diagnosed with PTSD despite experiencing fewer traumatic events. However, it remains unknown why this sex difference exists in PTSD prevalence. Our research focuses on elucidating the molecular mechanisms and sex dimorphic components involved in fear memory formation. In particular, we have recently shown that ubiquitin-proteasome-mediated protein degradation regulates fear memory formation in the amygdala of but males and females. However, surprisingly, none of our proteomic analyses have identified immediate early gene (IEG) protein products as targets of degradation-specific ubiquitin signaling following fear learning in either sex, despite the well described increases in IEGs, such as c-fos, Egr1, Arc and Npas4, in the amygdala

and other brain regions following fear conditioning. Recently, a seminal paper found that the nuclear localized protein, Midnolin, could shuttle non-ubiquitinated proteins to the proteasome for degradation in cells in vitro, though this has never been studied in the brain. Here, we found that fear conditioning increases Midnolin expression in the amygdala of males, but not females. Despite this, siRNA-mediated knockdown of Midnolin in amygdala led to increased fear behavior in male rats but a decrease in females. Current experiments are further investigating Midnolin's role as an alternative, sex-specific pathway critical for IEG clearance and fear memory formation.

13. **Chronic adolescent stress attenuates morphine-induced antinociception and central amygdala activity in adult male and female rats,** Hannah Fulenwider, Virginia Commonwealth University

    Stress exposure during critical developmental periods has been shown to increase the risk for an array of psychological and physiological pathologies in adulthood, including substance use disorder and chronic pain. Although incompletely understood, studies have suggested that stress alters opioid responses which in turn is associated with increased nociceptive and decreased antinociceptive responsivity. Studies demonstrating stress-induced attenuation of morphine antinociception have been conducted in male rodents and used stress exposure models that resulted in physical injury limiting generalization of the available findings.

    Objectives: The aim of the current study was to characterize the effects of chronic adolescent psychosocial stress on morphine-induced antinociception and neuronal activation in adult male and female rats. Male and female Wistar rats were exposed to chronic adolescent stress and tested for either antinociceptive response to morphine or morphine-induced expression of cFos in the central nucleus of the amygdala and periaqueductal grey in adulthood. Antinociceptive response to morphine was assessed with the tail-withdrawal test and morphine-induced cFos expression was assessed with immunohistochemistry. A history of chronic adolescent stress attenuated morphine-induced antinociception and neuronal activation in the central amygdala and these effects did not differ between males and females. Future studies will attempt to identify the role of the central amygdala mu opioid receptor system in these processes in efforts to identify more effective targets for pain management in individuals with stress histories.

14. **Diversity of mitochondrial morphology across hippocampal circuits**, Mayd Alsalman, Virginia Tech, Poster Number: 1

    Mitochondria produce energy that is required for neuronal function, including synaptic transmission and plasticity. Neuronal mitochondria

are morphologically diverse across cell types and subcellular compartments; however, the significance of these differences on neuronal function is unclear. We previously reported that hippocampal subregion CA2 has a unique mitochondrial transcriptional signature compared to neighboring hippocampal subregion CA1. This suggests that mitochondria may function differently in CA2 neurons compared to CA1 neurons. Despite receiving similar inputs, synapses in CA2 and CA1 dendritic layers exhibit distinct plasticity profiles. We hypothesize that mitochondrial morphology differs across CA1 and CA2 dendritic layers to match their circuit specific forms of plasticity. To address this, we sparsely labeled mitochondria in CA1 and CA2 and compared the average size and diameter of mitochondria across dendritic layers. Building on our previous results, we expect to see that CA2 SLM harbors larger and more elongated mitochondria than CA1 SLM. Larger dendritic mitochondria may produce more energy to support the enhanced plasticity at CA2 SLM synapses compared to CA1 SLM synapses. Future immunostaining for fission and fusion factors may reveal mechanisms underlying circuit-specific differences in mitochondrial morphology. Mapping the diversity of mitochondrial morphology across circuits will help us understand the role of mitochondria in supporting cell-specific functions, such as propensity for synaptic plasticity. It may also uncover the basis for differential circuit susceptibility to brain disorders.

15. **Investigating the Distinct Roles of the Anterior and Posterior Insula in Gating of Nociceptive Stimulus using Low-Intensity Focused Ultrasound**, Gabriel Isaac, Virginia Tech, Poster Number: 20

    Fibromyalgia (FM), a condition affecting over 8 million individuals in the US, is characterized by widespread musculoskeletal pain and a heightened risk for major depressive disorder. FM is widely acknowledged to be driven by hyperactivity in the insular cortex, a key pain processing center whose activity is strongly correlated with pain perception. Prior work examining FM has also demonstrated a disruption in the gating of nociceptive stimuli—a process that normally leads to reduced cortical activation in response to rapidly repeated painful stimuli. These findings suggest a generalized insular mechanism that begins to explain the development of chronic pain in FM. The insula can be parsed into the posterior insula (PI) and anterior insula (AI), which differ both structurally and functionally. The PI receives peripheral pain signals before relaying them to the AI. In the AI, these signals are integrated with expectations, awareness, and emotional responses, assigning significance to the painful stimuli. While differences between the AI and PI suggest distinct functions in pain processing, their specific roles in regulating nociceptive gating remain unexplored. To bridge this gap, we employed Low-Intensity

Focused Ultrasound (LIFU)–a novel technique for modulating neuronal activity–to selectively inhibit the AI and PI during a nociceptive gating task. Each participant received 20 pairs of brief, identical heat stimuli to the dorsum of their dominant hand both before and after undergoing either an active sham or 3-minutes of LIFU stimulation to either the anterior insula (AI) or posterior insula (PI). Differences in nociceptive gating were measured via continuous electroencephalography (EEG) recordings and subjective pain ratings before and after the intervention. Through the application of LIFU, we seek to causally interrogate the specific roles of the insular subregions in pain modulation, offering insights into potential therapeutic interventions for FM and chronic pain conditions.

16. **Enhancing Double-Blind Clinical Trials in Neuromodulation: A Novel Application of 3D-Printed Thermoplastics and High-Density Gel Matrices in Low-Intensity Focused Ultrasound**, Aditya Kapoor, Virginia Tech, Poster Number: 22

    Low-intensity focused ultrasound (LIFU) is emerging as a promising non-invasive neuromodulation technique for modulating various cortical and subcortical brain regions with high spatial precision. However, the advancement of LIFU into clinical applications is hampered by the challenge of conducting double-blind clinical trials, primarily due to the audible component which may confound neuromodulatory effects. This study introduces a novel method to mitigate non-specific auditory effects and facilitate experimenter blinding using 3D-printed thermoplastics combined with high-density gel matrices. We employed thermoplastic polyurethane (TPU) and polylactic acid (PLA) to create visually identical discs with varying acoustic properties, integrated into gel matrices, to serve as either active or sham couplants in LIFU experiments. The acoustic characteristics of these discs were empirically tested using a focused ultrasound transducer at 500 KHz within a controlled acoustic chamber. We assessed the pressure attenuation and beam profile distortion using a calibrated needle hydrophone. Subsequent variability testing on multiple prints of the selected best treatment and active sham discs validated the consistency of acoustic properties and presented minimal fluctuations in the manufacturing process. Our findings demonstrate that the SHAM disk resulted in a 94% pressure attenuation and the verum disk (still being analyzed in-gel condition) resulted in a 7% pressure attenuation (without being imbedded in the gel) despite being visually identically, thus providing an effective way to double blind studies utilizing LIFU. This advancement in creating identical verum and sham pucks now allows for effective double blinding and aids in future clinical human studies, allowing for more reliable neuromodulation studies.

17. **Application of the human 3D Neuron-Glial Brain Assembloid reveals the differential role of ApoE isoforms in the pathogenesis of Alzheimer's disease**, Eliana Sherman, University of Virginia, Poster Number: 54

    Exploration of the pathophysiology of Alzheimer's disease (AD) has been hampered by the lack of systems that accurately recapitulate the full profile of disease progression. We have developed a three-dimensional (3D) assembloid model with iPSC-induced neurons, astrocytes, and microglia derived from AD subjects to investigate the pathophysiology, protein-protein interactions, cellular mechanisms, and interventional strategies for AD. In the current study, we aim to elucidate the different ApoE isoforms in their contribution to tau pathological propagation, including the risk variant ApoE4 and the protective variant ApoE2 in comparison to ApoE3.

18. **Characterizing the Mcoln1-/- mouse as a model of early Age-related Macular Degeneration**, Jonathan Miller, James Madison University, Poster Number: 38

    Retinal degenerative diseases (RDDs) are a diverse group of retinal disorders that cause visual impairment. While RDD prevalence is high, little is known about the molecular mechanisms underlying the pathogenesis within many of these disorders. Here we use transcriptome analysis to elucidate the molecular mechanisms that drive early onset photoreceptor neuron function loss in the mouse model of the RDD Mucolipidosis type IV (MLIV). MLIV is a lysosomal storage disorder resulting from loss of function mutations in the MCOLN1 gene. MCOLN1 encodes a lysosomal cation channel, the transient receptor potential channel mucolipin 1 (Trpml1). To identify changes in gene expression during onset in MLIV we used a genetic mouse model (Mcoln1−/−) which recapitulates clinical attributes of the human disease. We conducted transcriptome analysis in 6-week old control and Mcoln1−/− mice under normal 12:12 light cycle as well as low and high light stress conditions. These data will be valuable to the vision research community for identifying differentially expressed in early onset MLIV potentially leading to new insights into the pathophysiology of this RDD.

19. **Molecular Mechanisms of Autophagy in Substance Use Disorder: Uncovering a Novel Cocaine Receptor**, Maged Harraz, University of Maryland School of Medicine

    Dopamine reuptake inhibition by cocaine, involving direct inhibition of the dopamine transporter (DAT) activity, is one of the principal mechanisms mediating the drug's behavioral actions. However, cocaine is a relatively weak inhibitor of DAT activity. We and others have shown more potent actions of cocaine, suggesting the existence of

other mechanisms for cocaine actions. We demonstrate that autophagy, or self-eating, selectively targets DAT for degradation in response to low levels of cocaine, suggesting that autophagy regulates dopamine reuptake inhibition. Further, we used a chemoproteomic approach to show that BASP1 is a high-affinity cocaine-binding protein. Knocking down BASP1 in the striatum inhibits [3H]cocaine binding to striatal synaptosomes and cocaine-induced hyperlocomotion, suggesting that BASP1 is a pharmacologically relevant receptor for cocaine. Our findings uncover a novel molecular mechanism for the behavioral actions of cocaine.

20. **Low-intensity focused ultrasound to the human insular cortex differentially modulates the heartbeat-evoked potential: a proof-of-concept study,** Andrew Strohman, Virginia Tech

    The heartbeat evoked potential (HEP) is a brain response time-locked to the heartbeat and a potential marker of interoceptive processing. The insula and dorsal anterior cingulate cortex (dACC) are brain regions that may be involved in generating the HEP. Low-intensity focused ultrasound (LIFU) is a non-invasive neuromodulation technique that can selectively target sub-regions of the insula and dACC to better understand their contributions to the HEP. These results demonstrate the ability to modulate HEP amplitudes via non-invasive targeting of key interoceptive brain regions. Our findings have implications for the causal role of these areas in bottom-up heart-brain communication that could guide future work investigating the HEP as a marker of interoceptive processing in healthy and clinical populations.

21. **Mechanisms of Ethanol Anxiolysis: The Role of Ninein Deletion on Ethanol and Anxiety-like Behaviors,** Emma Gnatowski, Virginia Commonwealth University

    Anxiety disorders serve as a predictor of developing Alcohol Use Disorder (AUD) with human subjects reporting stress and anxiety as drivers of ethanol consumption. The Miles laboratory previously identified Ninein (Nin) as a candidate gene underlying ethanol's acute anxiolytic-like properties in BXD recombinant inbred mice, using the light-dark box (LDB) transition model of anxiety. We have obtained global Ninein deletion mice and hypothesize deletion of Nin will decrease basal anxiety, increase ethanol anxiolysis, and increase ethanol consumption. Ninein may be a novel contributor to mechanisms underlying ethanol's anxiolytic properties and ethanol withdrawal-induced anxiety. Understanding the role of Ninein in these behaviors may contribute to future treatment of AUD. Ongoing experiments are investigating selective Nin deletion in the central amygdala (CeA) on anxiety-like and ethanol-related behaviors.

22. **Human perception of clusters in Gaussian-distributed fields of dots,** Anthony Cate, Roanoke College

    When objects in the same spatial neighborhood are perceived to form Gestalt groups, this has effects on a range of cognitive processes ranging from visual attention to enumeration. However, a century of perceptual grouping research has focused almost exclusively on human-designed patterns of objects that are often evenly-spaced, symmetrical, and otherwise regular. In contrast, data mining and other statistical methods work by identifying clusters of data points that conform to random probability distributions. This study investigated how human observers identified visual clusters in displays of randomly-distributed dots. Participants viewed different types of Gaussian (normally distributed) dot fields on a computer display, and indicated which dots appeared to form clusters using a mouse interface. Geometric and graph theory measures were calculated to describe the group of chosen dots and to compare it with the parameters of the dot field as a whole, to identify which features participants attended to when distinguishing cluster dots from the perceptual background. Across different types of dot fields, participants selected a region where the dot density increased markedly from the surrounding area (representing a maximal second derivative of density with respect to location), even if other regions had denser clusters of dots. These results provide a lawful principle for predicting human cluster perception in random conditions.

23. **Effects of aging on memory brain state dynamics,** Isabelle Moore, University of Virginia

    Healthy older adults typically show impaired episodic memory -- memory for when and where an event occurred -- but intact semantic memory -- knowledge for general information and facts. We hypothesize that these effects arise from an increased tendency to engage in a 'retrieval state,' a brain state in which attention is focused internally in an attempt to access prior knowledge. Engaging in a retrieval state can impair subsequent memory. We conducted multivariate pattern analyses of scalp electroencephalographic data while participants were explicitly directed to encode or retrieve object images. We find that whereas young and middle-aged adults show increased encoding state engagement over the course of the stimulus interval, older adults show inconsistent maintenance of an encoding state. These findings suggest that the temporal dynamics of memory brain states differ across the lifespan with possible implications for the ability to maintain versus flexibly shift between memory states.

