UCONN HEALTH

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DEPARTMENT OF NEUROSCIENCE ANNUAL RETREAT

PROGRAM & AGENDA

Tuesday, May 20, 2025
8:00 am – 5:00 pm

Registration begins at 8:00 am

The Jackson Laboratory
10 Discovery Drive, Farmington, CT

UCONN HEALTH

May 20, 2025

Dear UConn Neuroscience Community,

Welcome to the 2025 Annual Neuroscience Program Retreat! We are thrilled to hold the retreat, despite all the challenges, this time with the generous support of The Jackson Laboratory for Genomic Medicine.

We would like to draw your attention to a few important things:

1. The address of The Jackson Laboratory is 10 Discovery Drive, Farmington, CT 06032. There is free parking for those visiting from off campus. If you have assigned parking at UConn Health, please park in your assigned area.

2. **You must bring your university I.D.** to allow The Jackson Laboratory staff, security and other retreat participants to identify you.

3. The facility is air-conditioned. You might want to bring a sweater or light jacket in case you find the temperature unpleasant.

4. **Please print or carry an electronic copy of the program brochure with you to the meeting**. Only a printed agenda for the day is available at the check-in desk.

5. Free public Wi-Fi will be available courtesy of The Jackson Laboratory, no password required.

Oral presentations: Oral presentations will be held in the auditorium. Speakers **must** upload their files before the session by noon on May 19. Detailed information has been sent to the oral presenters.

Poster presentations: There will be two poster sessions. Session A will be before lunch, Session B immediately after. All posters should remain on display during both sessions. However, each presenter is required to stand by their poster during only one preassigned session. **If you are a poster presenter, please check which session you have been assigned to.**

Presentation awards: Presentations by students and postdocs will be judged for awards. You will be evaluated on the quality of your work, clarity of presentation, knowledge of the subject, and responses to questions. Winners will be announced immediately before the closing remarks.

If you have any suggestions for improving the event for next year, please let us know. We hope you will enjoy the day.

Sincerely,

Byoung-Il Bae, Ph.D.
Assistant Professor

Alice Burghard, Ph.D.
Assistant Professor

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2025 UConn Neuroscience Retreat Program

Date: Tuesday May 20, 2025

Location: The Jackson Laboratory for Genomic Medicine (10 Discovery Dr, Farmington, CT 06032)

Starts	Ends	Event	Location
8:00 AM	8:45 AM	Registration	Hall
8:45 AM	8:50 AM	Welcome address	Auditorium
8:50 AM	9:50 AM	**Symposium A** (4 oral presentations) *Moderator: Yulan Xiong*	Auditorium
9:50 AM	10:05 AM	Coffee break	Hall
10:05 AM	10:35 AM	**Outstanding alumni seminar** *Drew Kiraly, MD, PhD* *Wake Forest University School of Medicine* *"Targeting neuroimmune signaling pathways to reduce pathological opioid use behavior"*	Auditorium
10:35 AM	11:45 AM	**Poster session A** (15 posters)	Hall
11:45 AM	12:50 PM	Lunch	Cafeteria
12:50 PM	2:00 PM	**Poster session B** (15 posters)	Hall
2:00 PM	2:10 PM	Vendor recognition presentation	Auditorium
2:10 PM	3:10 PM	**Symposium B** (4 oral presentations) *Moderator: Sebnem Tuncdemir*	Auditorium
3:10 PM	3:25 PM	Group picture	Hall
3:25 PM	3:40 PM	Coffee break	Hall
3:40 PM	4:40 PM	**Keynote address** *Amita Sehgal, Ph.D.* *University of Pennsylvania* *"Unlocking the metabolic mysteries of sleep"*	Auditorium
4:40 PM	5:00 PM	Presentation of awards and closing remarks	Auditorium

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KEYNOTE SPEAKER

“Unlocking the metabolic mysteries of sleep”

Amita Sehgal, PhD

John Herr Musser Professor of Neuroscience
Director of the Chronobiology and Sleep Institute
Investigator, Howard Hughes Medical Institute
University of Pennsylvania School of Medicine

Dr. Amita Sehgal is a pioneer and leader in the field of circadian biology and sleep research, renowned for her groundbreaking work in elucidating the molecular and genetic mechanisms that govern rhythmic behavior and its impact on physiology.

Dr. Sehgal grew up in India and earned her BSc from Delhi University and MSc from Jawaharlal Nehru University in New Delhi. She then pursued her PhD with Dr. Moses Chao at Cornell University, where her interest in science deepened while studying a human neuronal growth factor. She completed postdoctoral training at Rockefeller University in the laboratory of Dr. Michael Young, a future Nobel laureate and pioneer in circadian biology. It was during this postdoctoral fellowship that Dr. Sehgal was first exposed to the study of circadian rhythms, setting the foundation for her distinguished career.

Her laboratory has been instrumental in advancing our understanding of the circadian clock and sleep, using the fruit fly *Drosophila melanogaster* as a powerful genetic model, as well as mice. Her team discovered the *timeless* (*tim*) gene, a core component of the circadian clock, and characterized how clock genes function in autoregulatory feedback loops to generate ~24-hour behavioral rhythms. Beyond circadian timing, Dr. Sehgal has pioneered *Drosophila* sleep research, identifying novel sleep genes (e.g., *jetlag*, *sleepless*) and neural circuits regulating sleep, and revealing conserved cellular functions of sleep across species. Importantly, her research also addresses the homeostatic regulation of sleep to identify genes and circuits that generate sleep need and implement recovery, linking sleep with metabolism and healthy aging.

She is a Howard Hughes Medical Institute Investigator and a member of the National Academy of Sciences and National Academy of Medicine, reflecting her exceptional contributions to neuroscience.

Homepage: https://www.med.upenn.edu/sehgallab/

OUTSTANDING ALUMNI SPEAKER

“Targeting neuroimmune signaling pathways to reduce pathological opioid use behavior”

Drew D. Kiraly, M.D., Ph.D.

Associate Professor Department of Translational Neuroscience & Department of Psychiatry

Co-Director Center for Addiction Research

Attending Physician, Department of Psychiatry
Wake Forest University School of Medicine

Dr. Kiraly is interested in the role of the immune system and gut microbiome in the pathophysiology of mental illness. He uses a combination of animal models and human research data to understand the underpinnings of psychiatric pathologies.

He graduated from the MD/PhD program at UConn School of Medicine in 2013. He did his research in the lab of Dr. Betty Eipper in the Neuroscience Department where he studied NMDA receptor signaling and addictive-like behavior in mice. After a residency in psychiatry at Icahn School of Medicine at Mount Sinai, NY, he worked as a postdoctoral fellow in the laboratory of Eric Nestler, at the same institution. He is a board-certified physician with a specialty diplomate of the American Board of Psychiatry and Neurology.

He started his current position at Wake Forest, NC in 2022 and works predominantly in his research lab, but also sees patients as an attending physician in the department of Psychiatry. He gained tenure in 2024 and has received awards for Neuroscience mentorship and excellence in research in the same year.

His Laboratory of Translational Psychiatry bridges research into the molecular causes of mental illness to patient-oriented studies investigating biomarkers of seizure activity in epilepsy patients. His research investigates the role of the neuroimmune response and the gut microbiome in the development and propagation of addictive disorders, depression, and autism spectrum disorder and he strives to use this line of research to help improve therapies for patients.

Lab website: https://www.kiralylab.com/

Symposium A: Talk No. 1

MAPK signaling regulates quiescence of cerebellar granule cell precursors and expansion of the cerebellar hemispheres

Martina Miranda[1], Qiuxia Guo[1], Kerry Morgan[1], Thomas Perry[1], James Y.H. Li[1,2]

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Granule cells (GCs) are the most abundant neuronal population in the mammalian brain, comprising more than 80% of total neurons in humans. In the postnatal cerebellum, granule cell precursors (GCPs) are highly proliferative, and dysregulation can lead to medulloblastoma, the most common type of childhood brain tumor. Despite extensive studies, the events preceding GCP differentiation remain unclear, largely because previous studies commonly treat GCPs as a homogeneous cell population. Using single-cell RNA sequencing and spatial gene expression analysis, we have identified five distinct GCP populations, including Shh-responding transit-amplifying cells and quiescent mitogen-activated protein kinase (MAPK)-responding cells, in the postnatal mouse cerebellum. Genetic fate-mapping experiments showed the quiescent subpopulation of GCPs contributed to the central lobe and the lateral-most regions of the cerebellum, which display late-onset growth, suggesting that this population acts as a reserve for the postnatal expansion of the cerebellar hemispheres. We demonstrated that quiescent GCPs were resistant to antimitotic treatment and later contributed to the replenishment of granule neurons. To address the molecular regulators underlying the transition between quiescence and transit amplification, we altered MAPK activities in a GCP-specific manner. Strikingly, constitutive expression of MAPK MEK1 in GCPs enriched quiescent stem cells and resulted in larger hemispheres, whereas blockage of MAPK activity through expression of a dominant negative downstream effector, Etv4-DN, led to a decrease in this population and smaller hemispheres. Since *Etv4/5* is essential for maintaining quiescence, we hypothesized that the cerebellum would have poorer regenerative outcomes in the Etv4-DN. We determined that *Etv4/5* is important to maintain GCP proliferation while recovering from antimitotic treatment. Together, our new findings suggest that the balance between rapid division and quiescence is crucial for the normal development and regeneration of the mammalian brain.