Abstracts for posters

#1: **Diversity of mitochondrial morphology across hippocampal circuits,** Mayd Alsalman, Virginia Tech

Mitochondria produce energy that is required for neuronal function, including synaptic transmission and plasticity. Neuronal mitochondria are morphologically diverse across cell types and subcellular compartments; however, the significance of these differences on neuronal function is unclear. We previously reported that hippocampal subregion CA2 has a unique mitochondrial transcriptional signature compared to neighboring hippocampal subregion CA1. This suggests that mitochondria may function differently in CA2 neurons compared to CA1 neurons. Despite receiving similar inputs, synapses in CA2 and CA1 dendritic layers exhibit distinct plasticity profiles. We hypothesize that mitochondrial morphology differs across CA1 and CA2 dendritic layers to match their circuit specific forms of plasticity. To address this, we sparsely labeled mitochondria in CA1 and CA2 and compared the average size and diameter of mitochondria across dendritic layers. Building on our previous results, we expect to see that CA2 SLM harbors larger and more elongated mitochondria than CA1 SLM. Larger dendritic mitochondria may produce more energy to support the enhanced plasticity at CA2 SLM synapses compared to CA1 SLM synapses. Future immunostaining for fission and fusion factors may reveal mechanisms underlying circuit-specific differences in mitochondrial morphology. Mapping the diversity of mitochondrial morphology across circuits will help us understand the role of mitochondria in supporting cell-specific functions, such as propensity for synaptic plasticity. It may also uncover the basis for differential circuit susceptibility to brain disorders.

#2: **The Impact of Buprenorphine on Astrocyte Communication after Traumatic Brain Injury in Rats**, Rana Ansari, Virginia Commonwealth University

Traumatic brain injury (TBI) carries significant health implications across diverse populations, with varied markers and indicators reflecting its heterogeneous pathology and impact on the central nervous system's major glial cells. Astrocytes, integral components of the central nervous system, form connections through gap junctions, notably facilitated by the connexin43 (Cx43) protein. The molecular expression changes in astrocytes following diffuse traumatic brain injury, particularly under the influence of opioid analgesics like buprenorphine (Bup), remain poorly understood. This study investigated alterations in Cx43 expression in adult rats 1 day after induced mild traumatic brain injury, with or without Bup administration. Immunodetection of the Cx43 protein within three brain regions—the hippocampus, cortex, and thalamus—was conducted, followed by statistical analysis. No statistically significant differences were observed across the three regions or between the saline and Bup-treated groups. However, noteworthy trends toward a statistically significant

increase in Cx43 expression in the midbrain, particularly in the saline-treated group, were observed. This observation may be attributed to the inherent heterogeneity of astrocytic populations in distinct brain regions, leading to different phenotypic changes with regard to pathology. Furthermore, the observed decreased expression of Cx43 in the Bup-treated group suggests Bup-related changes, consistent with previous findings impacting astrocyte morphology and glial fibrillary acidic protein (GFAP) expression. This study sheds light on the intricate molecular dynamics within astrocytes following TBI and Bup administration, providing valuable insights into potential therapeutics. However, future directions include observing Cx43 expression at various time points after injury for more conclusions to be made about the role of the protein after TBI.

#3: **The Expression of Ionotropic Receptors Impacts the Sensitivity of Drosophila Larval Cool Cells under Hypertonic Conditions**, Hua Bai, Virginia Tech

Drosophila melanogaster exhibits multiple highly sophisticated temperature-sensing systems, enabling its effective response and navigation to temperature changes. Previous research has identified three dorsal organ cool cells (DOCCs) in fly larvae, consisting of two A-type and one B-type cell that displays distinct calcium dynamics. When subjected to hypertonic conditions, A-type DOCCs maintain their responses to cool temperatures, while the responses of B-type DOCCs are greatly diminished or completely eliminated. The activation of both A-type and B-type DOCCs depends on the same three members of the ionotropic receptor (IR) family: IR21a, IR93a, and IR25a. A-type DOCCs exhibit a higher somal level of IR93a than B-type DOCCs. In larvae with Ir93a overexpression, B-type DOCCs exhibit a comparable level of IR93a expression to A-type cells. Under hypertonic conditions, B-type calcium responses to cool temperatures are also increased, similarly to A-type responses. These findings suggest that IR expressions may alter receptor responses to environmental stimuli, and B-type DOCCs may serve as a pivotal integrator for temperature and tonicity.

#4: **Transgenerational Effects of Ethanol Exposure in Caenorhabditis elegans**, Ava Berckmueller, Amenah Holt, Aryan Bangalore, Virginia Tech

Alcohol Use Disorder (AUD) is one of the most prevalent diseases in today's world, however, limited research has been done to understand the transgenerational effects of alcohol on these models and whether a parent's previous exposure to alcohol influences future generations' alcohol preference. The project's objective, therefore, is to examine how alcohol affects behaviors across generations using the C. elegans model, thereby exploring the heritability of these responses and shedding light on the intergenerational effect AUDs pose on offspring of these responses. C. elegans is an ideal model for this experiment due to its fast life cycle, homology to the human genome, and its use to research other neurological disorders. We conducted chemotaxis assays and preference index tests to

observe ethanol impacts on C. elegans' locomotion, as motor and cognitive impairments are evident in humans when they are intoxicated. We plan to continue observing the F1, F2, and F3 generations by measuring differences in locomotion. If we see an effect in subsequent generations, it will support our hypothesis that these effects can be passed onto generations. If we do not see an effect in the generations, we will have confirmed that locomotion is not affected by transgenerational alcoholism. The importance of this experiment lies in its potential to showcase the use of this organism for translational research into Alcohol Use Disorders (AUDs) in humans. Such research could help bridge the gap in our understanding of the intergenerational effects of alcohol consumption.

#5: **Effects of complex I deficiency and PINK1/ Parkin mediated mitophagy on neurogenesis of the subventricular zone in Ndufs4 KO mouse model of Leigh syndrome**, Sahitya Ranjan Biswas, Virginia Tech

Leigh syndrome is one of the most common forms of heritable mitochondrial disease with pediatric presentation. Most research on Leigh syndrome is focused on understanding the pathogenesis of characteristic brain lesions, while very little is known about the effects of chronically disrupted mitochondria on neurogenesis which may contribute to the observed psychomotor regression. To address this, we have used a well-established mouse model of Leigh syndrome lacking the Ndufs4 gene (Ndufs4KO) which is essential for complex I assembly. We crossed these mice with those lacking PINK1 and Parkin, to further investigate whether these mitophagy genes influence neural stem cell (NSC) functioning during mitochondrial damage. We performed immunohistochemistry (IHC) on P24 brain sections to label Sox2 (uncommitted progenitors) and DCX (neuroblasts) cells in the subventricular zone (SVZ). Although Ndufs4KO and PINK1 NDUFS4 DKO mice had significantly decreased brain weight and SVZ volume as compared to WT, we did not observe any significant differences in Sox2+, DCX+ and double positive cell density among the groups. However, Ndufs4KO and PINK1 NDUFS4 DKO mice had significantly reduced committed neuroblasts in their NSC pool, indicating mitochondrial damage may negatively affect NSC differentiation. Interestingly, PINK1 KO mice had a significantly higher double +/Sox2+ cell ratio as compared to WT. We also performed IHC on Parkin NDUFS4 DKO mice. Although Parkin operates at the same pathway as PINK1, mice Parkin NDUFS4 DKO mice had similar SVZ volume, brain weight, and no discernable differences in cell density or double +/Sox2+ cells compared to WT at P30. In conclusion, complex I deficiency negatively impacts SVZ neurodevelopment in young adolescent rodents. Future work will establish if this reduction in neuroblasts is truly due to a differentiation defect, establish a timeline for these deficits, and understand if PINK1 functions outside of the Parkin pathway in vivo.

#6: **Developmentally regulated "don't eat me" signaling in the lateral cortex of the inferior colliculus during peak pruning**, Maddie Book, James Madison University

The lateral cortex of the inferior colliculus (LCIC), a multisensory midbrain structure, is organized into two distinct regions: modular zones which receive somatosensory inputs, and a surrounding matrix which receives auditory inputs. This interfacing of afferent streams with its compartmental structure develops during an early postnatal critical period (postnatal day 0-12). Microglial cells (MGCs) are known to serve a variety of developmental functions, including a prominent role in synaptic remodeling. Such sculpting is thought to involve a balance between MGC recognition of "eat me" tags to remove unnecessary connections, coupled with "don't eat me" tags that serve to protect contacts being actively utilized. The classic complement signaling cascade (C3-CR3) has been implicated in unimodal systems in identifying and pruning exuberant connections, while CD47-SIRP-α interactions detect active synapses that should be avoided and maintained. MGCs expressing the SIRP-α receptor recognize CD47 "don't eat me" tags selectively expressed on connections that are highly utilized, thereby protecting them from subsequent engulfment. Whether CD47-SIRP-α signaling is involved in shaping multisensory circuits during early critical periods of development remains unaddressed. The present study investigates CD47 and SIRP-α expression in relation to the developing modular-matrix framework of the LCIC. Immunocytochemical experiments in a developmental series of GAD67-GFP and CX3CR1-GFP mice show robust CD47 and SIRP-α matrix expression leading up to the LCIC critical period peak, prior to its downregulation shortly thereafter. SIRP-α and CX3CR1-positive microglia were largely non-overlapping, providing further evidence suggesting significant microglial heterogeneity in the multisensory midbrain during its early critical period of development. These findings implicate CD47-SIRP-α signaling in the selective sculpting of newly established LCIC circuits, and that deficits in such signaling may yield multisensory network maps that are subject to over-pruning.

#7: **Examination of cannabidiol oil on C. elegans behavior and cellular stress,** Claire Branscome, Virginia Tech

With the continuous rise of medical marijuana as a valid treatment option for disorders such as chronic pain or anxiety, as well as the decriminalization of the scheduled substance in multiple states across the country for recreational use, this research attempts to reveal a link between cannabidiol supplementation and increased dopaminergic functioning. In humans, disruptions of dopamine pathways are associated with neurological movement and mood disorders. Using C. elegans as a model organism, we will demonstrate the effects of cannabidiol oil on learning and inflammation. If worms are administered CBD tincture synchronous with OP50 E. Coli in Luria broth then behavior expressed by C. elegans in the behavior assay will be inhibited and the biomarker will reveal a

decrease in cellular stress. C. elegans were selected as the model organism because their nervous system has been thoroughly mapped, and known to contain a dopamine system and endocannabinoid receptors. Upon completion of experimentation, significant results will provide more insight into the novelty of using cannabinoids in a therapeutic setting. Non-significant results will guide future research on the side effects of cannabinoid usage. Overall, lack of research and FDA approval of cannabidiol oil could pose risks and have unexpected side effects to consumers.

#8: **Investigating the role of MCU in powering circuit-specific forms of structural plasticity and social behavior**, Mikel Cawley, Virginia Tech

CA2 is a subregion of the hippocampus that is critical for social memory. Deficits in social memory are found in patients with Schizophrenia (SCZ), and understanding the molecular mechanisms supporting CA2's ability to encode social memory may identify novel targets to advance therapeutic interventions. Gene network analyses from SCZ patients and mouse models of SCZ have converged on deficits in bioenergetics. We recently uncovered a subpopulation of mitochondria in CA2 distal dendrites enriched with the mitochondrial calcium uniporter (MCU) complex, facilitating mitochondrial Ca2+ uptake. Different types of neurons express different amounts of MCU, but the functional significance of this on mitochondrial bioenergetics is unknown. The overall hypothesis is that MCU-enriched mitochondria in CA2 neurons sustain high levels of bioenergetics, which is required for synaptic function and social memory formation. CA2 distal dendrites receive entorhinal cortical (EC) inputs, which relay social information. Our previous data show that MCU deletion in CA2 (MCU cKO) neurons blocks long-term potentiation (LTP) at the EC-CA2 synapses. However, the link between MCU and LTP is unclear. Loss of MCU at EC-CA2 synapses may prevent morphological changes in spines required for LTP and prevent synaptic activity required for social memory. My preliminary data show a trending decline in average spine density in MCU cKO mice compared to CTL. However, it remains unclear whether MCU deletion leads to a loss in plasticity by failing to power structural changes to the dendritic spine. I expect the loss of LTP at the EC-CA2 synapses to disrupt social memory circuit function. My preliminary data show no impairment to sociability in MCU cKO mice and demonstrate the feasibility of directly testing social recognition memory. Deficits in spine morphology or social memory may result from diminished mitochondrial bioenergetics and MCU loss. Neurons expressing more MCU on the inner mitochondrial membrane may enhance the kinetics of mitochondrial Ca2+ uptake. MCU enrichment may provide a mechanism to increase the capacity for ATP production in mitochondrial subpopulations. Using a fluorometric assay, I measured mitochondrial calcium uptake using a Ca2+-sensitive indicator to test for changes in calcium kinetics. My preliminary data show that pharmacologically blocking the MCU pore with

RU360 prevents calcium uptake in isolated mitochondria compared to CTLs. In future studies, I will test the relationship between MCU enrichment and mitochondrial bioenergetics by overexpressing MCU at increasing dosages in neurons to assess calcium kinetics, enzymatic activity, and oxidative phosphorylation. Collectively, these experiments will inform whether differences in MCU expression alone can modulate mitochondrial calcium kinetics and whether MCU enrichment at the EC-CA2 synapses is required to maintain spine morphology and social memory in the CA2.

#9: **Role of extracellular matrix in Alzheimer Disease-associated social memory impairment**, Lata Chaunsali, University of Virginia

Alzheimer disease (AD) is characterized by a progressive loss of memory and cognition. AD pathology includes the deposition of beta-amyloid plaques and hyper-phosphorylated tau tangles. However, accumulating evidence suggests that alterations in the brain extracellular matrix (ECM) are also a significant core pathological feature of AD. In several parts of the brain including the cerebral cortex and hippocampus, ECM condenses to form highly organized structures, known as perineuronal nets (PNNs). PNNs surround not only the majority of parvalbumin-expressing GABAergic inhibitory neurons but also the excitatory pyramidal neurons in the hippocampal CA2 area. PNNs are known to stabilize synapses and regulate neuronal plasticity, thereby implicated in memory and cognition. Recent studies have shown alterations in the PNNs in various neurological disorders including AD. Since PNNs are involved in learning and memory functions, we hypothesized that memory deficits and cognitive decline associated with AD may be due to the altered ECM and PNNs. We immunostained PNNs in the 5XFAD model of AD, and observed significant alterations in PNNs in the CA2 region of the hippocampus at a surprisingly early age. To elucidate the mechanism of PNN disruption, we assessed the ECM remodeling machinery and observed upregulated gene expression of several matrix remodeling enzymes as matrix metalloproteinase (MMP) in the AD mouse model. We further examined the link between PNN disruption and memory impairments and observed impairment of social memory in the AD model in which PNNs were disrupted. Finally, by blocking MMP activity using a broad spectrum MMP inhibitor (GM6001 or Illomastat), we were able to prevent PNN disruption and consequently retains the social memory in 5XFAD mice. This finding positively correlates behavioral deficits to PNN depletion, suggesting that disrupted PNNs in the hippocampus CA2 area are associated with social memory dysfunction in the AD mouse model. Preventing PNN disruption by inhibition of ECM remodeling agents may have a positive outcome in AD-associated memory deficits.

#10: **Wandering with the wanderer: vagal modulation of glucose tolerance and cardiac function**, Nicholas Conley, University of Virginia

The dorsal motor nucleus of the vagus (DMV) represents a pivotal brain region implicated in parasympathetic autonomic control. In this study, we

investigate molecularly-defined subtypes within the DMV and their potential functional roles. Our findings reveal that activation of the entire DMV or a specific DMV subtype marked by Calb2 significantly improves glucose tolerance during a glucose challenge. Additionally, our investigation demonstrates that DMV stimulation induces significant bradycardia while concurrently reducing anxiety-like phenotypes, as measured in the elevated plus and open field mazes. These findings underscore the multifaceted role of the DMV in modulating physiological and psychological responses, offering novel insights into anxiety-related conditions and potential therapeutic interventions targeting metabolic disorders.

#11: **Investigating the electrophysiological features of post traumatic epilepsy in rat hippocampus**, Michael Dexheimer, Virginia Commonwealth University

Epilepsy occurs as a result of moderate to severe traumatic brain injury (TBI) in 10-20% of cases. Some risk factors fo post traumatic epilepsy (PTE) are known, but the mechanisms by which PTE arises are not. Loss of mossy cells and interneurons within the dentate gyrus (DG) has been implicated in the development of hippocampal (HC) seizures, including after TBI, potentially via increased hyperexcitability within the microcircuitry of the HC as a consequence of lost inhibitory control over entorhinal afferent inputs. Here, we use a fluid percussion injury model (FPI) of PTE in rats and laminar high channel count silicon probes to interrogate the microcircuitry of the HC. We aim to characterize changes in the post-synaptic response to entorhinal input within the DG as well as its downstream influence on the internal HC microcircuitry. We quantify and describe the morphology of distinct electrophysiological events in the DG after TBI compared with sham injury. However, challenges of electrophysiological interpretation arise when the normal architecture of the HC is altered due to pathological changes such as TBI. In order to address these challenges, we generate current source density (CSD) representations of local field potentials in conjunction with independent component analysis (ICA). This method allows us to define and classify distinct patterns of post-synaptic activity in the DG and CA1 sub-region that, in conjunction with anatomical localization of our probe, reveal the distinct combinations of entorhinal inputs contributing to these post-synaptic patterns. This may allow us to reveal pathway-specific alterations within the temporal lobe after TBI that could lead to PTE. Our preliminary results show examples of post-injury morphological changes in post-synaptic electrographic events (“dentate spikes”) in the DG that may reflect pathological alterations in the patterns of entorhinal inputs to the HC. This technique demonstrates a use of ICA to improve interpretability of CSD representations when altered parameters are unknown.

#12: **Auditory and Somatosensory Multimodal Pre-Pulse Inhibition in Control and Complement Receptor 3 Knockout Mice**, Alyssa Diana, James Madison University

During early developmental critical periods, microglial cells (MGCs) play a key role in synaptic pruning events. MGCs utilize classical complement cascade signaling to identify and target nonessential connections for subsequent degradation. Compromised complement signaling results in under-pruning and lack of refinement of newly established sensory maps that may contribute to autism-related behavioral phenotypes. To date, little is known about microglial influences on developing multisensory networks, although multisensory processing deficits are among the most reliable indicators of neurodevelopmental disorders. The lateral cortex of the inferior colliculus (LCIC) is a midbrain structure that receives and integrates inputs of multisensory origin. Previous work from our lab suggests complement signaling involvement in the shaping of LCIC circuits during an early critical period. The LCIC is a pivotal structure for pre-pulse inhibition (PPI), a phenomenon in which a pre-pulse stimulus results in the reduction of a startle to a subsequent stronger stimulus. PPI paradigms have classically used unimodal stimuli as pre-pulse cues. The aim of the current study was to explore the effect multisensory (auditory and somatosensory) pre-pulse cues have on PPI in control (C57BL/6J, n = 50) and complement receptor 3 knockout (CR3KO, n =20) mice. Both groups were tested at four and seven weeks of age (+/- 1 day). Pre-pulses for both modalities were 100 ms in duration and occurred 200 ms before the startle-eliciting stimulus (SES, 15 ms). Auditory pre-pulses were band-limited white noise, and vibratory pre-pulses were 380 Hz. Multimodal pre-pulses consisted of both auditory and vibratory stimuli delivered simultaneously before the SES. Higher intensity auditory pre-pulses (70 dB and 80 dB) were used to identify ceiling effects. Pre-pulse inhibition was quantified conventionally as inhibition of the startle response expressed as a percent. Results indicate auditory and somatosensory pre-pulses inhibit the startle response by about 20-40%. Each pre-pulse had inhibition of the SES greater than baseline, but less than the ceiling response trials. Preliminary analyses suggest potential differences between wild-type mice and those with compromised complement signaling. Our findings suggest multisensory PPI may serve as a novel tool for assessing the functionality of newly established multisensory circuits, as well as the potential consequences of altered early network refinement. During early developmental critical periods, microglial cells (MGCs) play a key role in synaptic pruning events. MGCs utilize classical complement cascade signaling to identify and target nonessential connections for subsequent degradation. Compromised complement signaling results in under-pruning and lack of refinement of newly established sensory maps that may contribute to autism-related behavioral phenotypes. To date, little is known about microglial influences on developing multisensory networks, although multisensory processing deficits are among the most reliable indicators of neurodevelopmental disorders. The lateral cortex of the inferior colliculus (LCIC) is a midbrain structure that receives and

integrates inputs of multisensory origin. Previous work from our lab suggests complement signaling involvement in the shaping of LCIC circuits during an early critical period. The LCIC is a pivotal structure for pre-pulse inhibition (PPI), a phenomenon in which a pre-pulse stimulus results in the reduction of a startle to a subsequent stronger stimulus. PPI paradigms have classically used unimodal stimuli as pre-pulse cues. The aim of the current study was to explore the effect multisensory (auditory and somatosensory) pre-pulse cues have on PPI in control (C57BL/6J, n = 50) and complement receptor 3 knockout (CR3KO, n =20) mice. Both groups were tested at four and seven weeks of age (+/- 1 day). Pre-pulses for both modalities were 100 ms in duration and occurred 200 ms before the startle-eliciting stimulus (SES, 15 ms). Auditory pre-pulses were band-limited white noise, and vibratory pre-pulses were 380 Hz. Multimodal pre-pulses consisted of both auditory and vibratory stimuli delivered simultaneously before the SES. Higher intensity auditory pre-pulses (70 dB and 80 dB) were used to identify ceiling effects. Pre-pulse inhibition was quantified conventionally as inhibition of the startle response expressed as a percent. Results indicate auditory and somatosensory pre-pulses inhibit the startle response by about 20-40%. Each pre-pulse had inhibition of the SES greater than baseline, but less than the ceiling response trials. Preliminary analyses suggest potential differences between wild-type mice and those with compromised complement signaling. Our findings suggest multisensory PPI may serve as a novel tool for assessing the functionality of newly established multisensory circuits, as well as the potential consequences of altered early network refinement.

#13: **Starvation resistance and increased food consumption in serotonin transporter (dSert) mutants in Drosophila melanogaster**, Abigail Forrest, Eastern Mennonite University

The monoamine serotonin is known to impact human behaviors such as sleep cycles, eating, anxiety, and depression. The reabsorption of serotonin back into the presynaptic neuron is mediated by the serotonin transporter (SERT), the target of citalopram, a selective serotonin reuptake inhibitor (SSRI) used to treat anxiety and depression. We turned to the model organism Drosophila melanogaster to better understand the mechanisms by which serotonin and SSRIs influence behavior. We created a 3.9 kb deletion within the Drosophila SERT (dSert61) gene and tested its effect on larval and adult behaviors. dSert61 larvae consumed 64.4% ($p=0.0044$) more food than controls, a result also noticed in the dSert16 deletion allele (74.7%, $p=0.0004$) and the dSert61/dSert61 trans-heterozygote (87.9%, $p=0.0003$). Adult dSert flies show a dramatic resistance to starvation, a trait possibly linked to increased food consumption. When 10-day-old female adults were starved on 1% agar, dSert61 flies survived 51.1% longer ($p<0.0001$) and dSert16 flies survived 8.9% longer ($p=0.001$). dSert61/dSert16 hybrids lived 66% ($p<0.0001$) longer when compared to Canton-S controls. The effects of citalopram on

starvation resistance and adult feeding are ongoing. The results in feeding and starvation give us the opportunity to study the role of serotonin in these behaviors.

#14: **The Effects of an Acidic Pollutant on the Nervous System of C. elegans**, Ariana Garrastegui Segarra, Virginia Tech

Climate change has resulted in changes to the incidence and intensity of heat waves. These have been linked to neurological and neurodegenerative pathologies, including Alzheimer's, Parkinson's, and Motor Neuron Diseases. While there does seem to be a pattern between global warming and the prevalence of neurodegenerative diseases in literature reports, the direct link between them is yet to be established (Bongioanni et al., 2021). We hypothesize that long-term exposure to varied levels of acidic pH in water reduces associative learning in C. elegans. We test this by exposing C. elegans to three pH levels (7, 4.3, and 3.5) for 7 consecutive days to mimic prolonged exposure to acidic rain. We use an associative learning paradigm to test the learning ability and feeding behavior of the test group against the control. We anticipate as the acidity increases, the associative learning in C. elegans will decrease. The results of the experiment would provide meaningful applications in understanding environmental impacts on the nervous system and encourage further inquisition toward strategies to combat resultant effects.

#15: **Altered Hippocampal Network Dynamics in a Lateral Fluid Percussion Injury model of Post-traumatic Epilepsy**, Adam Gibson, Virginia Commonwealth University

Epilepsy is one of the most common neurological diseases globally, it is increasing in rate, and has one of the highest disease burdens of all neurological disorders in the United States. Post-traumatic epilepsy (PTE) is a significant long-term complication of moderate-to-severe traumatic brain injuries (TBI), comprising as much as 20% of all structural epilepsies. However, there are currently no effective early biomarkers or therapies to prevent epilepsy after TBI in high risk individuals, in part because the changes in the post-TBI brain which lead to PTE are unknown. Pathological findings within the temporal lobe are common following TBI, raising the question of whether temporal lobe networks are particularly susceptible to injury and the development of PTE. There is a well-characterized loss of neuronal mossy cells within the hilar dentate gyrus (DG) of the hippocampus (HC) following both TBI in humans and in the lateral fluid percussion injury (FPI) rodent model of PTE. The dentate gyrus is thought to exert a gating function on signals carried by perforant path (PP) afferents from the entorhinal cortex which are relayed by the DG to CA3. The precise coordination of CA3 and entorhinal temporoammonic pathway (TA) inputs onto CA1 drives oscillatory and spiking activity in CA1. The loss of DG hilar mossy cells following injury may lead to the propagation of hyperexcitability through CA3 and CA1, and ultimately back to the entorhinal cortex as well as to a wide cortical distribution, over time causing further injury and

potentially precipitating the spontaneous late seizures which are the hallmark of PTE. Our preliminary data from acute recordings under anesthesia demonstrate the feasibility of using naturally occurring dentate spike events as a measure of postsynaptic DG response to entorhinal perforant path input in FPI-induced and sham-injured rats. We found examples of altered hippocampal network dynamics 4 weeks after FPI, including increased dentate spiking activity and exaggerated synchronization between dentate spikes and multi-unit firing patterns in CA1. We have also found that chronically implanted awake, behaving animals exhibit dentate spikes following FPI. We plan to monitor the independent components of these networks over time during post-traumatic epileptogenesis.

#16: **Dorsal Motor Nucleus of the Vagus Can Induce Bradycardia and Reduce Anxiety-like Behaviors**, Patricia Castro-Martinez, University of Virginia

Parasympathetic efferent neurons from the Dorsal Motor Nucleus of the Vagus (DMV) innervate the heart as well as the abdominal and thoracic organs through the vagus nerve. While both the nucleus ambiguous (nAMB) and the DMV have cardiovagal neurons innervating the heart, whether the DMV can exert the same level of control as the nAMB has remained a subject of controversy. Previous research has made the study of this challenging due to off-target effects of whole vagal stimulation and the previous inability to specifically target DMV neurons.In order to overcome this obstacle and examine the functional roles of the DMV in cardiac modulation, we used AAV1-CreOn/FlpOn-hm3Dq. Utilizing genetic targets intrinsic to the DMV, specifically Chat and Phox2b, we expressed the recombinases Cre and Flp respectively. Using a Cre-ON Flp-ON viral construct and limiting our injection to the DMV, we effectively targeted the population of interest.

Upon injecting the hm3dq receptor ligand Clozapine-n-oxide (CNO) we activated DMV neurons and observed robust bradycardia. We then examined the behavioral effects of this inducible bradycardia through the open field, plus maze and nestlet shredding paradigms. We noted a significant increase of explorative behavior and decrease of anxiety-like behaviors marked by increased open time in the open field and increased time in open arms in the elevated plus maze. No increase in nestlet shredding was observed and anecdotally, the mice were also much easier to handle.Notably, when co-administering Methylatropine (MA), a blocker of peripheral muscarinic signaling, the bradycardia was abolished, and subsequent anxiolytic-like effects previously seen in the CNO treated subjects were largely prevented. This indicates that heart rate, and potentially anxiolytic effects of the DMV require peripheral muscarinic signaling.

These results seem to support the James-Lange theory of emotions, stating that changes in physiological biomarkers such as heart rate precede our emotional states. Ultimately, the experimentation supports that the DMV

contains parasympathetic efferent cardiovagal neurons that when excited, produce a significant drop in heart rate, and consequently show signs of anxiolysis.

#17: **Biochemical Characterization of the Retina-specific Transcription Factor CRX,** Aparna Gupta, Ali Carl, James Madison University

The retina is a layered neuronal tissue lining the back of the eye that converts photons of light into what we perceive as vision. This process requires the coordination of distinct classes of retinal neurons, such as photoreceptors (PRs). PRs initiate signaling by absorbing photons of light and converting their energy into a biochemical signal. The Cone-rod homeobox (CRX) gene encodes a PR-specific transcription factor protein. Previous experiments in our lab demonstrate the importance of the CRX protein and its DNA binding domain (DBD) for cone and rod PR function and development. However, there is little research on the protein's transactivation domain (AD) portion. The AD of CRX has been previously shown to be 100% disorganized, with little homology for its known protein structures. It has been predicted that the AD of CRX is used for protein-to-protein interaction and is intrinsically disordered. Therefore, our research goal is to purify CRX and investigate the optimal conditions that allow specific interactions with the CRX AD. Using a bacterial system, we expressed a his-tagged full-length human CRX protein. We then used fast protein liquid chromatography (FPLC) for nickel or cobalt affinity protein purification. We have developed a potential method to purify CRX out of the insoluble pellet and are working to characterize the structure of the protein from this fraction. We further simulated the dynamics of the CRX DBD with and without the AD to understand if this region has any effect on the conformation of the DBD overall. Our ongoing study will provide further research on the role of CRX and how other proteins alter or bind to its structure, possibly contributing to the diseased retina.