Support: This work was supported by grants from the NIH to James Y.H. Li (R01 NS106844 and R01 NS120556).

Symposium A: Talk No. 2

Astrocyte heterogeneity during acute demyelination is modulated by TIMP-1.

Anirudhya Lahiri, Pearl Sutter, Zaenab Dhari, Jake Lustig, Lucille Papile, Evan Lombardo, Erica Lavoie, Stephen Crocker

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Multiple sclerosis (MS) is a complex autoimmune disease in which demyelinating lesions in the central nervous system (CNS) result in progressive and accumulating disability. Understanding the underlying mechanisms of demyelination are hypothesized to generate salient information that can be used to foster remyelination, which would potentially reverse existing damage and abrogate disease progression. Astrocytes display substantial phenotypic heterogeneity and functional diversity which in response to disease, injury, or infection can elicit beneficial or detrimental functions. Tissue inhibitor of matrix metalloproteases (TIMP)-1 is an extracellular protein known to be robustly produced by astrocytes in response to demyelinating injury. It has also been cited as a potential marker of reactive astrocytes since its expression in the healthy adult CNS is typically very low to below detection. Previous work from our lab has shown that mice lacking TIMP-1 exhibit delays in developmental myelination and deficits in remyelination following injury in the adult CNS. Of note, we had also determined that astrocytosis, the reaction of astrocytes to injury, also differed in TIMP-1 knockout mice, which has led us to investigate whether TIMP-1 may have a role in determining the astrocyte diversity in the CNS. To explore this possibility, we adopted an astrocyte classification scheme using cell surface markers in which astrocytes can be sorted into five distinct subtypes (named A-E), based on the cell surface expression of three proteins CD51, CD63 and CD71. Using this flow cytometry based technique we examined the subtypes of astrocytes in the wild type (WT) and global TIMP-1 knockout ($TIMP^{KO}$) mice in response to cuprizone induced demyelination. Astrocytes isolated from unlesioned (baseline) mice, CD51-CD71-CD63- (subtype “A”) was most abundant (50% percent of total live astrocytes) followed by CD51-CD71-CD63+ (subtype “E”; 34%) and CD51+ CD71-CD63- (subtype “C”, 2.5%), and these proportions did not differ between WT and $TIMP^{KO}$ mice. However, with demyelination, the abundance of subtypes A and C significantly decreased by approximately 2 and 5-fold, respectively in the $TIMP^{KO}$ mice when compared to WT. Concurrently, subtype E was significantly elevated by 4 fold in the $TIMP^{KO}$ mice. Presently, we are developing transcriptomic profiles of these different populations to better understand how these differences may contribute to pathological outcomes. Taken together, our data suggest that the expression of TIMP-1 can influence the relative proportions of definable astrocyte subpopulations during injury which may direct the heterogeneity of reactive astrogliosis.

Support: This was supported by NIH grant R01-NS131327 to SJC.

Symposium A: Talk No. 3

Corneal Reboot: Switching Sensation Back On

Christian Tallo, Paola Bargagna-Mohan, and Royce Mohan

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The global increase in regional wars and indiscriminate use of chemical toxins, such as sulfur mustard (SM), poses a potential threat also for homeland security. Like SM, its chemical analog nitrogen mustard (NM) causes severe corneal injury and loss of sensory function. This is an unmet medical need as treatments have yet to be developed. Schwann cells ensheathe axons and promote their regeneration after injury. However, the role of corneal Schwann cells (cSCs) in injury paradigms has not been investigated before. Here we have investigated the dynamics of the corneal sensory network in the NM model of corneal injury and also aimed to illuminate potential therapeutic strategies for axonal regeneration. Towards this, we developed a micellar small molecule drug formulation, RM4404, that targets a novel druggable protein identified from our recent single cell transcriptomic study of cSCs. Transgenic mice harboring the proteolipid protein 1 promoter enhanced green fluorescent protein (Plp1-eGFP) gene to fluorescently mark cSCs, were exposed to topical 0.2% NM under sedation. Mice were treated twice daily with topical application of either RM4404 or the vehicle, Kolliphor HS-15, from days 0-7 (early treatment) or days 8-14 (delayed treatment). Corneal mechanosensitivity was assessed with an esthesiometer. Following sensory scoring, mice were euthanized, and corneal flat-mounts were immunostained with β3-tubulin antibodies for axonal visualization and digital images collected on a Leica thunder microscope were employed for quantification using FIJI Neuroanatomy software. NM injury led to significant degeneration of both cSCs and axons, both remaining markedly diminished through 14 days post-injury (P=0.0025; P=0.0283). However, early RM4404 treatment significantly enhanced the regeneration of cSCs and axons at 14 days compared to vehicle controls (P=0.0317 for cSCs, P=0.0159 for axons). This significantly enhanced regeneration for cSCs and axons versus vehicle controls was also true for the delayed treatment group (P=0.0012 for cSCs; P=0.0006 for axons). Mechanosensory testing showed sensory deficits for the vehicle and delayed treatment groups at 7 days post-injury, and this deficit remained in the vehicle-treated mice at 14 days post-injury (P=0.0015), while delayed RM4404-treated mice exhibited significant recovery of corneal sensitivity to levels comparable with uninjured controls (P=0.7627). Early RM4404-treated mice also outscored vehicle treated mice in mechanosensory testing at 7 days post-injury and 14 days-post injury (P=0.0119; P=0.0022). When comparing early versus delayed RM4404 treatment groups, cSCs and axons in the delayed treatment groups had significantly more regeneration than early (P=0.0031 for cSCs; P=0.0016 for axons). This superiority of delayed versus early RM4404 treatment was also pervasive for mechanosensory scoring (P=0.046). These findings demonstrate that cSCs are particularly vulnerable to DNA-damaging agents like NM but retain the capacity to regenerate, especially from surviving cSCs in the corneal periphery, thereby promoting trophic

support for axonal regeneration. As the delayed treatment approach yielded successful axonal regeneration and sensory recovery, this potentially offers a wide therapeutic window for clinical management of eye injuries. Importantly, this study provides the first evidence that pharmacological targeting of cSCs offers a promising avenue for therapeutic management of chemical injuries to the corneal sensory system.

Support: John A. and Florence Mattern Solomon Endowed Chair; R21EY035976; R21EY031113

Symposium A: Talk No. 4

Targeting miR-200 Family miRNAs Protects BV2 Microglia Cells Against OGD/Reoxygenation Injury by Restoring the Tgfβ2/Zeb1 Signaling Pathway

Sanjeev Kumar Yadav, PhD[1], Daylin Gamiotea Turro, PhD[1]; and Rajkumar Verma PhD[1]

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Body of the abstract: Our prior work demonstrated the upregulation of miR-200 family miRNAs specifically miR-141 & miR-200c after stroke in mice models. Moreover, we found a significant upregulation of miR-141 in stroke patient's blood samples and validated the potential of targeting miR-141 to mitigate ischemic stroke damage. In this study, we aimed to validate the upregulation of miR-200c in stroke patients plasma samples and evaluate the potential of inhibiting miR-200c alone or in combination with miR-141 to mitigate stroke damage in oxygen-glucose deprivation (OGD) in microglial BV2 cells. Total RNA (including miRNAs) was isolated from plasma samples of stroke patients and healthy controls and miR-200c expression was assessed by qPCR. In vitro, an oxygen-glucose deprivation (OGD) model in BV2 cells was used to assess the potential of inhibition using antagomiRs of miR-200c alone and in combination with miR-141 on cell viability, cytotoxicity, apoptosis and inflammation via MTT, LDH assays, and qPCR for gene expression. TargetScan web portal was used to obtain the potential targets of miR-200c/141 and explored the molecular mechanism behind this neuroprotection. miR-200c was significantly upregulated in stroke patients compared to healthy individuals, confirming the current study's translational potential. We found a gradual increase in miR-200c expression after 1h, 2h, 3h, and 4h (>5 folds) OGD followed by 24h reperfusion (R). Inhibition of miR-200c or miR-141/200c cluster after 4h OGD/24h R showed a synergistic effect on cell viability and cytotoxicity. Inhibition of the miR-141/200c increased anti-apoptotic gene Bcl2 and reduced the pro-apoptotic (Bax) and pro-inflammatory genes (Il-1β, Il6, and Tnf-α) in BV2 cells following OGD/R. According to TargetScan analysis, Smad2 is a direct target of miR-200c, Tgfb2 is targeted by miR-141, and Zeb1 is commonly targeted by both miR-200c and miR-141-3p; the expression of these genes significantly decreases following OGD/R. The inhibition of miR-141/200c restored the levels of Tgfβ2, Smad2 & Zeb1. Cluster miR-141/200c can act as a biomarker and a prominent therapeutic target for stroke treatment. In vitro, the inhibition of miR-200c alone or together with miR-141-3p protects from OGD-induced injury in microglial BV2 cells by reducing neuroinflammation and apoptosis. Overall, the Tgfβ2/Zeb1 pathway is involved in the cluster miR-141/200c mediated neuroprotection.