#18: **Acetaminophen and ibuprofen do not have a direct effect on nerve activities in the central nervous system in crickets**, Henry Holley, Virginia Tech

Acetaminophen and ibuprofen are both over-the-counter painkillers. However, they function through different pathways. Ibuprofen, a nonsteroidal anti-inflammatory drug (NSAID), targets the cyclooxygenase (COX)-1 and COX-2 enzymes and hinders prostaglandin synthesis, providing anti-inflammatory effects in peripheral tissues. Unlike ibuprofen, acetaminophen is not an NSAID. Although it possesses analgesic and antipyretic properties similar to NSAIDs, it lacks peripheral anti-inflammatory effects. Studies suggest that acetaminophen may inhibit the COX pathway in the central nervous system. Recent studies also report that systemic administration of ibuprofen exerts a therapeutic effect on post-traumatic stress disorder (PTSD), but there are also studies suggesting that NSAIDs have no significant effect on pain with a neuropathic component. Thus, it is worth investigating if acetaminophen and/or ibuprofen might have direct effects on nerve activities.

In this study, using PowerLab (ADInstruments, Inc) we measured the spontaneous nerve activity in the central nervous system of crickets before and after Intraperitoneal administration of acetaminophen (0.75mg/kg, 7.5mg/kg, and 75mg/kg) and ibuprofen (0.3mg/kg, 3mg/kg, and 30mg/kg). We also measured the nerve activity response to cercal stimuli before and after administration of acetaminophen or ibuprofen.

After comparing spontaneous nerve activity before and after the administration of three doses of each drug. We did not find any significant difference in spontaneous nerve activity (n=12). Interestingly, the nerve activity response to cercal stimuli did not significantly differ before and after drug administration either. Our study suggests that although it is documented that acetaminophen and ibuprofen may exert effects on the central nervous system, it is unlikely to have a direct affect one nerve activity.

#19: **Responses of different Drosophila species to temperature changes**, Ainul Huda, Virginia Tech

Temperature is a critical environmental variable affecting most animals' distribution, survival, and reproduction. Although temperature receptors have been identified in many animals, how these receptors respond to temperature is still unclear. Using an automated tracking method, we applied the adult thermotactic two-choice assay to examine nine Drosophila species' movement and temperature preferences. The ability or inclination to move varied among these species and at different temperatures. Distinct species preferred various ranges of temperatures. Wild-type D. melanogaster flies avoided the warmer temperature in the warm avoidance assay and the cooler temperature in the cool avoidance assay. D. biarmipes and D. mojavensis did not avoid warm temperature, like the mutant disrupting the warm receptor in arisal heating cells that guide animals to avoid warm temperature in the two-choice assay. D. bipectinata and D. yakuba did not avoid warm or cool temperatures in the respective assays. This phenomenon replicates mutants disrupting cool receptors expressing in aristal cooing cells. These results will benefit further research exploring molecular mechanisms of temperature responsiveness of the specific warm and cool receptors.

#20: **Investigating the Distinct Roles of the Anterior and Posterior Insula in Gating of Nociceptive Stimulus using Low-Intensity Focused Ultrasound,** Gabriel Isaac, Virginia Tech

Fibromyalgia (FM), a condition affecting over 8 million individuals in the US, is characterized by widespread musculoskeletal pain and a heightened risk for major depressive disorder. FM is widely acknowledged to be driven by hyperactivity in the insular cortex, a key pain processing center whose activity is strongly correlated with pain perception. Prior work examining FM has also demonstrated a disruption in the gating of nociceptive stimuli—a process that normally leads to reduced cortical activation in response to rapidly repeated painful stimuli. These findings suggest a generalized

insular mechanism that begins to explain the development of chronic pain in FM. The insula can be parsed into the posterior insula (PI) and anterior insula (AI), which differ both structurally and functionally. The PI receives peripheral pain signals before relaying them to the AI. In the AI, these signals are integrated with expectations, awareness, and emotional responses, assigning significance to the painful stimuli. While differences between the AI and PI suggest distinct functions in pain processing, their specific roles in regulating nociceptive gating remain unexplored. To bridge this gap, we employed Low-Intensity Focused Ultrasound (LIFU)–a novel technique for modulating neuronal activity–to selectively inhibit the AI and PI during a nociceptive gating task. Each participant received 20 pairs of brief, identical heat stimuli to the dorsum of their dominant hand both before and after undergoing either an active sham or 3-minutes of LIFU stimulation to either the anterior insula (AI) or posterior insula (PI). Differences in nociceptive gating were measured via continuous electroencephalography (EEG) recordings and subjective pain ratings before and after the intervention. Through the application of LIFU, we seek to causally interrogate the specific roles of the insular subregions in pain modulation, offering insights into potential therapeutic interventions for FM and chronic pain conditions.

#21: **Inducible knockout of autism gene Itgb3 and MORF3 labeling of excitatory pyramidal neurons during early postnatal development of the cerebral cortex**, Anna Kalinowski, James Madison University

Autism spectrum disorder (ASD) is characterized by repetitive behaviors, deficits in communication, and overall impaired social interaction. Mutations in integrin β3 (Itgb3) are associated with ASD. Previous work causing Itgb3 loss-of-function during specific early timeframes suggests that Itgb3 might function during early postnatal development in the cerebral cortex. However, to date, no genetic tool has been devised that can conditionally remove Itgb3 from cortical pyramidal neurons in a temporally precise way during this early period of postnatal development. We utilized the Nex1-CreERT2 driver line crossed to the floxed Itgb3 mouse line to allow for temporally precise conditional removal of Itgb3. Informed by prior studies utilizing this line, we hypothesized that a single injection of tamoxifen causes a significant induction of Nex1-CreERT2 (as measured by the tdTom fluorescent reporter), and that it causes a reduction in Itgb3 mRNA. To determine the induction efficiency, we used in situ hybridization to detect Itgb3 mRNA transcripts, and followed this with immunohistochemistry to detect the tdTom fluorescent reporter. Results show that the induction is effective at postnatal day 1 and 7, and that it occurs between 6 and 72 hours. To our knowledge, these results are the first to show induction of Nex1-CreERT2 (including the full deletion of mRNA expression) during the first postnatal week and in only 3 days. Additionally, we piloted the MORF3 reporter to allow for single-cell morphological reconstruction and analysis. Combined with the Nex1-CreERT2 mouse line,

MORF3 appears to be a genetically-driven, high signal-to-noise, and low-density reporter of excitatory pyramidal neurons. In conclusion, we demonstrate inducible knockout of Itgb3, the precise timing needed for induction of the Nex1-CreERT2 mouse line, and single-cell MORF3 labeling during early postnatal development of the cerebral cortex.

#22: **Enhancing Double-Blind Clinical Trials in Neuromodulation: A Novel Application of 3D-Printed Thermoplastics and High-Density Gel Matrices in Low-Intensity Focused Ultrasound**, Aditya Kapoor, Virginia Tech

Low-intensity focused ultrasound (LIFU) is emerging as a promising non-invasive neuromodulation technique for modulating various cortical and subcortical brain regions with high spatial precision. However, the advancement of LIFU into clinical applications is hampered by the challenge of conducting double-blind clinical trials, primarily due to the audible component which may confound neuromodulatory effects. This study introduces a novel method to mitigate non-specific auditory effects and facilitate experimenter blinding using 3D-printed thermoplastics combined with high-density gel matrices. We employed thermoplastic polyurethane (TPU) and polylactic acid (PLA) to create visually identical discs with varying acoustic properties, integrated into gel matrices, to serve as either active or sham couplants in LIFU experiments. The acoustic characteristics of these discs were empirically tested using a focused ultrasound transducer at 500 KHz within a controlled acoustic chamber. We assessed the pressure attenuation and beam profile distortion using a calibrated needle hydrophone. Subsequent variability testing on multiple prints of the selected best treatment and active sham discs validated the consistency of acoustic properties and presented minimal fluctuations in the manufacturing process. Our findings demonstrate that the SHAM disk resulted in a 94% pressure attenuation and the verum disk (still being analyzed in-gel condition) resulted in a 7% pressure attenuation (without being imbedded in the gel) despite being visually identically, thus providing an effective way to double blind studies utilizing LIFU. This advancement in creating identical verum and sham pucks now allows for effective double blinding and aids in future clinical human studies, allowing for more reliable neuromodulation studies.

#23: **The Effect of unc-53 on C. elegans Apoptosis,** Evan Kauffman, Eastern Mennonite University

Caenorhabditis elegans, or C. elegans, is a nematode model organism useful for biological research for many reasons. A fast replication cycle, invariant cell lineage, and transparent body make genetic crosses and fluorescence imaging easy. This project aims to investigate the role of the C. elegans gene unc-53 in the programmed cell death of neurons along the ventral nerve cord (VNC) of the nematode. Apoptosis is interesting because it is a last resort for cells dealing with immunological stress or cancer (Arvantis et al., 2013). Because of the invariant number of neurons in the nematode, ventral nerve cord and mechanosensory neurons were counted

to assess the effect of ced-3 knockouts and ced-3/ced-4 overexpression on apoptosis. After crossing these mutants with unc-53 knockout mutants, the impact of unc-53 on apoptosis could be evaluated in overexpressed and underexpressed models.

The C. elegans being studied were genetically modified to express green fluorescent protein (GFP) in either the VNC neurons or the mechanosensory neurons. The animals were observed under UV light using a fluorescent microscope. For the VNC neurons, wild-type animals were compared to animals with nonfunctional ced-3 and double-mutants with nonfunctional ced-3 and nonfunctional ced-53. The number of neurons for each worm was counted visually, and the number of neurons for each strain was averaged. Based on preliminary results, ced-53 does not have a significant effect on apoptosis compared to the ced-3 knockout strain.

Arvanitis, M., Li, D., Lee, as detailed in Arvanitis et al. (2013), holds significant implications for the fields of cancer and immunity. The lessons we learn from this model organism can potentially shed light on these complex areas of study, underscoring the relevance and importance of our work.

#24: **Investigating the potential role of adaptive immunity in dopaminergic neuron vulnerability after mild traumatic brain injury,** Colin Kelly, Virginia Tech

Parkinson's disease (PD) is the most common motor deteriorating neurodegenerative disease, resulting in 90,000 diagnoses each year in the U.S. alone and over 10 million current diagnoses worldwide. Traumatic brain injury (TBI) is a known risk factor for PD development later in life, with chronic neuroinflammatory processes drawing growing interest. A brain injury may trigger activation of neuroinflammatory signaling pathways that are similarly activated in PD patients to increase susceptibility to neurodegenerative pathologies, though the exact mechanisms by which TBI increases this risk remain unknown and understudied. Previous studies have demonstrated that specific subtypes of T cells can mediate inflammation and neurodegeneration in preclinical models of PD in response to α-synuclein, the protein implicated in PD pathology. In addition, human PD patients display the T-cell antigen presenting molecule MHC-I in dopaminergic (DA) neurons, which are susceptible to degeneration. T cells release cytokines and interferons, like interferon gamma (IFN-y), which can maintain a pro-inflammatory feedback loop and trigger MHC-I expression, leading to DA neuron death. Our transcriptome analysis of DA neurons from the striatum of mice revealed upregulation of genes related to neuroinflammation, peripheral immune signaling, and IFN-y, and dysregulation of genes known to play a role in PD, 90 days post-mild TBI (mTBI). Additionally, we also see significant DA neuron cell death in the substantia nigra 90 days post-injury, with resulting alterations in neurotransmitter concentrations in the striatum of these brains. Future directions of this study will determine how mTBI impacts neurodegenerative properties in a combinational mTBI + preclinical PD

model, and if T-cell dependent signaling pathways are directly responsible for the sensitivity of DA neurons after injury.

#25: **Input-specific organization of NMDA receptors in the thalamus,** Rabeya Khondaker, Virginia Tech

Functional diversity among synapses contributes to circuit-specific physiology and pathophysiology in the nervous system. Synapse diversity is generated, in part, by differential expression of neurotransmitter receptors and their associated protein complexes. GluN2 subunits of the N-methyl-D-aspartate receptor (NMDAR) generate functionally diverse glutamatergic synapses across neuron populations through cell-type-specific expression patterns. GluN2 subunits also exhibit input-specific function in some neuron populations, but the subcellular and subsynaptic organization of NMDARs in native brain tissue remain poorly understood. We investigated GluN2 synaptic localization in thalamocortical (TC) neurons expressing all four GluN2 subunits: GluN2A, GluN2B, GluN2C, and GluN2D. Utilizing super resolution imaging and knockout-validated antibodies, we revealed subtype- and input-specific GluN2 localization at corticothalamic (CT) and sensory inputs to TC neurons in C57Bl/6J mice aged P28-P32. GluN2B was localized to the greatest proportion of glutamatergic synapses, followed by GluN2A and GluN2C, and GluN2D was localized to the smallest proportion. GluN2B subunits were preferentially localized to VGLUT1-positive CT inputs over VGLUT2-positive sensory inputs. On the contrary, GluN2A and GluN2C were preferentially localized to sensory inputs over CT inputs. GluN2D was weakly associated with both CT and sensory inputs. These data suggest that input-specific NMDAR subunit organization likely contributes to the established functional distinctions between CT and sensory synapses in the thalamus. Moreover, these data establish TC neurons as a model to study regulatory mechanisms that control NMDAR localization in a subtype- and input-specific manner.

#26: **The Role of Brain Stem 5HT1A Receptors in the Protective Hypothermic Response to Hypoxic Stress,** Catherine Kirkhorn, James Madison University

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) (Paterson et al. 2006). The nucleus raphe pallidus (NRP), an area rich in serotonin (5HT1A) receptors, mediates cardiovascular responses to stress (Patterson et al 2006, Brown et al 2011) and may also coordinate protective thermoregulatory responses to hypoxic stress. It is hypothesized that activating inhibitory 5HT1A receptors at the NRP in rats with 8-OH-DPAT will exacerbate the suppression of sympathetic premotor neurons, leading to a greater regulated hypothermic response to hypoxic stress when compared to a control. Conversely, injections of the 5HT1a receptor antagonist (WAY-100635) should attenuate this response. A surgically implanted cannula targeting the NRP allows microinjection of drugs while an abdominal

thermoprobe allows for constant recording of core temperature (Tc). After recovery from surgery, rats are housed in a thermocline which quantifies selected ambient temperature (STa). Exposure to 60 minutes of hypoxic (6% O2) stress causes protective reductions in both Tc and STa with ACSF injection while 8-OH-DPAT injections exacerbates these responses. WAY injection did not change the thermoregulatory responses. These data suggest that the 5HT1a receptor is involved in mediating the protective hypothermic response. It was expected that WAY would block the hypothermic response, but it was muted, perhaps due to the short half-life of WAY or a concentration of WAY too low to antagonize this receptor. These results suggest that altered 5HT1a receptors at the NRP may contribute to SIDS. Determining the neural paths that coordinate the protective hypothermic response is essential to the etiology of SIDS and may help prevent its occurrence.

#27: **Vasoactive intestinal peptide-expressing interneurons are impaired in SCN8A Epileptic Encephalopathy,** Shrinidhi Kittur, University of Virginia

SCN8A Epileptic Encephalopathy (SCN8A EE) is a severe epilepsy syndrome caused by gain-of-function mutations in the SCN8A gene which encodes the voltage gated sodium channel Nav1.6. Nav1.6 is expressed in both excitatory and inhibitory neurons, and the balance of excitation and inhibition is critical in understanding seizure networks. There are three main subtypes of inhibitory neurons: parvalbumin (PV), somatostatin (SST), and vasoactive intestinal peptide (VIP) interneurons. Previous work from our lab shows that PV and SST inhibitory interneurons are impacted by Scn8a mutations and contribute to the seizure phenotype of SCN8A EE. RNASeq data shows that Scn8a is also expressed in VIP interneurons, which inhibit other inhibitory interneurons and have a disinhibitory influence on the cortical network, yet they have not been studied in the context of SCN8A EE. Disinhibition leads to decreased inhibitory drive in cortical networks, and aberrant disinhibition may lead to excessive cortical excitation consistent with the seizure phenotype seen in SCN8A EE. Here, we use whole-cell patch clamp electrophysiology to examine the effects of mutant Nav1.6 on VIP interneurons. We show that VIP interneurons from Scn8a mutant mice are intrinsically hyperexcitable and exhibit significantly greater spontaneous firing frequencies than their wild-type counterparts. Additionally, we observe an increased persistent sodium current in Scn8a mutant VIP interneurons, a hallmark of gain-of-function SCN8A mutations that may underlie hyperexcitability. These novel results indicate an increase in disinhibition in the cortical network in SCN8A EE and highlight a potential role for VIP-INs in the network dysfunction of this disorder.

#28: **Hippocampal perineuronal net plasticity is associated with astrocyte dysregulation,** Piotr Kraszewski, University of Virginia

The CA2 region of the hippocampus, an area characterized as a “hub” for social memory circuitry, has a high abundance of perineuronal nets (PNNs) – specialized extracellular matrix structures that regulate brain plasticity.

BTBR mice, a model of social memory dysfunction, display increased CA2 PNNs that coincide with a shift in astrocyte populations, where GFAP+ astrocytes were selectively diminished with no change in total astrocyte number. Reducing PNNs to control-like levels in BTBR mice improves social memory and rescues GFAP expression in astrocytes, suggesting a link between excessive PNNs and astrocytic function. One possibility is that PNN composition impacts astrocytic plasticity. One of the key PNN components, chondroitin sulfate proteoglycans, are sulfated mainly at the 4 (C4S) and 6 (C6S) positions, which have differing effects on PNN plasticity. We observed a marked increase in C6S intensity and a decrease in C4S intensity in the CA2 of BTBR mice. However, BTBR mice with control-like levels of PNNs maintained high C6S expression, suggesting that C6S+ PNNs are not responsible for shifts in glial populations. Since evidence suggests that astrocytes produce sulfotransferase enzymes, an alternate possibility is that dysregulated astrocytes in BTBR mice result in excessive C6S+ PNNs. Using viral inhibition of astrocytic calcium signaling in control mice, we found an increase in C6S, but not C4S, deposition and mislocalization onto CA2 PNNs similar to what we observe in BTBRs. This suggests that astrocytic calcium regulates C6S PNN-associated sulfation patterns, although its effects on social memory function remains unknown.

#29: **Multiple glial subtypes interact and compensate for the loss of nearby glial function,** Kevin Krause, University of Virginia

Glia play multifaceted roles in the nervous system including the secretion of trophic factors, maintenance of ionic and nutrient balances, and the promotion of synapse formation. Proper glial development and morphogenesis is required to establish the necessary neuron-glia associations that underlie these functions. Previous work in Drosophila has shown that when the secreted neurotrophin Spätzle 3 (Spz3) is knocked down in cortex glia, there is a loss of cortex glial ensheathment of neuron cell bodies, increased neuronal cell death, and defects in animal behavior. Interestingly, otherwise healthy glial cells adjacently located to the cortex aberrantly infiltrate and extend processes into the cortex in this condition and begin to interact with the unwrapped neurons. Here we explored whether these other glia—namely astrocytes, ensheathing glia, and subperineurial glia—infiltrate this region in order to functionally compensate for the loss of normal cortex glial morphology and function, such as neuronal support or the clearance of neuronal debris. In order to quantify the relationship between the extent of neuronal cell death in the cortex and the amount of aberrant infiltration by each glial subtype, we utilize the image analysis software Imaris combined with a machine learning Fiji plugin called Labkit to segment these volumes. We show that aberrantly infiltrating glia functionally compensate for this loss of morphology by clearing dying neuronal debris, yet their own normal functions appear to be remarkably preserved. This work illustrates that other glial cell types in the Drosophila CNS can adapt to improper glial

function and take on the role of cortex glia to properly clear dying neuronal cell bodies while still maintaining their own roles in synaptic maintenance (astrocytes), neurite clearance after injury (ensheathing glia), and maintaining the blood-brain barrier (subperineurial glia).

#30: **Proprioceptive control of rapid and flexible limbs movements during Drosophila landing,** Wayne Kuo, Virginia Tech

Proprioception, the sense of self-movement and body position, is vital for highly coordinated movement. However, the neural mechanisms underlying proprioceptive control of behavior remain poorly understood. Here, we developed a novel behavioral assay using the fruit fly, Drosophila melanogaster, to determine how proprioceptive feedback coordinates limb movements. We constructed a high-resolution 3D kinematics analysis paradigm to track the leg joints, wings, and body of tethered, flying flies. We will use this assay to dissect how the central nervous system integrates leg proprioceptive information to coordinate a behavioral transition from flight to a stable landing. We induced landing behavior by elevating a platform to contact the flying fly's legs, mimicking the contact that happens during landing. Flies were more likely to land when the distal tibia-tarsus (TTa) joint was contacted compared to the proximal coxa-trochanter-femur (CTF) joints. Additionally, stimulation at TTa joint led to landings with lower latency compared to stimulation at CTF joint. We further examined how behavioral state modulates landing by comparing the behavior of starved and fed flies. We found that starvation increased flies' likelihood of landing in response to contact of the CTF joint. Overall, our study reveals that flies exhibit distinct landing responses in response to contact of different leg joints, suggesting that limb mechanosensory cues can influence landing coordination, but may do so in different ways. This assay will enable novel insights into how proprioceptive feedback rapidly coordinates ethologically important behaviors.

#31: **The nociceptive withdrawal response in intact, unanesthetized rats exhibits strong dependence on initial posture but weak dependence on stimulus location**, Claire Larson, James Madison University

Animals adopt numerous survival behaviors in response to aversive stimuli. One of these behaviors, the nociceptive withdrawal response (NWR), consists of the removal of an animal's limb from the affected site when presented with a noxious stimulus. Preliminary studies in our laboratory have identified three distinct components of the NWR – early extension, rapid flexion, and rapid extension. These studies have suggested that posture may be critical in influencing the animal's NWR when the animal is in a weight-bearing stance. Despite these findings, how the NWRs of intact, non-human mammals rely on stimulus location and how this pattern may be influenced by the animal's initial posture and stimulus location remains unclear. The specific aim of our research was to investigate the NWR in intact, unanesthetized rats in a weight-bearing posture when presented with noxious heat stimuli at various locations on the sole of the foot and

circumferentially around the leg. We hypothesized that as stimulus location changed, the direction of the NWR direction of the rat's hind limb would depend on both stimulus location and initial posture. To accomplish this, adult Sprague-Dawley rats were anesthetized and marked with 2 mm circles at six locations on the left hind leg, which defined rotation around the toes, ankle, knee and hip. Following recovery, the rats were then presented with localized heat stimuli that targeted specific rostra-caudal locations on the plantar surface of the foot and anterior and posterior surfaces of the lower leg. Hind limb movement was tracked laterally using a high-speed (500 fps) video. Rotation of the toe, ankle, knee, and hip joints was calculated based three adjacent marks. Based on preliminary results, we found that there was limited effect of stimulus location on the three phases of response but a clear dependence on the initial location of the foot prior to stimulation. Overall, the NWR appears designed to preserve postural stability and sufficiently remove the leg from the noxious stimuli, rather than accurately direct withdrawal movements away from the stimuli.

#32: **Microglial depletion impairs social memory function and diminishes hypothalamic afferents to the hippocampal CA2 region**, Sabrina Lee, University of Virginia

Deficits in social memory, or the ability to recognize and remember individuals, are a feature of many neuropsychiatric illnesses and can severely impede quality of life. The hippocampal CA2 subregion and its connecting regions are critical for social memory function, although the specific cellular mechanisms that regulate these connections remain unknown. One contender is microglia, the brain's resident macrophages, which are known to participate in modulating synaptic connectivity through pruning and trophic support. Here, we examined whether microglia regulate afferents to the CA2 region that are associated with social memory function. We first ablated microglia with colony stimulating factor 1 receptor inhibitor PLX and found significant social memory impairments in mice with no microglia, demonstrating the necessity of microglia to social memory circuitry. Using immunohistochemistry, we then examined excitatory afferents from mature (zinc transporter 3 [ZNT3]) and immature (3R-TAU) neurons in the dentate gyrus, excitatory afferents from the supramammillary nucleus (SUM) of the hypothalamus (vesicular glutamate transporter 2 [VGLUT2]) and entorhinal cortex (vesicular glutamate transporter 1 [VGLUT1], and cholinergic afferents from the basal forebrain (vesicular acetylcholine transporter [VAChT]) in the CA2 region of control and PLX-treated mice. Optical intensity analyses revealed no change in most of the afferents in the CA2 region, including ZNT3, 3R-TAU, VGLUT1, and VAChT. However, we found a significant decrease in VGLUT2 intensity, suggesting that elimination of microglia selectively diminishes SUM-CA2 connections. Future research is focused on elucidating the dynamic interactions between microglia and synaptic connections within the SUM-

CA2 circuit, investigating the mechanisms by which microglia regulate this connection and its influence on social memory function.

#33: **Navigating the Navs: Applying RNA-seq Tools to Understand Differential Isoform Expression of UNC-53 in C. elegans**, Meredith Lehman, John Jantzen, Eastern Mennonite University

The development of higher organisms requires that cells and cellular processes navigate complex environments to reach their final destinations. The Neuron Navigator (Nav) family of genes encode conserved intracellular signaling protein NAVs (NAV-1,2,3) that function to integrate extracellular guidance cues to changes in the cytoskeleton. The C. elegans homolog of NAV2, UNC-53 (uncoordinated-53), is encoded by the unc-53 gene. Like the Navs more generally, the unc-53 genetic locus is complex, encoding several short and long isoforms, subject to alternative splicing, and driven by at least three promoters. The precise spatiotemporal pattern and relative importance of the various isoforms of unc-53 and the Navs is not well understood. This project employs a custom bioinformatics approach to identify and compare the various unc-53 isoforms from FACS sorted neurons derived from L1 and L4 staged animals (Sun & Hobert 2022) and from adult tissues: neurons, muscle, intestine and hypodermis (Kaletsky et. al 2018). In each case, RNA-Seq data from publicly available databases was obtained and processed via a dual-output bioinformatics workflow in Cyverse. The workflow included five main steps: processing raw data, read alignments, transcriptome reconstruction, expression quantification, and differential expression analysis. For qualitative analysis, RNA-Seq data was visualized after alignment with HISAT2 using the UCSC genome browser. We are currently employing more quantitative approaches using EdgeR4.0 in addition to STAR and RSEM to determine isoform prevalence.

#34: **Interactions between positive allosteric modulation of muscarinic acetylcholine receptors and orexin receptor antagonism in cognitive flexibility in rats (Rattus norvegicus),** Mutian Li, William & Mary

In Alzheimer's Disease (AD), one important function affected is cognitive flexibility, the ability to adjust to different rules according to context. Previous research has shown independent effects of orexin and acetylcholine (ACh) on cognitive flexibility. Nonetheless, there are strong neural connections between orexin and ACh, suggesting potential interactions between these neurotransmitters. In the present project, rats received drug treatments to manipulate their orexin and ACh at different timepoints and then their cognitive flexibility was tested. First, rats were exposed to VU0453595, an M1 PAM (positive allosteric modulator of the M1-subtype muscarinic receptors), which upregulates ACh. Then, following injections of the orexin-1 receptor antagonist, SB-334867, rats were tested through tasks that required them to follow different rules to get rewarded. We hypothesized that the M1 PAM will improve task performance, whereas the orexin antagonist will impair performance. We also hypothesized that the orexin antagonist can block the positive effects

of lower dose M1 PAM. Results from this study can better inform treatment of cognitive flexibility in AD.

#35: **Regional astrocyte changes following diffuse traumatic brain injury and buprenorphine administration in rats**, Radina Lilova, Virginia Commonwealth University

Secondary sequelae from traumatic brain injury (TBI) can result in glial alterations. Our previous studies showed regional morphological astrocyte changes following central fluid percussion injury (cFPI) and buprenorphine (bup) administration, however, what these morphological changes indicate remains enigmatic. Therefore, this study aimed to investigate the influence of bup on astrocyte protein expression post-cFPI. At 4w post-cFPI and saline or bup treatment, cortical, hippocampal, and thalamic tissue from adult male rats was assessed for protein levels of the intermediate filament, GFAP, the volume reducing channel, SWELL1, and the ion channel associated with astrocyte cell swelling, TRPM4. Using multi-factor analyses across injury, region, and treatment we found regional, injury-dependent differences in all three proteins. There was a trend toward increased GFAP protein levels following cFPI compared to sham. There were interactions among injury, region, and treatment in which bup reduced GFAP in the cortex and hippocampus following cFPI, but not the thalamus or in sham animals. There were significant regional differences in both SWELL1 and TRPM4. There was a significant interaction between region and treatment and a trend toward interactions among region, injury, and treatment for SWELL1, in which TBI animals treated with bup had lower SWELL1 in the cortex but higher in the thalamus. There was an interaction among region, injury, and treatment for TRPM4 in which TBI animals treated with bup had lower TRPM4 in the cortex.

This work was funded by NINDS grants R01NS096143.