Support: This work was supported by NIH (1R21NS114981-01A1) and UConn OVPR (to Rajkumar Verma)

Symposium B Talk No. 1

Elucidating the role of non-imprinted genes in Dup15q syndrome neuronal phenotypes

Deepa Anjan Kumar, Tiwanna M. Robinson, Marwa Elamin, Eric S. Levine

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Dup15q syndrome is a neurodevelopmental disorder caused by maternal duplication or triplication of chromosome 15q11-q13 region and is characterized by developmental delay, seizures, and autism. Of the duplicated genes in this region, *UBE3A,* which encodes a ubiquitin ligase, is only expressed from the maternal allele in neurons and is thought to be the main contributor for Dup15q syndrome phenotypes. However, overexpressing *UBE3A* alone in mouse models fails to accurately recapitulate behavioral phenotypes, indicating a role for other duplicated genes in the region. By using patient specific neurons derived from induced pluripotent stem cell lines and isogenic CRISPR-corrected control lines, we can gain a better understanding of the pathophysiology underlying this syndrome. We have shown that human Dup15q neurons exhibit a hyperexcitability phenotype characterized by increased action potential firing frequency and altered spontaneous excitatory and inhibitory synaptic activity. Overexpression of *UBE3A* alone fails to mimic all cellular phenotypes in Dup15q, indicating a role for other duplicated genes in this region. Non-imprinted genes in this region include a cluster of $GABA_A$ receptor subunit genes (*GABRB3, GARBA5,* and *GABRG3),* and *HERC2*, another ubiquitin ligase, all of which are associated with neurodevelopmental disorders. To evaluate the roles of these genes, we have normalized the expression of *GABRB3* and *HERC2* in Dup15q neurons using antisense oligonucleotides (ASO) and performed electrophysiological recordings at early developmental timepoints. Results indicate a role for *GABRB3* and *HERC2* in increased inhibitory synaptic transmission, since normalizing the expression of these genes early in neuronal development using ASOs prevented this phenotype. Identifying the roles played by non-imprinted genes in this region is important for developing more effective therapies and for generating improved mouse models of Dup15q syndrome.

Support: Grants from the NIH and the Eagles Autism Foundation.

Symposium B: Talk No. 2

Heterozygous NLGF Model of Alzheimer's Disease – Physiological Properties of Neocortical Pyramidal Neurons

Katarina D. Milicevic[1,2], Yan M.D. Zhu[1], Violetta O. Ivanova[1], Riqiang Yan[1], and Srdjan D. Antic[1,2]

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The heterozygous NLGF mouse model of Alzheimer's disease (AD) replicates key aspects of human pathology, including late-onset amyloidosis and mild cognitive and histological changes, making it a more representative model of the human condition compared to its homozygous counterpart. In this study, we performed electrophysiological, voltage imaging, and proteomic analyses of heterozygous NLGF mice in both young (<100 days) and aged (>200 days) cohorts. We successfully combined the NLGF pathology with expression of a genetically encoded voltage indicator (GEVI) in parvalbumin-positive (PV+) GABAergic interneurons. Physiological assessments using whole-cell recordings and multi-site voltage imaging revealed that, relative to healthy littermates, heterozygous NLGF mice exhibited increased input resistance, elevated h-current, and enhanced input/output action potential firing. However, no differences were observed in spontaneous epileptiform discharges evoked by the K^+ channel blocker 4-aminopyridine (4-AP), nor in the response to the gap junction blocker carbenoxolone (CBX). Notably, in the presence of the GABAA receptor antagonist gabazine, evoked voltage transients propagated significantly faster (i.e., with shorter time delays) in the AD group compared to healthy controls. This effect was consistently observed using both GEVI- and voltage-sensitive dye imaging. Experimental groups were initially stratified by genotype and later refined based on the presence of cortical amyloid plaques, with results presented for both classifications. Proteomic profiling using UPLC-MS/MS revealed increased expression of metabotropic (mGluR-7, mGluR-3) and ionotropic (AMPA) glutamate receptors, ankyrin, the CaMKIIβ subunit, and voltage-gated channel components (P/Q-type Ca^{2+} channels and Na^+ channel β-subunits). In contrast, levels of neural cell adhesion molecule-2, Syntaxin-7, and Synaptotagmin-11 were reduced, suggesting alterations in synaptic and membrane excitability. Together, these findings provide the first integrated electrophysiological, voltage-imaging, and proteomic characterization of heterozygous NLGF mice, revealing key neurophysiological changes associated with AD pathology.

Support: This research was funded by the National Institute of Neurology and Stroke award (NS138991-01) to SDA; Cure Alzheimer's Fund award (#65539) to SDA and RY; the National Institute on Aging grant (R21-AG064554) to SDA and RY; the IBACS Grant to KDM, and Grant #4242 "NIMOCHIP" Science Fund RS to P. Andjus.

Symposium B / Talk No. 3

Two-photon calcium imaging of the mouse prefrontal cortex during a two-armed bandit task reveals functionally distinct cell types.

Alexander Mitchell[1], Katarina Kalajzic[1,2], John Stout[1], Peyton Fletcher[1], and Timothy Spellman[1, *]

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Cognitive flexibility is an important facet of higher-order brain function that allows organisms to adapt their behavior to changing circumstances. Regrettably, cognitive flexibility is impaired in many neurological and psychiatric diseases including Alzheimer's Disease and Substance-Use Disorders, and these deficits often carry and especially high burden on patients' and families' quality-of-life. One element of cognitive flexibility is response to uncertain and evolving environments, and a good experimental paradigm for measuring the ability to adapt to such environments is the 2-armed bandit (2AB), in which animals must make judgments about uncertain information to maximize reward. Many facets of cognitive flexibility are confirmed to involve the prefrontal cortex (PFC), although much debate remains over the exact identity of the cell types involved in the process of generating flexible action in the brain.
Here we report the initial findings of a study in which animals were trained to perform a head-fixed variant of the 2AB. These animals also received injections of an adeno-associated virus containing to express the calcium indicator GCaMP8f, which allowed for real-time two-photon imaging of calcium dynamics of the PFC during task performance. These images were then aligned to task performance variables across multiple sessions. This is the first instance known to use real-time calcium imaging of the PFC during the 2AB. Although this work is only in its initial stages, we can initially report success in building the data pipeline and tentative support for the claim that specific cells in the PFC maintain reward-dependent activity across multiple days. Our hope is that this work will further elucidate the role of the PFC in representing and generating cognitive flexibility and thus provide refined targets for treating neuropsychiatric disorders.

Support: Financial Support provided by the National Institute of Mental Health, University of Connecticut Health Center, and University of Connecticut.

Symposium B / Talk No. 4

Evaluation of Various Monoamine Transport Inhibitors and the Triple Reuptake Inhibitor Diclofensine in Reversing the Behavioral Effects Induced by the Dopamine Depleting Agent Tetrabenazine in Male and Female Rats

Gayle A. Edelstein, Alev Ecevitoglu, Jenelle Miller, John D. Salamone

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Major depressive disorder (MDD) is characterized by anhedonia, low mood, anxiety, and motivational dysfunctions including psychomotor slowing, apathy, avolition, fatigue, deficits in reward seeking and exertion of effort. Women are twice as likely to suffer from MDD and are more frequently affected by fatigue and psychomotor slowing compared to men. This could be due to sex differences in neurochemical signaling and circuitry which could have important implications for variations in treatment response. These variations in treatment response are often overlooked despite these known sex differences. Monoamine transport (MAT) inhibitors are the most common treatments for depression, but across various drugs there are many different profiles of action on distinct MAT proteins. SSRIs are the most widely prescribed antidepressants, but clinical and preclinical evidence indicates that they are relatively ineffective at treating motivational dysfunction symptoms. Rodent tests of effort-based choice are used as models for assessing motivational dysfunction symptoms as well as assessing potential therapeutic effects of various antidepressants. One such task is the tetrabenazine (TBZ) model on the fixed ratio 5 (FR5)/ chow choice task. The FR5/chow choice task provides rats with a choice between lever pressing five times to receive a preferred high-carbohydrate pellet vs approaching and consuming the less preferred but freely available laboratory chow. At baseline, rats exhibit high levels of lever pressing and low levels of chow intake, and TBZ administration induces a low-effort bias in which animals switch from high lever pressing behavior to low lever pressing, and low chow consumption, to high chow consumption. TBZ is used clinically for Huntington's Disease and Tardive Dyskinesia and patients often report depressive-like side effects, validating the use of TBZ on the FR5 / chow choice task to model motivational dysfunction symptoms. The present study assessed the ability of various MAT inhibitors for their ability to reverse the low-effort bias induced by TBZ in male and female rats. Additionally, a novel class of antidepressants, triple reuptake inhibitors (TRIs), which block the dopamine transporter (DAT), norepinephrine transporter (NET), and the serotonin transporter (SERT) are currently being studied as they are thought to attenuate a greater variety of symptoms while limiting the number of unwanted side effects. However, the order of relative binding affinity for DAT, NET, and SERT, to achieve the greatest therapeutic effect, remains up for debate. Previous work and current results show that compounds that inhibit DAT reverse the behavioral effects of TBZ while compounds that inhibit SERT and NET fail to do so. Therefore, it is hypothesized that for a TRI to be most effective at attenuating motivational dysfunction symptoms, the relative order of binding affinity should be DAT>NET>SERT. Results from the current study illustrate differences in effects of various MATs and a TRI as well as differences in efficacy of treatment between male and female rats.

Support: IBACS, University of Connecticut Research Foundation

Poster Session A / No. 1

Characterization of cell type-specific synaptic protein expression and function in hypocretin/orexin neurons

William Armstrong[1], Sara Bernardo[1], James Salvatore[1], Steven Yang[2], Samer Hattar[2], David C. Martinelli[3], Alexander C. Jackson[1]

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Hypocretin/orexin (H/OX) is an excitatory neuropeptide expressed exclusively in a subpopulation of neurons within the lateral hypothalamic area (LHA), which are critical regulators of the wake state. Across vertebrate phylogeny, H/OX neuron dysfunction results in defects in sleep-wake architecture. In humans, loss of H/OX signaling results in the sleep disorder narcolepsy, which is characterized by excessive daytime sleepiness, fragmented sleep and, in the most severe cases, cataplexy. Despite a large body of work describing the circuitry and behavioral role of H/OX neurons, little is known about the molecular building blocks that comprise their wake-promoting excitatory synapses. Through single-cell RNA sequencing, we identified a suite of molecular markers that, within the LHA, are uniquely and robustly expressed in H/OX neurons. One of these markers is *C1ql3*, a transcript encoding complement component 1q-like protein 3 (C1QL3), which in other regions of the brain is known to be an important synaptic organizing protein. We hypothesize that C1QL3 may also be crucial in the integrity of H/OX excitatory synapses and contribute to their role in the regulation of sleep-wake states. To address this hypothesis, we first confirmed *C1ql3* mRNA expression in virtually all H/OX neurons using fluorescence *in situ* hybridization (FISH) in both male and female adult mice. We likewise demonstrated C1QL3 protein localization in H/OX soma and axons through immunohistochemistry, including its subcellular localization relative to synaptic proteins using STED microscopy. Next, we conditionally knocked out (cKO) *C1ql3* from H/OX neurons by virally expressing Cre recombinase in the LHA of *C1ql3*$^{flox/flox}$-mVenus mice. We found that, compared to uninjected littermate controls, *C1ql3* H/OX-cKO mice had significantly diminished H/OX-IR puncta in the locus coeruleus (LC), an important wake-promoting target of H/OX neurons. This is consistent with previous work in which *C1ql3* cKO in other regions of the brain resulted in diminished excitatory synapses formed onto targets. To determine if this diminished connectivity resulted in behavioral deficits, we measured locomotor activity across the day and night using wheel-running, and observed decreased wheel-running activity specific to the dark phase, mirroring the phenotype of H/OX knockout mice. These results support our hypothesis that C1QL3 may be an important regulator of both H/OX synaptic function and wake-related behavior.

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Poster Session A / No. 2

Investigating Molecular Mechanisms of Optic Nerve Regeneration Through Spatial Transcriptomics

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Retinal ganglion cells (RGCs) are central nervous system (CNS) projection neurons responsible for transmitting visual information from the eye to the brain. Unlike embryonic RGCs, which could regenerate axons after injury, adult mammalian RGCs fail to regenerate their axons, resulting in permanent vision loss following optic nerve injury or disease. This inability to regenerate axons has been linked to both intrinsic neuronal changes and extrinsic environmental factors, particularly guidance molecules. To investigate these factors, we were able to employ spatial transcriptomics using the 10x Genomics Visium platform to spatially map the transcriptomic landscape of embryonic mouse optic nerves at developmental stages E16 and E18. We utilized the Seurat bioinformatics package in R to analyze spatial gene expression data, identifying molecules that exhibited gradient patterns. We calculated absolute correlation coefficients (|r|) and performed Spearman rank correlation tests with Benjamini-Hochberg corrections for multiple comparisons to validate significant spatial gradients. Preliminary analyses have successfully identified clear gradients of key axon guidance molecules, including Netrin-1, Slits, and ephrins. Spatial feature plots, heatmaps, and gradient vector fields were employed to visualize and confirm these gradients along proximal-distal sides of optic nerve. Understanding these spatially regulated molecular pathways during embryogenesis enhances our knowledge of embryonic regeneration and provides potential therapeutic targets for adult CNS regeneration. Future studies will extend this approach to adult injured optic nerves to uncover additional therapeutic avenues. Integration of higher-resolution single-cell spatial transcriptomics (Xenium) and proteomics will further elucidate the mechanisms underlying optic nerve regeneration.

Poster Session A / No. 3

Investigating the role of microglial KLF2 in Alzheimer's Disease

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Alzheimer's Disease (AD) is the leading cause of dementia in the population. Accumulation of amyloid beta (Aβ) plaque and phosphorylated tau neurofibrillary tangles are the classic hallmarks of the disease. Microglia are responsible for protecting the central nervous system (CNS) and maintaining homeostasis. They are found to be critical players in AD pathogenesis. Single-cell RNA sequencing and GWAS studies have revealed heterogeneity in the microglial population, with unique signature, based on the progression of the disease. In contrast to the healthy brain, microglia undergo transcriptomic and morphological changes from ramified homeostatic state to an amoeboid phagocytic state termed disease-associated microglia (DAM).

Our previous publication on microglial-specific inhibition of beta-site amyloid precursor protein cleaving enzyme (BACE1), the rate-limiting enzyme facilitating beta-amyloid production and plaque formation, has identified the transition state in samples obtained from AD patients and 5XFAD mice. This has resulted in upregulation of phagocytic genes, enhanced amyloid clearance, and improved cognitive functions in mice. Among various genes, **Kruppel-like factor-2 (KLF2)**, a zinc finger transcription factor that is downregulated in AD, exhibited an elevation in the transition state microglia compared to other states, making it a promising characteristic for transition state.

KLF2 has been widely studied in peripheral immune cells and brain endothelium but the amount of research done in microglia is limited. Similarly, very little is known about transition state microglia in the field. Here, we aimed to explore the effect of KLF2 levels on cellular functions such as phagocytosis. BV2, mouse microglial cell line, was used for over-expressing KLF2 in-vitro via electroporation of KLF2 plasmid followed by treating the cells with Hylite^{TM} Fluor 488 Aβ which showed enhanced phagocytosis. Additionally, we have used fluorescence in-situ hybridization (FISH) and a combination of FISH and immunohistochemistry (IHC) to locate KLF2 expressing microglial cells in 4-month-old 5XFAD brain slices as a part of characterization. Currently, we are at a very preliminary stage of our project. In future, we plan to perform other cellular experiments like cytokine-chemokine array, western blot analyses on various markers, cell proliferation assay and chip seq to identify the genes and pathways getting affected by the levels of KLF2. For in-vivo studies, we are developing a microglial- specific KLF2 overexpression model using FLEx (flip-excision) approach which will be used for further characterization of transition state microglia, understanding KLF2 driven pathways, and IHC, FISH, and single -cell RNAseq on isolated microglial cells to look at different aspects of the disease pathology. Behavioral experiments on these mice will allow us to determine the effect on cognitive functions as well.

Together, the proposed experiments will help us answer the unknown in the field. Our goal is to understand functional aspect of microglial KLF2, its role in the microglial transition, and experimental evidence of whether overexpression of KLF2 ameliorates AD symptoms. Future studies and more work will need to be done in developing therapies against various microglial targets regulated by KLF2 or KLF2 itself to treat AD patients.

NS074256 to R.Y., **AG046929** to R.Y.