#36: **Exploring P2RY12 as a marker for widespread harvesting of microglia in the early postnatal multisensory midbrain**, Sophia Lindauer, James Madison University

Encompassing the purely auditory core of the midbrain inferior colliculus (IC) are shell nuclei that receive multisensory afferent arrays. Our lab has been instrumental in characterizing the development and organization of one of these regions, its lateral cortex (LCIC). We've defined an early postnatal critical period (P0 through P12) when its compartmentalized structure emerges and its multimodal inputs segregate, each targeting discrete LCIC zones (somatosensory: modules, auditory: matrix). Recently, we've implicated microglial cells (MGCs) as being important players in the refinement and selective pruning of developing LCIC afferent patterns. Not only are MGCs present in the developing LCIC at critical timepoints, but it appears that subsets of microglia with unique expression profiles occupy spatially distinct LCIC zones, and perhaps perform different developmental functions. As a next-step for probing the extent of MGC heterogeneity in the nascent LCIC and characterizing specific MGC subpopulations, we aim to

identify a more ubiquitous marker for LCIC microglia that would facilitate widespread harvesting of its resident microglia. P2RY12 is a G-protein coupled, purinergic receptor considered to be highly specific for microglia. The present study examines P2RY12 labeling in GAD67-GFP and CX3CR1-GFP neonatal mice to assess the breadth of its expression in the developing LCIC. Results indicate widespread P2RY12 expression throughout all major IC subdivisions, and in particular its lateral cortex. In comparison to other markers previously vetted in our lab (e.g. Iba1, CX3CR1, CD11b, TMEM119, SIRP-α), P2RY12 is without question the most broadly expressed. Such findings are promising for anticipated studies that plan to utilize P2RY12Cre lines to specifically harvest microglia for transcriptomic level cluster analyses.

#37: **The Relationship Between Purkinje Cells and Nuclei Cells in Mouse Models for Cerebellar Movement Disorders**, Alyssa Lyon, Virginia Tech

The cerebellum plays an important role in movement. The cerebellum is comprised of inhibitory Purkinje cells (PC), which synapse onto cerebellar nuclei(CN) cells to relay their output to the rest of the brain (1). Cerebellar dysfunction leads to a diverse set of disorders, including dystonia, ataxia, and tremor (2). Specifics about the relationship between PC and CN spiking activity during these disorders remain unclear. Knowing this information is crucial when understanding the disorders and developing therapeutics, especially since CN is downstream of PC. Evidence suggests PC dysfunction can cause dystonia, making it a potential therapeutic target. The current project investigates neural signal changes in mouse disease models by measuring single-neuron spike train properties in PC and CN. Because of the inhibitory nature of the PC, we hypothesize an inverse relationship between PC and CN firing rate.

Using an existing database of single-neuron recordings, we measured spike train properties in PC and CN in various cerebellar disease mouse models to investigate the relationship between them. Also from the database are recordings of optogenetically induced, acute changes in PC spike signals. To determine which changes in PC spike pattern parameters best predict changes in CN, the recordings were processed using a custom MATLAB code to analyze six different parameters relating to spike properties. A linear regression model was used to observe the relationship between PC and CN spike properties in cerebellar disease. Results didn't support our hypothesis, because there was no inverse relationship between PC and CN firing rate. However, in disease models, we found significant, positive relationships between PC and CN measurements for firing rate irregularity (CV, skewness, and interburst pause(IBP)). As for the optogenetic results, the FR was the only parameter with a significant inverse relationship between PC and CN. This may indicate that FR is the most susceptible to the synchronized activity driven by optogenetic manipulation, as opposed to the parameters in naturally occurring, long-term disease models. Overall, results from the disease model spike activity suggest CV,

skewness, and IBP are the best parameters to predict CN spike activity based on changes in PC spike activity in disease models. Also, optogenetic results allow us to observe the direct effect PC FR has on CN FR through different manipulations. This is crucial information because it provides a better insight into how disease-causing spike properties are propagated through the cerebellar circuit. Normalizing these spike properties will help develop novel therapeutics.

#38: **Characterizing the Mcoln1-/- mouse as a model of early Age-related Macular Degeneration**, Jonathan Miller, James Madison University

Retinal degenerative diseases (RDDs) are a diverse group of retinal disorders that cause visual impairment. While RDD prevalence is high, little is known about the molecular mechanisms underlying the pathogenesis within many of these disorders. Here we use transcriptome analysis to elucidate the molecular mechanisms that drive early onset photoreceptor neuron function loss in the mouse model of the RDD Mucolipidosis type IV (MLIV). MLIV is a lysosomal storage disorder resulting from loss of function mutations in the MCOLN1 gene. MCOLN1 encodes a lysosomal cation channel, the transient receptor potential channel mucolipin 1 (Trpml1). To identify changes in gene expression during onset in MLIV we used a genetic mouse model (Mcoln1−/−) which recapitulates clinical attributes of the human disease. We conducted transcriptome analysis in 6-week old control and Mcoln1−/− mice under normal 12:12 light cycle as well as low and high light stress conditions. These data will be valuable to the vision research community for identifying differentially expressed in early onset MLIV potentially leading to new insights into the pathophysiology of this RDD.

#39: **The Role of Brain Stem 5HT1A and GABAA Receptors in the Thermoregulatory Response to Handling Stress,** Halle Miller, James Madison University

Sudden Infant Death Syndrome, or SIDS, is characterized by the unexplained death of an infant in the first year of life. It has been established that babies who die of SIDS have altered serotonergic neuronal function when compared to non-SIDS babies. Specifically, 5HT1A receptor binding at the nucleus raphe pallidus (NRP) is decreased (Paterson et al. 2006). These sympathetic premotor neurons mediate cardiovascular stress responses through 5HT1A and GABAA receptors (Brown et al. 2011). This project focuses on the ability of these receptors to mediate thermoregulatory stress responses in rats. It is hypothesized that the activation of these inhibitory receptors will attenuate the usual short term hyperthermic response to handling stress, while blocking them will exacerbate it. A surgically implanted cannula targeting the NRP allows for microinjection and an abdominal thermoprobe allows for core temperature (Tc) readings. A 5HT1A agonist and antagonist as well as a GABAA agonist and antagonist were administered at the NRP. The Tc and selected ambient temperature (STa) of the rats were measured following handling and

microinjection. Handling resulted in a short-term increase in Tc (1.26 ± 0.05℃) but no significant change in STa in the control group. Injection of the GABAA agonist muscimol attenuated this response, with a Tc change of 0.94 ± 0.23℃. The modification of the 5HT1A receptor had no effect. This suggests that GABA receptors at the NRP may play a role in the thermoregulatory response to handling stress.

#40: **The Effect of Diet-Induced Obesity on Cognitive Performance in Rodent Model**, Haley Minnery, Washington and Lee University

Escalating obesity rates in the United States are a major public health concern. By 2022, 21 states had an adult obesity rate over 35% (Centers for Disease Control). While a plethora of health conditions are associated with obesity, ranging from metabolic disorders to cardiovascular disease, the connection between obesity and cognitive function is not fully understood. However, recent investigations are beginning to identify a relationship between diet-induced obesity and deficits in spatial learning, memory, and impulse control. The current study utilizes a diet-induced obesity model to assess cognitive changes in rats. Rats were divided into control and high fat high sugar (HFHS) experimental groups. The HFHS diet is comprised of a 32% sucrose solution and 60% fat chow, while control rats only receive standard 17% fat chow. The rats were put through a variety of behavioral tests, including: the Barnes Maze (spatial learning) and a delayed choice T-Maze (impulsivity). In the study, no significant impairments in spatial learning were observed; HFHS rats performed comparably to control rats. The delayed choice T maze yielded mixed results. However, HFHS rats were found to exhibit a longer average first choice latency (the time it takes for the rat to pick an arm of the cognitive maze), suggesting a potential deviation from normal cognitive function. In addition to behavioral analysis, the total distance the rat moved through the maze was recorded using FIJI. These findings contribute to current investigations of the relationship between cognitive impairments and obesity and emphasize the importance for further studies in this field to inform preventive strategies and interventions.

#41: **Perineuronal net plasticity in age-related cognitive function**, Jacqueline Moats, University of Virgnia

Aging is accompanied by several non-pathological brain changes that, even in the absence of disease, can deleteriously influence cognitive capacities. Our present society has an increasing aging population, generating a need to elucidate the basis behind age-related cognitive decline. Among the brain regions affected by aging, the hippocampus is notable for its role in cognitive function. Evidence suggests that the condensed form of extracellular matrix known as perineuronal nets (PNNs) play an important part in memory. PNNs form a lattice-like structure that surround certain neurons and limit their plasticity. PNNs are composed of multiple components, including chondroitin sulfate proteoglycans (CSPGs), which are primarily sulfated in the 4 (C4S) or 6 (C6S) positions. These sulfation

patterns affect PNN function, where C4S sulfation patterns are inhibitory to plasticity while C6S sulfation patterns are permissive to plasticity. In the aging brain, PNNs shift to a higher C4S to C6S ratio, although the impact of this PNN plasticity shift on cognitive function is unknown. I focused first on the CA2 region of the hippocampus, an area critical for social memory function. Using pan-PNN marker Wisteria floribunda agglutinin, I did not find any changes in PNN abundance in the CA2 between young (3-months old) and aged (18-months old) mice. However, I found that aged mice have greater C4S intensity compared to young mice, suggesting that PNN plasticity may be altered in the aged brain. Ongoing studies are exploring PNN plasticity throughout the hippocampus to evaluate how it relates to age-related cognitive decline.

#42: **Characterization of Metachromatic Leukodystrophy Disease Model**, Emily Moran, Virginia Commonwealth University

Metachromatic leukodystrophy (MLD) is a lysosomal storage disorder that is due to an inherited deficiency of the lysosomal enzyme arylsulfatase A (ARSA; EC 3.1.6.8). Arylsulfatase A catalyzes the first step in the degradation of the sphingolipid 3-O-sulfogalactosylceramide, known as sulfatide. The prognosis is severe, with death resulting, in the majority of cases, a few years after diagnosis. An arylsulfatase A–deficient knockout mouse has been generated that mimics some features of human MLD. Recent characterization of this model includes abnormal node confirmations, increased pathology of myelin, and increased expression of activated morphology in both microglia and astrocytes which both showed further activation corresponding to progressive deterioration of the CNS between 7 months and 14 months. These structural pathologies are consistent with poor balance and impaired locomotion as established by rotorod experiments. Additionally, using an open field strategy the mice spent more time in the center of the field consistent with decreased anxiety. Novel object experiments showed no indication of memory deficits. Together, our structural and functional analyses provide a detailed molecular and behavioral characterization of the MLD mouse. Presently, we have designed a breeding strategy that will generate a double mutant mouse consisting of the MLD mouse on the background of a mouse that synthesizes a reduced level of sulfatide. This double-transgenic mouse will test the efficacy of substrate targeting as a potential therapeutic strategy for the treatment of this devastating disease.

#43: **Drosophila Larvae Cool Cell 3D Calcium Imaging Analysis using TACI,** Trisha Naidu, Virginia Tech

Drosophila melanogaster, commonly known as the fruit fly, have cells capable of responding to environmental temperature fluctuations. Specifically under cooler conditions, three dorsal organ cool cells (DOCCs) have been found to help the fly larvae sense suboptimal temperature conditions, with two classified as type-A and one as type-B .However, imaging these cells in live larvae presents challenges due to the difficulty in

controlling organismal movements. To address this issue, our lab developed a new ImageJ plugin called TrackMate Analysis of Calcium Imaging (TACI) which enhances the accuracy of neuron tracking while accommodating motion in all directions. TACI takes into consideration not only the x and y axes but also the z-axis, enabling identification of the maximum fluorescence value across all the z-positions a neuron appears in and uses it to represent the neuron's intensity at the corresponding time point. By using TACI which factors in z-planes, I have been able to efficiently analyze the DOCCs in fly larvae even when there are neurons that are overlapping in the lateral direction. This efficient method of analysis has allowed for a clear visualization of the Drosophila larvae cool cell temperature responses.

#44: **Neuromelanin Contrast Ratio's Inverse Relationship with Locus Coeruleus Volume and Its Impact on Attentional Ability**, Joshua Neal, Virginia Tech

The locus coeruleus (LC) has been previously identified as pertaining to broad arousal in the nervous system, especially in terms of the production of norepinephrine. A metabolic by-product of norepinephrine, neuromelanin, has been associated with the prevalence of neurodegenerative disorders and attention performance in older adults but has limited study in younger age groups, where concern on sufficient neuromelanin deposit for study has been previously noted. Volume estimations of the LC are also in need of additional studies in more generalized populations, as prior studies have focused on neurodevelopmental populations and post-mortem direct examinations. Within the two measures of the LC, both exhibited a significant relationship to inattentive scores, supporting prior understandings of arousal and its relationship to proper attention functioning. The presence of this relationship within the right portion of the LC aligns with evidence for hemispheric effects on attentional processing. The inverse relationships between LC's contrast ratio and volume may carry mechanistic implications for understanding the functional and structural roles of LC in terms of neural signaling and norepinephrine production, thereby influencing attentional behaviors. Such mechanistic interpretations (ie. that norepinephrine-producing neurons may function most effectively when in closer proximity in a smaller LC, or that a smaller LC may more effectively protect such neurons for neurodegeneration over the lifespan) should be examined further in subsequent studies.

#45: **A Novel Feeding-Activated Arcuate Neuron Subtype That Controls Body Weight, But Not Feeding,** Roberta Onoharigho, University of Virginia

The arcuate nucleus (ARC) is a critical hypothalamic region for the control of metabolism and feeding. Previous studies show that a subgroup of inhibitory ARC neurons, which express the vesicular inhibitory amino acid transporter gene (Slc32a1) and the leptin receptor gene (Lepr), control metabolism but not feeding. However, the identity of these neurons remains elusive. We identified a candidate neuron subtype in the ARC,

marked by the expression of the phosphoinositide interacting regulator of the transient receptor potential (Pirt) gene. We hypothesize, based on their molecular profile, that PirtARC neurons are activated by feeding to drive thermogenesis. We used the Pirt-Cre transgenic mouse to investigate the role of PirtARC neurons in energy homeostasis. To validate Cre activity, we expressed a reporter virus (AAV8-hSyn-FLEX-TVA-P2A-GFP-2A-oG) in the ARC of male and female Pirt-Cre mice (n=4; 6 injections, 50 nL each) and compared GFP (green fluorescent protein) fluorescence to Pirt expression by RNA fluorescence in situ hybridization (RNA FISH). In the absence of Cre expression, we failed to detect GFP expression, confirming the Cre dependency of the AAV. However, in Pirt-Cre mice, we found that 77% (380 of 495) of ARC Pirt RNA+ neurons were GFP+ and that 79% (380 of 483) of GFP+ ARC neurons were Pirt RNA+, thereby validating the Pirt-Cre mouse for genetically targeting PirtARC neurons. Next, to investigate whether PirtARC neurons respond to metabolic states, we subjected male and female C57BL/6J mice to ad libitum feeding, 18-hour fasting, and 18-hour fasting then 2-hour refeeding (n=4 mice per condition). Using RNA FISH, we assessed the co-localization of Pirt mRNA with Fos mRNA (neuronal activity marker) across the metabolic states. We found that the percentage of PirtARC neurons co-expressing Fos mRNA is higher in ad libitum-fed mice than in fasted mice ($p=0.0135$; one-way ANOVA followed by Tukey's multiple comparison test), though neither group differed significantly from post-fast re-fed mice ($p > 0.05$). This indicates that PirtARC neurons are more active in fed mice than fasted ones, consistent with our hypothesis. Finally, to investigate whether PirtARC neurons regulate food intake, we expressed the intersectional chemogenetics (AAV-CreON/FlpON-hM3Dq-HA) in the ARC of male and female Pirt-Cre::Slc32a1-Flp mice (n=6; 6 injections, 100 nL each) to activate PirtARC neurons. We intraperitoneally injected the artificial muscarinic receptor ligand, clozapine N-oxide (CNO; 1 mg/kg), or saline vehicle in ad libitum-fed mice during the 12-hour light cycle and measured food intake after 30 minutes, 1 hour, 2 hours, and 4 hours on alternating days (CNO, 0.11 ± 0.05 g food/4 hrs.; saline, 0.11 ± 0.04 g food/4 hrs.; Two-way ANOVA, CNO vs saline, $p > 0.05$ for all time points). Unlike other ARC inhibitory neurons, we found that activating PirtARC neurons does not increase food intake. Together, our results show that PirtARC neurons are a novel subtype of feeding-activated, non-orexigenic neurons in the arcuate hypothalamus.