Poster Session A / No. 4

Klf9 and experimental factor TF Exp1 promote retinal ganglion cell survival and axon regeneration in an overlapping subset of RGCs after optic nerve injury i*n vivo*

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Retinal Ganglion Cells (RGCs) are prototypical Central Nervous System (CNS) projection neurons which are responsible for transmitting visual information to the brain. As CNS projection neurons, they fail to regenerate axons after injury, leading to permanent loss of vision in humans due to many conditions including trauma, ischemia, and glaucoma. There are currently no approved clinical therapies which induce neuroregeneration. The neuronal intrinsic factors which contribute to regeneration failure remain poorly understood, and while certain pre-clinical therapies targeting the proclivity of neurons to regenerate have been explored, these generally lead to sparse long-distance regeneration from a limited subset of RGCs. Kruppel-like factor 9 (Klf9) has previously been identified as a developmentally upregulated suppressor of axon growth in RGCs, with knockdown by anti-Klf9 shRNA-expressing AAV2 leading to long distance-axon regeneration after ONC. Here, we performed single-cell RNA sequencing (scRNA-seq) of RGCs following optic nerve crush (ONC) injury and treatment with Klf9 knockdown (KD) to determine the RGC subtypes which respond to Klf9 KD and analyzed the downstream gene networks which are differentially regulated by said treatment following injury. Through our analysis of Klf9, we also identified a transcription factor (TF Exp1) which is significantly negatively correlated with Klf9 expression throughout development but whose expression rises along with Klf9 following injury. Through experimental upregulation of TF Exp1, we determined that it is sufficient to induce long-distance regeneration of axons following injury comparable to knockdown of Klf9. Further, we found that experimental manipulation of both Klf9 and TF Exp1 leads to regeneration in both intrinsically photosensitive (ip) RGCs, previously identified as a target of regenerative therapies, and XRGCs following injury.

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Poster Session A / No. 5

Characterization of the Endocannabinoid System in Human Neurons

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The endocannabinoid (eCB) system is an important physiological system that is involved in processes such as learning and memory, neuronal development, nociception, inflammation, appetite, stress and emotional regulation. This system has been shown to be dysregulated in several neuropsychiatric disorders. However, the eCB system is not widely studied as a therapeutic target for symptoms of these disorders. The role of eCBs has been well studied in rodents, however, it remains a challenge to study impairments of the system in the context of neuropsychiatric disorders that are known to have eCB dysfunction. Differences between species create a barrier to recapitulating disease phenotypes in animal models. Additionally, drug therapies in rodents have poor predictive power for efficacy in human diseases. Human embryonic and induced pluripotent stem cells provide an alternative model for studying the eCB system. They are capable of indefinite proliferation and can differentiate into a wide range of cell types, including neurons. This system is a minimally invasive human model suitable for long-term and pharmacological study. There is strong support for CB1R expression, the presence of the eCBs 2-AG and AEA, and the predominant biosynthetic and metabolic enzymes for these ligands in stem cell-derived neurons. However, studies have yet to characterize the functional consequences of eCB signaling in these neurons. We used a well-established human embryonic stem cell line (H9) to determine physiological consequences of eCB system activity. Using patch clamp electrophysiology, our preliminary data show that activation of CB1Rs can alter intrinsic neuronal properties. We have also shown that this activation could decrease spontaneous and evoked synaptic currents. Because the eCB system has been shown to be dysregulated in disorders such as autism spectrum disorder (ASD), future studies will compare how these physiological responses to CB1R activation may change in neurons derived from ASD patients and whether therapeutic modulation of this system can restore normal function during development.

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Poster Session A / No. 6

Locus coeruleus output modulates cortical taste encoding

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The noradrenergic locus coeruleus (LC) is one of the most extensive neuromodulatory systems in the brain, known for its involvement in arousal, learning, and sensory processing. Our previous research revealed a new role of the LC in feeding suppression, yet the neural circuit mechanisms underlying this effect have not been fully explored. We hypothesize that LC activity may impact feeding partly through alterations in taste perception. The primary gustatory cortex (GC), a crucial brain region for taste processing, receives dense axonal projection from the LC. To examine the impact of LC output on GC taste encoding, we conducted miniscope calcium imaging in the GC while simultaneously activating LC axons using optogenetics. We found that brief activation of LC axons produces diverse changes in GC neurons' taste-evoked responses. Principal component analysis revealed that taste stimuli are represented in a palatability-relevant manner in GC's coding space, and the activation of LC axons shifts GC taste responses towards a more aversive direction. This result suggests that increased LC output to the GC can potentially decrease perceived palatability. Our findings may provide insight into how taste processing is flexibly regulated to promote adaptive feeding decisions.

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Poster Session A / No. 7

Presynaptic Scaffold CLA-1 and Autophagy Mediator ATG-9 Cooperate to Maintain Synaptic Transmission and Neuronal Health

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Macroautophagic (hereby, autophagy) is a cellular process used to sequester damaged organelles, misfolded or aggregated proteins, and other unwanted cytoplasmic materials into double-membrane vesicles known as autophagosomes for degradation. ATG9 (AuTophaGy-related protein 9) plays a key role in this process by expanding the autophagosome membrane as the cargo gets engulfed. Abnormal ATG9 accumulation is observed in multiple neurodegenerative disorders, including Alzheimer's disease (AD) and Parkinson's disease (PD), suggesting that ATG9-mediated autophagy may play a neuroprotective role. However, the molecular mechanisms of how ATG9 supports neuronal function are poorly understood. Recent studies in *C. elegans* revealed that CLA-1, a presynaptic scaffold protein, regulates the sorting of ATG-9-containing vesicles, with loss-of-function (*lf*) mutations in *cla-1* resulting in abnormal ATG-9 accumulation in neurons. This suggests that scaffold proteins may be responsible for the regulation of ATG-9-mediated autophagy. However, the roles of CLA-1 and ATG-9 in synaptic transmission and neurodegeneration have not been investigated. In our lab, we sought to test the hypothesis that CLA-1's regulation of ATG-9-mediated autophagy is necessary for neuronal protection, and that loss of these proteins will result in functional deficiencies. In preliminary studies, we observed significant decreases in the amplitude of evoked postsynaptic currents and the frequency of miniature postsynaptic currents at the neuromuscular junctions in *cla-1(lf)* and *atg-9(lf)* mutants compared to wild type, and these synaptic phenotypes were not exacerbated in *cla-1(lf);atg-9(lf)* double mutant. In addition, we observed strong phenotypes of axonal degeneration in both *cla-1(lf)* mutants and transgenic animals with neuron-specific *atg-9* knockdown. These findings suggest that CLA-1 and ATG-9 function together to regulate synaptic transmission and maintain neuronal health. We are currently investigating whether and how CLA-1's physiological roles are related to ATG-9's roles in autophagy, and whether CLA-1 and ATG-9 function in a common molecular pathway to regulate synaptic transmission. By elucidating these mechanisms, our work may redefine roles of CLA-1 and ATG-9 in neuronal function and provide insights into the molecular mechanisms of neurodegenerative diseases.

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Poster Session A / No. 8

Elucidation of the Molecular Function of the Novel Parkinson's Disease-Causing Gene L10

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by motor symptoms such as tremors, bradykinesia, and postural instability. A hallmark of PD is the intracellular accumulation of α-synuclein. Mutations in the L10 have recently been linked to familial PD, yet its molecular function remains largely unknown. Through co-immunoprecipitation, we identified ubiquitin ligases as novel L10-binding partners. These ligases ubiquitinate α-synuclein, targeting it for proteasomal degradation. We hypothesized that L10 enhances α-synuclein degradation via these ligases. In cultured cells, overexpression of L10 promoted α-synuclein degradation and increased its ubiquitination. Yeast toxicity assays further demonstrated that L10 reduced α-synuclein aggregation in wild-type yeast, but not in ubiquitin ligase mutant strains. These findings suggest that L10 alleviates α-synuclein toxicity through specific ubiquitin ligases. Ubiquitin ligases typically adopt an autoinhibited conformation maintained by intramolecular interactions that mask their catalytic domains. We propose that L10 binding disrupts this autoinhibition, thereby activating the ligases and enhancing their ability to ubiquitinate α-synuclein. To further investigate this mechanism, we will perform in vitro ubiquitination assays. In addition, in vivo studies using ubiquitin ligase knockout and α-synuclein mutant mouse models will be performed to evaluate the physiological relevance of L10-mediated activation of ubiquitin ligases in α-synuclein clearance.

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Poster Session A / No. 9

Neuroanatomical and functional analysis of a lateral hypothalamic inhibitory projection to the DP and BNST

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The Lateral Hypothalamic Area (LHA) critically orchestrates innate behaviors by interfacing with complex neural networks. This study investigates how GABAergic neurons (LHA^{GABA}) within the LHA regulate distinct behavioral states via projections to pivotal brain regions. It aims to elucidate how LHA^{GABA} populations modulate reward-driven behaviors through connections to the dorsal pons (DP), and manage anxiety behaviors through projections to the bed nucleus of the stria terminalis (BNST). Employing neuroanatomical tracing and behavioral assays, the research enhances mechanistic insight into LHA^{GABA} functions, with profound implications for addressing neuropsychiatric conditions like PTSD and metabolic syndromes.

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Poster Session A / No. 10

CB_1 positive allosteric modulation reduces opioid – induced mechanical and cold allodynia

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Opioid-induced hyperalgesia (OIH) is a condition where prolonged use of opioids causes heightened sensitivity to various painful and nonpainful stimuli, including touch and temperature. OIH occurs via mu opioid receptor-dependent and -independent mechanisms, coinciding with drug tolerance. The endocannabinoid system, which interacts with the opioid system in pain regulation, may be a target for counteracting OIH and restoring normal pain processing. The goal of the present study was to assess the ability of the monoacylglycerol lipase (MAGL) inhibitor, JZL184, or the CB_1 selective positive allosteric modulator, ZCZ011, to reduce mechanical and cold allodynia induced by repeated morphine treatment. Under general anesthesia, female and male CD-1 mice (n=10; 5f/5m) were implanted with an osmotic minipump that delivered 60 mg/mL/day of morphine sulfate or sterile saline vehicle. Mechanical allodynia and acetone-induced cold allodynia were quantified over 7 days post-surgery. On day 7, JZL184 (40 mg/kg, i.p.), or ZCZ011 (40 mg/kg, i.p.) was administered 120 or 75 min before allodynia testing, respectively. Morphine induced both mechanical and cold allodynia, which became statistically significant, as compared to control, starting on day 4 post surgery ($p < 0.01$). The MAGL inhibitor JZL184 did not attenuate morphine-induced mechanical or cold allodynia ($p = 0.842$ and $p = 0.865$, respectively). In a separate cohort of mice, the CB_1 positive allosteric modulator ZCZ011 attenuated morphine-induced mechanical and cold allodynia ($p = 0.047$ and $p = 0.0003$, respectively). These data suggest that cannabinoid modulating drugs are a potential strategy for attenuating morphine-induced allodynia. Future studies will evaluate a wider dose range of JZL184 and ZCZ011 while investigating potential cannabinoid receptor mechanisms.

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Poster Session A / No. 11

Deficient MBP Synthesis and the Unfolded Protein Response in Senolytic-Induced Demyelination

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Aging is a primary risk factor for developing multiple sclerosis (MS). Because of this, treatments which can prevent the molecular consequences of aging, specifically cellular senescence, have garnered attention for their potential to affect disease related pathology in MS. Previous work from our lab has shown that senolytic treatment with dasatinib and quercetin (DQ) induced demyelination in C57BL6/J mice, and that *in vitro* DQ can inhibit oligodendrocyte (OL) differentiation. Because the phenotype of DQ-treated OLs was noted to resemble OPC populations in the MS brain, investigating the mechanisms by which dasatinib and quercetin impair the differentiation of OLs and CNS myelination could result in novel strategies to promote remyelination in the MS brain. Bulk RNA sequencing of naive mature OLs and OLs treated with DQ was performed to determine the effect of treatment on these cells. Comparative analyses and gene ontology determined that differentially expressed genes in DQ-treated oligodendrocytes were associated with biological processes myelination, RNA metabolism, and ER stress. Specifically, key myelination regulatory genes like FYN and MYRF were downregulated in DQ-treated OLs compared to untreated controls, indicating less propensity to myelinate. Moreover, RNA binding proteins, microtubule genes, and motor proteins essential for proper MBP transportation and translation were downregulated at the mRNA level compared to controls, suggesting that DQ-treated OLs may lack sufficient machinery to synthesize the important myelin protein. Furthermore, DQ treated OLs displayed increased expression of ER stress markers ATF4, DDIT3, and XBP1, indicating activation of the unfolded protein response (UPR). Together, this data provide evidence that activation of the UPR mechanisms can impair OL differentiation *in vitro* and CNS demyelination *in vivo*. Ongoing analyses will be exploring potential parallels between the transcriptomic signatures of DQ treated OLs with the transcriptomes of OPC and OL subpopulations identified in the human MS brain.

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Examining PTSD-like contextual memory discrimination deficits in male and female mice

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Post-Traumatic Stress Disorder (PTSD) is a mental health condition that develops after exposure to a traumatic event and causes long lasting symptoms of severe stress, and pathological changes in several brain structures including the hippocampus .PTSD researchers have focused on the hippocampus, as it plays a central role in fear processing, episodic memory, and contextual memory learning—all processes linked to PTSD symptomology. One of the most widely used rodent behavioral models to mimic PTSD-like symptoms in rodents is the contextual fear conditioning (CFC) paradigm. This paradigm behaviorally measures context discrimination by testing the encoding and retrieval of safe and unsafe contexts, an underlying memory deficiency in PTSD. In this paradigm, mice are exposed to a chamber and allowed to freely explore before one or more noxious foot shocks are administered. The mice's freezing levels, a defensive behavior in response to fear, are recorded using video software and quantified. When the animals are placed back into the same chamber, but this time without the shocks, they exhibit increased levels of freezing, indicating that the mice associated the context with shocks. However, if the mice are placed in a new environment not associated with shocks, they normally show lower levels of freezing, indicating that the mice are able to distinguish safe and unsafe contexts.

However, traumatic events, such as high intensity shocks or additional levels of stress before shock-context association, can impair context discrimination with elevated freezing in both the safe and unsafe contexts. Recent studies have expanded on this paradigm by showing that pre injecting Corticosterone (CORT), the main rodent stress hormone, creates additional levels of stress because the hippocampus has glucocorticoid receptors that bind this hormone and can elicit a long-lasting fear conditioning response, like that of high intensity shocks. To explore the effect of CORT on context discrimination and long-lasting fear responses, we tested 2 cohorts of mice, one male and one female. Mice pre-treated with CORT injections *i.p* prior to CFC showed non-specific freezing in both the shock-paired and novel contexts two weeks post-conditioning. In contrast, vehicle-injected mice and those receiving CORT without shocks exhibited context- specific freezing. Comparable exploration levels in Elevated Zero maze task indicate contextual discrimination deficits in CORT+ Shock mice rather than non-associative fear sensitization.

Preliminary analysis of cFos expression, a marker for neuronal activity, revealed that mice with discrimination deficits show biased activation in the granule cell layer of the hippocampal dentate gyrus as well as lateral entorhinal cortex, which have been previously implicated in initial encoding of salient experiences or suppression of anxiety-like behaviors. Future studies will test whether reducing activity in these areas can rescue discrimination of traumatic stress-related sensory cues.

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Poster Session A / No. 13

Failed remyelination alters demyelination-induced incontinence

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Multiple Sclerosis (MS) is a chronic, demyelinating disorder of the central nervous system (CNS), that leads to significant impairment of cognitive and motor functions. One of the earliest and often most debilitating symptoms of MS is dysfunction in control of urination (i.e. incontinence) which diminishes patients' quality of life and is the leading cause of hospitalization of MS patients. In the CNS, a "micturition circuit" communicates with bladder detrusor smooth muscles via sensory and motor neurons to regulate urination. However, the cause of this incontinence, and the role that myelination plays, particularly in the context of MS, has not been previously characterized. We have recently determined that in a cuprizone (CPZ) model of de- and remyelination, acute demyelination resulted in increased voiding behavior that was found to be subsequently reversed by remyelination. Remyelination in the CNS is mediated by oligodendrocytes and depends upon the transcription factor: myelin regulatory factor (MYRF). Mice with a conditional, oligodendrocyte-specific *Myrf* deletion have been previously reported to exhibit impaired myelination associated with impaired learning, and experience-dependent on neuronal plasticity. Based on these findings, we hypothesized that *remyelination is necessary to restore bladder control following acute demyelination*. To test this hypothesis, oligodendrocyte-specific myelin regulatory factor conditional knockout mice (*Pdgfra-cre*ER^{TM}, *Myrf*$^{fl/fl}$ (*Myrf*-cKO)) were treated with CPZ to induce demyelination and measured the voiding behaviors of these animals following the acute demyelination, and the voiding behavior of these animals was measured. We found that acute demyelination leads to increased voiding behavior in all tested genotypes. Importantly, incontinence, as measured by voiding behaviors, also improved in all genotypes following the withdrawal of CPZ, which permits remyelination to occur. These data suggest that chronic demyelination following acute demyelination is not sufficient to cause sustained incontinence. Given the merging data on the influence of myelin in learning and neuronal plasticity, we speculate that under chronically demyelinated conditions, compensatory mechanisms facilitate the recovery of functional bladder control. These data indicate that while demyelination can initiate a deficit in behavior (incontinence), a sustained loss of myelin can initiate neuroadaptive changes in the CNS, a concept clinically referred to as cognitive reserve. Further studies will delve into the nature of potential neuronal plasticity as a feature elevated by CNS demyelination.