#46: **mTBI in higher order animal models: Assessing changes in Yucatan Minipig sociability following diffuse central fluid percussion injury**, Mark Pavlichenko, Virginia Commonwealth University

Traumatic brain injury (TBI) is a major healthcare concern affecting millions. Symptoms of TBI include sensory sensitivity and social changes that may persist long-term. Due to similarities in cytoarchitecture, metabolism, and inflammation, higher order minipig models are advantageous for translational TBI research, however, there remains a shortage of

information regarding their behavioral sequela following injury. Therefore, in this study, we assessed changes in sociability and somatosensation in adult male and female Yucatan minipigs for up to one-week following a sham or central fluid percussion injury (cFPI). Specifically, the forced human approach task (FHAT) was done to investigate each animal's approachability/sociability with both known and unknown humans prior to and following cFPI. Von Frey test was also done to determine potential changes in sensitivity to somatosensory stimulus on the ear and tail. We found that while approachability to FHAT was not significantly impacted by previous exposure to the human or cFPI over the first week post-injury, FHAT was highly sensitive to additional external stress in our male cohort ($X2(1)=9.16$, $p=0.002$) with a significant interaction between injury and stress. Von Frey assessments showed that the ears, but not tails, in female ($n=7$) pigs were more sensitive to tactile stimulation than males ($n=14$) prior to injury ($U=17.5$, $p=0.016$). Sensory sensitivity of the ear was decreased in our male cohort following cFPI ($\chi2(9) = 21.829$, $p = 0.009$), which suggests that TBI has an effect on somatosensation of the ear in this model.

This work was funded by NINDS grants R21NS126611, R56NS128104

#47: **Spatiotemporal multi-omics analysis of the hippocampal-amygdala circuit during contextual fear memory consolidation**, Xiguang Xu, Virginia Tech

The consolidation of contextual fear memories requires the crosstalk among multiple brain regions, including the hippocampus and amygdala. Despite the advance in single cell transcriptomic techniques, tracing the dynamic gene expression across the hippocampal-amygdala circuit remains challenging. Here we integrated high resolution spatial transcriptomics with single nuclei multi-omics techniques to systematically investigate the spatiotemporally transcriptional programs across the hippocampal-amygdala circuit during the encoding and retrieval of contextual fear memory. It reveals brain region-specific gene expression changes during memory consolidation, especially in the subregions of hippocampus and amygdala. Furthermore, it showed cell-type specific epigenetic and transcriptional changes during the contextual fear memory consolidation. Our data provides new insight into the spatiotemporal and cell-type specific transcriptional programs during memory consolidation.

#48: **Investigation of Low-Intensity Focused Ultrasound (LIFU) for Neuromodulation of Anxiety**, Arthi Prabhakar, Virginia Tech Carilion School of Medicine

Anxiety disorders are the most common mental illness in the US, affecting 31.1% of adults at some point in their lifetime. Although several pharmacological treatments have been proven effective, many patients do not achieve remission. Neuromodulatory techniques such as transcranial magnetic stimulation and transcranial electric stimulation are limited due to their low penetrative power and poor spatial resolution; however low-intensity focused ultrasound (LIFU) overcomes these obstacles and provides millimeter resolution with deep focal lengths.

The right dorsal anterior insula (rdAI) and dorsal anterior cingulate cortex (dACC) are proposed modulators of anxiety responses. Previous studies have used the no, predictable, and unpredictable threat (NPU-threat) task to assess anxiety in the lab. It is also theorized that autonomic function may be maladaptive in anxiety, and the insula may control this response.

#49: **Miro Regulation of Neuron-Glia Interactions**, Kathleen Reuwer, University of Virginia

Glia support many aspects of neuronal cell function, including supplying metabolic support for neurons to keep up with their immense energy demand. Miro, a mitochondrial rho GTPase, is a mitochondrial outer membrane protein known for connecting mitochondria to motor proteins to aid in the transport along cellular architecture. Not surprisingly, mutations in Miro result in disrupted mitochondrial motility with mitochondria becoming sequestered in the neuronal soma, as well as perturbations to mitochondrial function, and have been implicated in neurodegenerative diseases such as Parkinson's and Alzheimer's pathology. Using Drosophila melanogaster, we investigate how Miro dysfunction affects the interaction between glia and other cells in the central nervous system (CNS), particularly focusing on cortex glia, a glial subtype that support neuronal cell bodies and neural stem cells (neuroblasts). Interestingly, global Miro knockout results in a unique degradation in these cortex glia in the thoracic region of the ventral nerve cord (VNC) of late stage larvae, a region that undergoes a second wave of neurogenesis at this time. Whether the loss of cortex glia and the onset of neuronal expansion in these mutants are linked remains to be explored. This unique loss of cortex glia provides a tool to investigate the role of glia in the metabolic support of neuronal cell bodies and neuroblast function. Employing cell-specific Miro RNAi knockdown and rescue constructs, we aim to uncover how cortex glia interact with surrounding cells such as neurons, neuroblasts, and other glial subtypes to adapt in the presence of mitochondrial dysfunction. Using structure-function analysis to rescue the global Miro knockout with human disease mutations lacking specific functional domains and molecular interaction sequences, we aim to delve deeply into the mechanistic role of Miro in these processes, and discover potential targets for restoring disrupted cell-cell interactions and neuronal degeneration associated with Miro loss in disease.

#50: **Mediodorsal Thalamus Projections to the Prefrontal Cortex and Their Roles in Cue Detection**, Kelly Runyon, Virginia Tech

A deficit in the capacity to use instructive cues to guide ongoing behavior, "cue detection", is a feature of many neurodegenerative and neuropsychiatric disorders. Previous work in humans and model species highlights that circuitry including the mediodorsal thalamus (MD) and its projections to the prefrontal cortex (PFC) are critical for the control of this cognitive process. How activity in this circuit is sculpted to support these functions remains unclear. Here, we describe a set of multi-disciplinary

studies designed to gain insight into this gap in our knowledge. First, using male and female wild-type mice and fiber photometry, we measured in vivo calcium dynamics (GcAMP7s) at both the local cell bodies (MD, n=4) and distal terminal fields (anterior cingulate cortex (ACC), n=4 and prelimbic cortex (PrL), n=4) in mice undergoing Pavlovian cue-reward pairing. In both the cell bodies of the MD and terminals in the PFC, we observed significant increases in activity in response to a conditioned light stimulus. Interestingly, presentation of the primary reward evoked a transient increase in Ca+2 only in MD cell bodies, suggesting that information about the learned value of a predictive cue is selectively relayed to the PFC. Additionally, in a serial reaction time task (SRTT) in which mice are required to use the cue to inform lever presses, we find a similar transient increase in MD activity to reward, especially on correct responses. Interestingly, both cortical targets show an overall decrease in activity at that same timepoint, suggesting a dampening of certain regions of the circuit while other regions are allocated more resources. These region and task-specific responses highlight the importance of uncovering the specific functions encoded by different parts of an important cognitive system.

#51: **Synaptic transmission alterations in a mouse model of Szt2 loss-of-function**, Mona Safari, Virginia Tech

Epilepsy, a chronic disease of the brain, affects 5 million people worldwide annually and 30% are resistant to the current therapies. In children, seizures often co-occur with intellectual impairment, cognitive disorder, and autism spectrum disorder. It is now understood that genetic factors account for at least 40% of all epilepsy cases. Notably, variants in at least 10 genes that regulate the mammalian/mechanistic target of rapamycin (mTOR) pathway cause seizures, intellectual disabilities, and cognitive impairments, collectively referred to as mTORopathies.

Seizure threshold 2 gene (Szt2) was previously identified in a screen as a gene whose loss-of-function (LOF) causes increased seizure susceptibility and lethality in mice. More recently Szt2 was found to negatively regulate mTOR signaling, and biallelic variants in the SZT2 gene were identified in children as causes of early-onset epileptic encephalopathies (EOEEs). Despite its significance, the function of Szt2 in the brain remains poorly understood. We hypothesized that biallelic Szt2 LOF shares key features of other mTORopathies, including altered mTOR signaling, dysregulated synaptic function, and altered neuronal morphology. Therefore, we assessed the synaptic and morphological changes in mice with biallelic Szt2 LOF. Our preliminary results suggest increased excitatory synaptic transmission and cortical thickness consistent with other models of mTOR hyperactivation.

#52: **Microglial-mediated perineuronal net accumulation in the hippocampal CA2 region coincides with social memory impairments**, Brenda Sanchez, University of Virginia

Studies have highlighted the hippocampal CA2 as a critical region in social memory circuitry. The CA2 has many distinct features, including an abundance of perineuronal nets (PNNs) — condensed extracellular matrix structures that ensheath particular neurons and serve as critical regulators of brain plasticity. Microglia, the brain's resident macrophages, participate in the structural remodeling of PNNs, but much remains unexplored about the specific aspects of PNNs that are modified by microglia and how this remodeling impacts social memory. In our current work, 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. Using Wisteria floribunda agglutinin (WFA), a broad marker of PNNs, we found increased WFA+ PNN intensity in the CA2 after microglial depletion but no overt changes in other hippocampal regions implicated in social memory, suggesting the CA2 may be more vulnerable to microglial-mediated PNN remodeling. We then investigated whether microglia remodel select aspects of PNN structures. Although we did not find differences in the chondroitin sulfate proteoglycan aggrecan, an essential PNN component, we observed increased glycosaminoglycan chains attached to chondroitin sulfate proteoglycans, in mice deficient in microglia, indicating that microglial elimination causes an accumulation of some, but not all, PNN components. Ongoing studies are exploring whether microglial repopulation reverses CA2 PNN accumulation and social memory deficits induced by microglial depletion.

#53: **Loss-of-function in the Drosophila serotonin transporter (dSert) gene changes sleep and activity and decreases life span**, Marciella Shallomita, Eastern Mennonite University

The monoamine serotonin is known to impact human behaviors such as sleep cycles, eating, anxiety, and depression. The reabsorption of serotonin back into the presynaptic neuron is mediated by the serotonin transporter (SERT), the target of selective serotonin reuptake inhibitors (SSRIs) used to treat depression. We turned to the model organism Drosophila melanogaster to better understand the mechanisms by which serotonin and SSRIs influence behavior. We created a gene knockout of the Drosophila SERT (dSert61) that removes the coding sequence and eliminates mRNA transcription. dSert61 flies show an increase of 144.5% ($p<0.0001$) in total sleep and 86.5% ($p<0.0001$) in average activity. dSert16, a 1.1 kb deletion in the promoter region, shows similar in responses in sleep and activity. dSert61/dSert16 hybrids phenocopy the individual dSert61 flies, verifying these two fly strains both these two alleles affect the same gene. Compared to Canton-S control flies, dSert61 and dSert16 have 15.1% ($p<0.0001$) and 4.2% ($p=0.008$) shorter life spans, respectively. The fly dSert ortholog will be used further to explore the mechanics of serotonin uptake and its connection to other complex behaviors.

#54: **Application of the human 3D Neuron-Glial Brain Assembloid reveals the differential role of ApoE isoforms in the pathogenesis of Alzheimer's disease,** Eliana Sherman, University of Virginia

Exploration of the pathophysiology of Alzheimer's disease (AD) has been hampered by the lack of systems that accurately recapitulate the full profile of disease progression. We have developed a three-dimensional (3D) assembloid model with iPSC-induced neurons, astrocytes, and microglia derived from AD subjects to investigate the pathophysiology, protein-protein interactions, cellular mechanisms, and interventional strategies for AD. In the current study, we aim to elucidate the different ApoE isoforms in their contribution to tau pathological propagation, including the risk variant ApoE4 and the protective variant ApoE2 in comparison to ApoE3.

Analysis of the Masteroid cultures after 2-4 weeks revealed accumulated β-amyloid deposition, tau pathology, neurodegeneration, astrogliosis, and microglial activation in the conditions challenged with oligomeric tau propagation and Aβ oligomers stress. Immunofluorescence labeling demonstrated abundant Aβ deposits (observed by Thiazine red or 4G8 antibody labeling), tau phosphorylation and aggregation, microglial activation (Iba1 labeling and TNF-α secretion), and neurodegeneration quantified using FluoroJade B, cleaved caspase 3, and LDH measurements. The ApoE4 variant and oTau seeding synergistically exacerbated neurodegeneration and gliosis. Concurrent proteomic and lipid mass spectrometry revealed novel cellular and molecular signal pathways in disease progression.

We have generated a novel 3D brain assembloid model of AD, which we term Masteroid. The Masteroid system recapitulates many features of AD pathology, including microglia/astrocyte gliosis, Aβ and tau pathology, and neurodegeneration. This innovative AD model offers an advanced platform to study the cellular and molecular mechanisms of disease progression and reveals the distinct signal pathways in which each ApoE isoform regulates the pathogenesis of AD and related tauopathies.

#55: **Heterozygous expression of a Kcnt1 gain-of-function variant has differential effects on SST- and PV-expressing cortical GABAergic neurons**, Amy Shore, Virginia Tech

More than twenty recurrent missense gain-of-function (GOF) mutations have been identified in the sodium-activated potassium (KNa) channel gene KCNT1 in patients with severe developmental and epileptic encephalopathies (DEEs), most of which are resistant to current therapies. Defining the neuron types most vulnerable to KCNT1 GOF will advance our understanding of disease mechanisms and provide refined targets for precision therapy efforts. Here, we assessed the effects of heterozygous expression of a Kcnt1 GOF variant (Y777H) on KNa currents and neuronal physiology among cortical glutamatergic and GABAergic neurons in mice, including those expressing vasoactive intestinal polypeptide (VIP), somatostatin (SST), and parvalbumin (PV), to identify and model the

pathogenic mechanisms of autosomal dominant KCNT1 GOF variants in DEEs. Although the Kcnt1-Y777H variant had no effects on glutamatergic or VIP neuron function, it increased subthreshold KNa currents in both SST and PV neurons but with opposite effects on neuronal output; SST neurons became hypoexcitable with a higher rheobase current and lower action potential (AP) firing frequency, whereas PV neurons became hyperexcitable with a lower rheobase current and higher AP firing frequency. Further neurophysiological and computational modeling experiments showed that the differential effects of the Y777H variant on SST and PV neurons are not likely due to inherent differences in these neuron types, but to an increased persistent sodium current in PV, but not SST, neurons. The Y777H variant also increased excitatory input onto, and chemical and electrical synaptic connectivity between, SST neurons. Together, these data suggest differential pathogenic mechanisms, both direct and compensatory, contribute to disease phenotypes, and provide a salient example of how a pathogenic ion channel variant can cause opposite functional effects in closely related neuron subtypes due to interactions with other ionic conductances.