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Poster Session A / No. 14

Sub-cellular Trafficking of P2X4 Receptors in Immune Cells Following Ischemic Stroke

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Ischemic stroke remains a major cause of death and disability in the United States, affecting nearly 800,000 individuals each year. While thrombolysis and mechanical thrombectomy offer effective acute treatments, these options are limited to only 5–15% of eligible patients, underscoring the need for alternative therapeutic strategies that support long-term neuroprotection and recovery. Following ischemic injury, neurons and glial cells release damage-associated molecular patterns (DAMPs), including ATP, which activate purinergic receptors such as P2X4, an ATP-gated cation channel expressed predominantly by myeloid cells (e.g., microglia, macrophages) and also on lymphocytes. Under physiological conditions, P2X4 receptors (P2X4Rs) are localized to endo-lysosomal compartments, where they regulate the release of neuroprotective molecules such as brain-derived neurotrophic factor (BDNF). However, during central nervous system pathologies, including ischemic stroke and neuropathic pain, surface expression of P2X4Rs is significantly upregulated, enhancing immune cell sensitivity to extracellular ATP. Despite increasing evidence implicating P2X4R in disease pathology, the mechanisms governing its subcellular redistribution during ischemic conditions remain poorly understood. Notably, P2X4R activation may also potentiate inflammasome signalling, exacerbating neuroinflammation. Previous work from our lab has demonstrated that short-term inhibition of P2X4R after stroke reduces inflammation and promotes tissue recovery, positioning P2X4R as a promising therapeutic target for modulating neuroimmune responses. We hypothesized that redistribution of P2X4R during ischemic stroke from endolysosomal compartments to the plasma membrane in immune cells modulates their activation. To test this hypothesis, we differentiated mouse primary bone marrow-derived macrophages (BMDMs) and stimulated them with extracellular ATP (50 μM) to monitor subcellular distribution of P2X4R and its effect on calcium influx. Immunofluorescence and live-cell confocal microscopy demonstrated a time-dependent increase in P2X4R on the plasma membrane following ATP stimulation. ATP-evoked Ca^{2+} influx analysis showed a robust increase in intracellular calcium levels, suggesting increased activation of immune cells. This Ca^{2+} influx was reversed by the P2X4R-specific antagonist NP-1815-PX, suggesting P2X4R's role in immune cell activation. These findings support dynamic regulation of P2X4R trafficking in immune cell activation and lay the groundwork for future in vivo studies examining stroke-induced P2X4R subcellular redistribution and its role in immune cell activation.

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Poster Session A / No. 15

The evolution of arousal micro-states during memory formation and consolidation

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Learning involves the formation of novel stimulus-specific associations in the brain. Much of the recent literature on learning and memory has revealed how these stimulus-specific associations are underpinned by the emergence of co-active neural ensembles during learning – ensembles which remain co-active during post-learning memory consolidation. However, the= process of learning is typically associated with non-stimulus specific changes in brain and= behavioral states. One crucial set of learning elicited, but not specific, changes are those in the= dynamics of arousal which have been experimentally linked to changes in pupil diameter and= CNS norepinephrinergic tone. Critically, the interaction between these non-stimulus specific= learning-elicited dynamics, and the stimulus-specific changes observed at the neural ensemble= level are yet largely unknown. Here we use 1) two-photon calcium imaging, 2) large-scale= electrophysiological recordings, 3) 3D virtual reality exploration, 4) real-time pupillometry and 5)= norepinephrine -specific fiber-photometry in awake behaving animals prior, during and after= learning of novel spatial environments. We present preliminary evidence regarding the= structure of the interaction between changes in micro-states of arousal and the spatial stimulus= specific activity of large populations of hippocampal place cells.

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Assessing the Effects of Dopaminergic Agents on Touchscreen Operant Performance in CD1 Mice

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Motivational dysfunction and deficits in exertion of effort are debilitating symptoms in several psychological disorders including major depressive disorder (MDD) and schizophrenia. It is vital to have animal models to study these effort-related symptoms to be able to assess potential therapeutic compounds. Effort-based decision-making tasks provide rodents with a choice between exerting a high level of effort for a more preferred reinforcement vs a less effortful behavior option for a less preferred alternative. These tasks have been used to identify treatments in rats that induce a low-effort bias, such as the dopamine D2 antagonist haloperidol, which shifted choice away from high-effort lever pressing in rats tested on the fixed ratio 5 (FR5)/chow choice task. Furthermore, research has focused on identifying treatments that enhance exertion of effort in motivation, such the atypical dopamine transport (DAT) inhibitor, GBR12909, which was assessed using a progressive ratio (PROG)/chow choice procedure. Although these tasks have been optimized for rat operant effort-based choice performance, it is important to develop similar procedures for mice, to have cross-species validations and open the possibility for additional studies on genetically altered mice. Bussey-Saksida touchscreen chambers developed for mice operant effort-based choice procedures provide mice with a choice between rearing up to panel press for a more preferred reinforcer (strawberry Ensure milkshake) vs. approaching and consuming a less preferred alternative (high-carbohydrate Bio-Serve pellets). Previous research has assessed the effects of haloperidol in CD1 mice on the fixed ratio (FR)1 / panel pressing choice procedure. The present study assessed the effects of haloperidol using a FR2 panel pressing/choice, and the effects of GBR12909 on PROG panel pressing/choice procedures in CD1 mice. Haloperidol administration (0.05-0.15 mg/kg) reduced panel pressing and produced a significant linear trend towards an increase in pellet intake as the dose increased. Additional analyses split the mice into high and low performance groups based on a median split of control panel pressing, which yielded a significant treatment by group effect on panel pressing. High performers showed a clear shift in effort-based choice, in which haloperidol significantly reduced panel pressing and increased pellet intake. Low performers had a significant though less robust reduction in panel pressing, and no change in pellet intake. Thus, haloperidol reduced the tendency for mice to work for food, however due to individual differences the effect of haloperidol varied on the more difficult FR2 schedule. In the second experiment, the DAT inhibitor GBR12909 (vanoxerine) increased selection of high-effort PROG lever pressing in most mice tested. These studies demonstrate that mouse procedures can be optimized in order to assess the effects of treatments that bi-directionally affect selection of high-effort options, which has implications for the use of mice in translational studies of motivational dysfunction.

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Poster Session B / No. 2

C. elegans Synaptobrevin-5 Cooperates with Synaptobrevin-1 to Mediate Synaptic Vesicle Fusion

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Synaptic vesicle fusion is mediated by the SNARE machinery, which consists of syntaxin and SNAP-25 in the presynaptic plasma membrane and synaptobrevin/VAMP in the synaptic vesicle membrane. Although mammals and *C. elegans* possess at least 7 and 6 synaptobrevin-like genes, respectively, previous studies have identified essential roles in synaptic vesicle fusion for only two mammalian genes (VAMP2 and VAMP1) and a single *C. elegans* gene (*snb-1*). Whether any of the other synaptobrevin-like genes contribute to synaptic vesicle fusion remains unclear.
In this study, we examined the potential role of *C. elegans* SNB-5 in synaptic transmission by analyzing miniature postsynaptic currents (minis) and evoked postsynaptic currents (ePSCs) at the neuromuscular junction. Compared to wild type, both mini frequency and ePSC amplitude were reduced by approximately 50% in a *snb-5* loss-of-function (*lf*) mutant and in a transgenic strain with motor neuron-specific *snb-5* knockdown. These phenotypes were fully rescued by panneuronal expression of human VAMP7, a SNB-5 ortholog, indicating functional conservation. The reductions in minis and ePSCs in the *snb-5(lf)* mutant were significantly smaller than those observed in a *snb-1(lf)* mutant, which exhibited approximately 90% reduction relative to wild type. Notably, the phenotypes of the *snb-1(lf);snb-5(lf)* double mutant were similar to those of the *snb-1(lf)* single mutant, suggesting that SNB-5 likely functions through SNB-1, possibly by modulating its expression or activity. Alternatively, synaptic vesicle fusion may involve the synergistic actions of two distinct SNARE complexes, with one containing SNB-1 and the other SNB-5. We are currently investigating these possibilities. The findings from this study may lead to a revised understanding of the roles of synaptobrevins in synaptic exocytosis.

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Poster Session B / No. 3

Delivery and augmentation of fibronectin levels promotes long distance axon regeneration and survival *in vivo*

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Trauma to the central nervous system from injury or disease, leads to the activation of glial cells, can induce infiltration of peripheral immune cells, and causes neuronal damage and cell loss. Unlike the peripheral nervous system, the CNS is unable to repair sufficiently after injury, and those axons that are damaged are unable to regenerate to their post synaptic targets. Retinal ganglion cells (RGCs) are the major projection neuron of the retina and are responsible for all the visual transmission from the eye to the brain. Upon injury to RGC axons, glial scar formation occurs, and RGCs undergo degeneration and cell death. This effect leaves those who have sustained traumatic optic neuropathies without a clinical treatment. Here, we generated a small recombinant peptide derived from fibronectin, that can interact with developmentally regulated, and injury regulated integrin receptors found on RGCs, and promote robust axon regeneration *in vivo*. This peptide not only promoted axon regeneration, but improved survival rates of RGCs after injury. Furthermore, when combined with other axon promoting treatments, we were able to improve the capacity of long-distance regeneration all the way to the chiasm. Finally, we identified the mechanism by which zymosan can promote CNS axon regeneration, through an increase in macrophage infiltration and fibronectin production. Together, this development of a small peptide, with a fundamental understanding of inflammatory driven CNS axon regeneration may provide an avenue for future therapeutics to treat CNS injury.