#56: **Inhaled Wildfire Smoke Particulate Drives Aberrant Proteinopathy-related Changes in the Adult Mouse Brain**, Mohammad Siddiqi, Virginia Commonwealth University

An increasing frequency and intensity of wildfires represents a significant public health concern and is expected to cause an annual U.S. mortality of >40,000 by the end of the 21st century. Wildfire smoke (WFS) releases a high concentration of PM2.5 (≤ 2.5 μm particulate matter), to include nanoparticulate, that spreads widely in the atmosphere and creates an unhealthy, toxic atmosphere over long distances. Respiratory complaints from WFS are very common and hospitalizations due to cardiovascular morbidities also increase in areas impacted by WFS; however, impacts to brain health and particularly neurodegenerative disease pathogenesis remains poorly understood. PM2.5 and nanoparticulate from engine combustion sources has shown an alarming increased risk for neurodegenerative and other neurological outcomes, raising the concern of a similar burden with WFS. To address this gap, this study aimed to investigate the pathobiological consequences following exposure to WFS particulate.

Together, this study provides new insights into the relationship between WFS and pathogenesis of neurodegenerative disease relevant proteinopathy and underscores the need to understand longer-term health implications and development of suitable interventions to protect against WFS exposure.

#57: **Mapping neural connectivity of strain-sensing proprioceptive sensory neurons in the leg of Drosophila melanogaster**, Himadri Sunam, Virginia Tech

Proprioception, the sense of the position and movement of one's body, is important for facilitating normal, coordinated movement. Dysfunction in

proprioception leads to various motor impairments. The sense of proprioception is mediated by diverse populations of proprioceptive sensory neurons that encode different body kinematics. Although proprioceptive feedback is crucial for accurate motor control, little is known about how downstream circuits in the central nervous system extract relevant features from such complex patterns of activity across diverse proprioceptor populations. Here, we describe a set of tools that will enable us to map the connectivity of proprioceptive neurons in the leg of the fruit fly, Drosophila melanogaster. In particular, we are investigating the sensory feedback provided by campaniform sensilla (CS), which are strain sensors embedded in the cuticle of the fly that are functionally analogous to the vertebrate Golgi tendon organs. Campaniform sensilla detects stress and strain of the fly's musculoskeletal system via mechanosensory neurons attached to flexible cuticular domes. These neurons project their axons into the fly's ventral nerve cord (VNC), where they form connections with interneurons and motor neurons. To understand CS connectivity, we first reconstructed CS axons in an electron microscopy volume of the Drosophila ventral nerve cord (VNC). Next, we used automated template matching tools to identify genetic driver lines that label neurons that are morphologically similar to our reconstructions. Subsequently, we dissected and imaged fly legs from these genetic driver lines to confirm whether they label CS neurons, and also to determine which CS neurons are labeled. We have thus far identified several genetic driver lines that label CS in various parts of the leg. We are now using intersectional genetic techniques to further refine expression of our genetic driver lines, with the goal of matching the location of a CS in the leg with its axonal projection in the VNC.

#58: **Uncovering the molecular logic of dendritic RNA localization to synapses**, Renesa Tarannum, Virginia Tech

The incredibly complex and highly polarized morphology of neurons requires tight regulation of when and where proteins are made. Neurons traffic thousands of RNA transcripts to distant processes (axons and dendrites) to make proteins locally and on-demand. RNA localization and activity induced local translation at the synapses are required for long lasting changes in neuronal connectivity that underlie learning and memory. Dysregulation of RNAs at synapses is a common cause of neurodevelopmental disorders and learning disability. The molecular mechanisms of how neurons orchestrate this multistep process of localizing thousands of RNAs to the dendrites and synapses for spatially restricted protein synthesis are not clearly understood. Single molecule imaging studies from developing neurons in culture suggest RNAs are trafficked as single independent low-copy-number ribonuclear particles (RNPs) with minimal colocalization across species. Biochemical studies using subcellular fractionation also identified large complex multi-meric RNA-protein (RNP) granules from neurons. Detecting co-assemble of

multiple mRNA molecules as multimeric RNPs in vivo has been technically challenging and constrained by limitations in imaging more than three molecules at once. Using smFISH coupled with high resolution multiplexed imaging, we have surpassed this limitation in fluorescence microscopy and simultaneously visualized and characterized the composition and colocalization of a dozen dendritic RNAs in intact hippocampal circuit. We found that dendritic RNAs show considerable heterogeneity in the distribution of sizes that vary per RNA population, consistent with multiple modes of RNA transport in low copy number RNPs as well as larger homotypic RNPs that are higher order complexes containing multiple copies of the same transcript. With regards to composition, we find that ~70%-90% of the dendritic RNPs investigated colocalize with one or more of the other RNPs. However, pairwise colocalization varied considerably from ~2-30% above chance across transcripts. Interestingly, when we performed hierarchical clustering on pairwise colocalization values to identify RNAs with shared co-assembly (heterotypic RNPs containing multiple types of RNAs), we discovered that RNAs clustered by abundance. Highly abundant dendritic RNAs more frequently colocalize with any of the other RNAs regardless of their abundance. Together, our data suggest multiple pools of dendritic RNP compositions exist, including low and higher copy number (homotypic) and multiple RNA species (heterotypic), which appears to be related to abundance. This heterogeneity may potentially reflect differences in the functional state of these RNPs, for example ribosome-bound RNPs that are actively translating vs translationally repressed reservoir pools of RNPs. Characterizing these diverse flavors of RNPs would pave the way to understanding the different mechanisms neurons utilize to achieve efficient and precise RNA localization for on-demand translation.

#59: **Assessing The Effect of Caffeine on Associative Butanone Learning in C. elegans**, Caleb Townsend, Virginia Tech

Caffeine is the most widely consumed drug worldwide, affecting alertness and physiological activity. Many college students rely on caffeine to improve their memory retention and focus during studying. As such, many experiments have attempted to assess caffeine's effect on cognitive abilities, particularly learning. However, there have been inconsistent results regarding the efficacy of caffeine on learning and memory. In humans, these inconsistencies might be due to psychological factors, such as the placebo effect. Thus, we aim to distinguish between the biological and psychological effects of caffeine by using a model organism that does not have the cognitive complexity for placebo effects. Our team identified C. elegans to assess the effect of caffeine on associative learning. C. elegans are an organism with a simple nervous system, and they can form memories associating environmental olfactory information with positive or negative conditions, such as starvation or the presence of food. This, in addition to them being easy to grow and maintain, makes C. elegans an

ideal model organism to study effects of caffeine exposure on learning. We are investigating the effects of acute caffeine exposure on C. elegans' associative memory by placing them in a caffeine or control solution immediately before training them to associate the scent of butanone with the presence of food. We will then perform a chemotaxis assay allowing them to travel towards butanone or ethanol (vehicle) to investigate how many retain a positive association with butanone. We predict that animals exposed to caffeine before the training period will retain this association at a greater rate than those not exposed to caffeine. This will be calculated with a Chemotaxis Index formula. After obtaining results, we will compare the rate at which caffeine-exposed and control C. elegans are attracted to butanone, and if the caffeine-exposed C. elegans are significantly more attracted to butanone, this would verify our hypothesis.

#60: **Investigating the Efficacy of a Targeted Therapy on Reducing Seizures in a Mouse Model of Epilepsy,** Jessica Urbanczyk, Virginia Tech Carilion School of Medicine

Kcnt1-related epilepsy is an infant-onset seizure disorder caused by genetic variants within the Kcnt1 gene which encodes the sodium-gated potassium channel KNa1.1. To date, there are no effective therapies for Kcnt1-related epilepsy. Given the genetic contributions to disease progression in Kcnt1-related epilepsy, gene therapies are an appealing therapeutic strategy, yet their efficacy and safety has not been assessed.

Our characterization of mice with gain of function mutations in KCNT1 revealed these mice experience significantly higher frequency of spontaneous seizures compared to control mice. Excitingly, targeting KCNT1 with a small molecule completely prevented seizures in Kcnt1RQ/RQ mice who previously had high frequency of seizures before treatment.

Our work reveals the therapeutic potential of a novel small molecule in the treatment of Kcnt1-related epilepsy. Future studies will seek to determine the efficacy of siRNAs that target Kcnt1 to prevent epileptic seizures.

#61: **Defining Molecular Mechanisms Underlying Thermoreceptors IR21a and IR68a**, Thomas Vaden, Virginia Tech

Temperature affects nearly every aspect of an organism's life, from general survivability to efficiency of biological functions. To navigate to preferred temperature ranges, animals use thermoreceptors: a diverse group of temperature sensitive molecules located in specialized sensory neurons. While several classes of thermoreceptors have been identified in countless life forms ranging from nematode to human, the molecular mechanism responsible for thermosensation is not well understood. This project focuses on two closely related thermoreceptors found in Drosophila melanogaster, IR21a and IR68a, a cool and warm receptor respectively, to elucidate the molecular mechanism underlying the response to temperature stimuli. We first utilized the computational protein modeling software AlphaFold to understand the structures of IR21a and IR68a. We

are currently generating fly lines containing chimeric IR21a/IR68a proteins using CRIPSPR/Cas9 technology. These fly lines will be analyzed using immunofluorescent staining, calcium imaging, and temperature preference assays. We expect to identify at least one fly line showing an altered response to temperature which will be further analyzed to determine critical amino acid residues, as well as residue interactions, required for thermosensation. If successful, this project will answer a fundamental question regarding sensory systems. It will also provide valuable insight about a key structure used by insect vectors of disease in host seeking, which will help develop methods to minimize the spread of infectious diseases harbored by these insects.

#62: **The Role of Brain Stem GABA-A Receptors in the Protective Hypothermic Response to Hypoxic Stress,** Carissa Vergeres, James Madison University

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 and thereby contribute to SIDS. Specifically, the nucleus of the raphe pallidus (NRP), which has an abundance of serotonin and GABA receptors on sympathetic nervous system (SNS) premotor neurons. Previous work proved that these neurons control a protective cardiovascular response to stress (Brown, et al., 2011) and are thought to also control thermoregulatory stress responses (Brown, et al. 2008). When oxygen levels decrease a mammal's core temperature (Tc) and selected ambient temperature (STa) will decrease as a protective response to lower metabolism and decrease oxygen consumption for survival. This hypothermic response to hypoxia (HH) may be mediated by SNS premotor neurons in the NRP. It is hypothesized that microinjection of GABA1A receptor agonists and antagonists will alter SNS premotor neuron activity at the NRP and therefore HH. Rats' Tc and STa are measured during 60 min hypoxic stress after microinjection at the NRP. The GABA1A receptor antagonist, muscimol (30mM), resulted in an exacerbated HH response vs control. These data suggest that the GABA1A receptors on the premotor neurons in the NRP play a role in the HH. Altered development of this brainstem locale and/or these receptors, may contribute to SIDS. Determining the mechanics of the NRP in the HH is essential to the etiology of SIDS and may help prevent its occurrence.

#63: **Ultrasonic Modulation of Insular Subregions Reveals Distinct Roles in the Gating of Nociceptive Stimulus,** Annie Walls, Virginia Tech

Fibromyalgia (FM), a condition affecting over 8 million individuals in the US, is characterized by widespread musculoskeletal pain and a heightened risk for major depressive disorder. FM is widely acknowledged to be driven by hyperactivity in the insular cortex, a key pain processing center whose activity is strongly correlated with pain perception. Prior work examining FM has also demonstrated a disruption in the gating of nociceptive stimuli—a process that normally leads to reduced cortical activation in response to rapidly repeated painful stimuli. These findings suggest a generalized

insular mechanism that begins to explain the development of chronic pain in FM. The insula can be parsed into the posterior insula (PI) and anterior insula (AI), which differ both structurally and functionally. The PI receives peripheral pain signals before relaying them to the AI. In the AI, these signals are integrated with expectations, awareness, and emotional responses, assigning significance to the painful stimuli. While differences between the AI and PI suggest distinct functions in pain processing, their specific roles in regulating nociceptive gating remain unexplored. To bridge this gap, we employed Low-Intensity Focused Ultrasound (LIFU)–a novel technique for modulating neuronal activity–to selectively inhibit the AI and PI during a nociceptive gating task. Each participant received 20 pairs of brief, identical heat stimuli to the dorsum of their dominant hand both before and after undergoing either an active sham or 3-minutes of LIFU stimulation to either the anterior insula (AI) or posterior insula (PI). Differences in nociceptive gating were measured via continuous electroencephalography (EEG) recordings and subjective pain ratings before and after the intervention. Through the application of LIFU, we seek to causally interrogate the specific roles of the insular subregions in pain modulation, offering insights into potential therapeutic interventions for FM and chronic pain conditions.

#64: **Developing a New Animal Model of AQP4 Autoimmunity,** Leon Zheng, Julie McVoy, Yoichiro Abe, Masato Yasui, Unsong Oh, Virginia Commonwealth University & Keio University

Preclinical research in neuromyelitis optica spectrum disorder associated with aquaporin 4 antibody (AQP4 NMOSD) is hampered 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. A limitation of the existing models of AQP4 NMOSD is that their requirement for adoptive transfer confounds interpretation of studies evaluating therapeutic effect, particularly those targeting anti-AQP4 T cells. In order to develop a new animal model of AQP4 NMOSD, a novel transgenic mouse strain containing a loxP flanked LacZ sequence disrupting exon 1 of the Aqp4 gene (AQP4.LacZ) was tested for the AQP4 expression and function with or without Cre recombinase transduction. Quantitative reverse transcriptase (qRT)-PCR, immunoblotting and immunohistochemistry confirmed the lack of Aqp4 transcript and protein in the AQP4.LacZ mouse. Analysis of transcript variant expression showed that the M1 transcript is the predominant Aqp4 transcript in wild type mice, and that both M1 and M23 transcripts are very low in the AQP4.LacZ mice. Calcein fluorescence quenching assay showed that 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. 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 the hypothesis that selectively restoring AQP4 expression in astrocytes in the AQP4 null mouse following immunization will result in the development of AQP4-directed autoimmunity and pathology in the same animal.

#65: **Neuroscience PhD Program at Virginia Tech**, Alicia Pickrell, Virginia Tech

Central Virginia Chapter of the Society for Neuroscience (CVCSN)

Virginia Tech School of Neuroscience warmly invites undergraduates, graduate students, postdocs, and faculty from all institutions in Virginia and the surrounding region to attend and present at the CVCSN 2024 conference