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Poster Session B / No. 4

Retrograde Transport in β-Glucocerebrosidase (GCase) Function and Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disease, however, there is currently no curative treatment. Mutations in *GBA1*, encoding the lysosomal enzyme β-Glucocerebrosidase (GCase), are the most common genetic risk factor for PD. Despite this well-established link, the mechanisms of how these mutations contribute to the disease are unknown. Proper membrane trafficking is vital to GCase function. GCase is synthesized in the endoplasmic reticulum (ER), transported to the Golgi, then moved to the lysosome where it is mature and active. Mutations in *GBA1* result in misfolded GCase that becomes retained in the ER, leading to loss of its function and accumulation of its substrates in the lysosome. Throughout the trafficking process, glycosylation modifications occur on GCase which are crucial for its function, resulting in various-sized forms of GCase occurring at different subcellular locations. To identify candidates for modifiers of GCase, we performed an unbiased siRNA knockdown screening. Alterations in genes implicated in retrograde transport were consistently found to shift GCase size. Further immunoprecipitation experiments revealed binding of the corresponding protein complex to GCase. Despite the important role of membrane trafficking in GCase function, little is known about its retrograde transport. As alterations in machinery required for this transport alter GCase modifications, our data suggests that GCase may undergo transport beyond its canonical anterograde processing. It is possible that this process is implicated in the ER retention of GCase and ER stress found in PD. This work further suggests protein interactions that have not yet been characterized. In future research, we will investigate the physical interaction of GCase and this complex, how GCase localization and function are affected by alterations to these proteins, and how these processes are implicated in PD pathogenesis.

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Poster Session B / No. 5

Depletion of oligodendrocytes in tamoxifen inducible mouse lines alters glial scar architecture

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Damage to central nervous system tissue from injury induces the activation and migration of several CNS cell types including microglia, astrocytes, and oligodendrocytes. Together, these cells encompass the glial scar, a protective barrier that prevents widespread damage. Although the glial scar may prevent continued damage, it also acts as an inhibitory barrier for the regeneration of CNS neurons. In our prior report, we identified that newly born oligodendrocytes at the site of injury, prematurely myelinate CNS axons, contributing to their failure to regenerate. To model CNS axon outgrowth, we utilize the optic nerve crush (ONC) injury model to completely sever all retinal ganglion cell (RGC) axons. This model recapitulates all aspects of CNS trauma as it is located within the CNS. Here we wanted to determine the effect oligodendrocytes have on glial scar architecture. We utilized the tamoxifen inducible oligodendrocyte driver CreER mouse lines to specifically excise out myelin regulatory factor (Myrf) preventing myelination and inducing oligodendrocyte cell loss. To accomplish this, we performed tamoxifen injections on 6-week-old mice, performed optic nerve crush injury to sever all RGC axons, and evaluated glial scar formation at 16-weeks of age. Although preliminary, we have identified reduction in myelin levels and CC1+ cells. Furthermore, we demonstrate differences in microglia and astrocyte localization to the glial scar injury site. Together these data demonstrate a model in which we can decrease myelin expression by oligodendrocytes, while simultaneously altering glial scar architecture.

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Poster Session B / No. 6

Epigenetic regulator DOT1L contributes to disease severity and neuropathology in globoid cell leukodystrophy

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Globoid cell leukodystrophy (GLD) is a rare and fatal demyelinating disease defined by one of many loss-of-function mutations in galactosylceramidase (*galc*). These mutations result in a rapid accumulation of psychosine (a neurotoxic lipid demonstrated to contribute to cell death), the formation of multinucleated globoid cells, cell death, and severe neuroinflammation. Disruptor of telomere silencing 1-like (DOT1L) is a histone methyltransferase that regulates the expression of various proinflammatory genes, many of which contribute to the neuroinflammation seen in GLD. We have previously reported elevated DOT1L expression in $CD11b^+$ immune cells in the brains of *twitcher* (*twi*) mice, an authentic mouse model of GLD. Additionally, single-cell RNA sequencing of microglia from *twi* mice determined that approximately one-third of all differentially expressed genes were genes known to be regulated by DOT1L. Thus, we hypothesize that DOT1L activity in the *twi* mouse brain contributes to neuroinflammation and neuropathology. To test this hypothesis, we administered a pharmacological inhibitor of DOT1L, EPZ5676 (EPZ), or vehicle (1:8 dilution of ethanol in PBS) to *twi* mice beginning on postnatal (p) day 14. We performed western blotting on protein isolated from treated or control twitcher mice for histone H3 lysine 79 dimethylation (H3K79me2), a histone mark unique to DOT1L, to verify that administration of EPZ effectively reduced DOT1L activity in the brain. To determine the impact of treatment on CNS pathology, we examined CNS myelination using transmission electron microscopy (TEM) and assessed compact myelination of axons using g-ratio analysis. This analysis revealed significantly reduced CNS demyelination in EPZ-treated animals. Clinically, mice treated with EPZ experienced a delay in symptom onset and exhibited greater weight gain than their vehicle-treated counterparts. Together, these data indicate that elevated DOT1L activity in the *twi* brain contributes to GLD neuropathology. This preliminary data therefore supports further investigation into the role of DOT1L in GLD and its potential for regulating the immunology and/or inflammatory environment of the GLD brain. Current and future studies will investigate how DOT1L influences transcriptional networks in $CD11b^+$ cells, including microglia, to define how this protein influences cellular functions and disease-related activities.

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Poster Session B / No. 7

C-X-C Motif Chemokine Ligand 14 Enhances Microglial Function – Implications for Alzheimer's Disease

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Alzheimer's disease (AD) is the most common cause of dementia worldwide (Knopman et al., 2021). Accumulation of extracellular amyloid beta (Aβ) leads to a cascade of neuropathological changes resulting in gliosis, neuroinflammation, and neuronal death (Selkoe et al., 2016). Previous work has shown that astrocyte-specific knockout (KO) of beta-site APP cleaving enzyme 1 (BACE1) significantly reduces Aβ pathology in a mouse model of AD (Zhou et al., 2023). Analysis of differentially expressed genes from single-cell transcriptomic studies on BACE1 KO astrocytes revealed a significant upregulation of C-X-C motif chemokine ligand 14 (CXCL14). CXCL14 is a secreted chemoattractant protein for peripheral immune cells such as macrophages and neutrophils (Lu et al., 2016). Interestingly, several studies have found that CXCL14 is upregulated around Aβ plaques (Chen et al., 2022; Mallach et al., 2024). However, the role of CXCL14 in AD is unknown. Here, we investigated the effect of CXCL14 on Aβ-treated microglia *in vitro*. We demonstrated that CXCL14 significantly increased microglial migration, uptake of Aβ, and phagocytic machinery involved in the degradation of Aβ. Additionally, we outline the development of a transgenic CXCL14 overexpression model, which we are crossing with 5xFAD AD mice to test the effect of CXCL14 on alleviating AD-related memory deficits and AD pathology *in vivo*. These studies could help identify CXCL14 as a novel therapeutic target for AD.

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Poster Session B / No. 8

Regulator of protein signaling 12 mediates tetrahydrocannabinol and neuropathic pain in mice

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Background: Agonist-stimulated G protein-coupled receptors (GPCRs), like the CB_1 and CB_2 cannabinoid receptors, activate intracellular signaling cascades that are normally opposed by a superfamily of "Regulators of G protein Signaling" (RGS proteins). RGS12 is one of the largest members of this regulatory protein family and is known to oppose kappa opioid receptor signaling; the analgesic effects of kappa agonists are seen to be potentiated in mice lacking *Rgs12* gene expression. The RGS domain of RGS12 associates with $G_{i/o}$ alpha subunits, an integral part of the signaling cascade following cannabinoid receptor activation. Moreover, RGS12 is expressed in many central and peripheral tissues that likely also express CB_1 and CB_2 receptors. Thus, it is plausible that RGS12 might also regulate the effects of cannabinoid drugs through its intracellular activity opposing GPCR-activated signal transduction. In the present study, we worked to determine whether RGS12 deletion affects various behavioral and physiological effects of Δ^9-THC.

Methods: Adult male and female RGS12 constitutive knockout or wildtype cagemate mice (C57BL/6J background strain) were administered Δ^9-tetrahydrocannabinol (up to 100 mg/kg, s.c. in 1:1:18 parts kolliphor:ethanol:saline) cumulatively, 50 min prior to testing for body temperature, catalepsy (bar test), and spinal antinociception (tail immersion in 56°C water).
Results: Δ^9-THC dose-dependently induced hypothermia (≥10 mg/kg), catalepsy (≥30 mg/kg), and antinociception (≥1 mg/kg) in both knockout and wildtype mouse cohorts. RGS12 deletion potentiated THC-induced spinal antinociception and hypothermia, where greater outcomes were observed in the RGS12 knockout mice.

Conclusions: RGS12 *knockout* mice showed both greater antinociception and hypothermia caused by THC. These data suggest that RGS12 modulates the effects of cannabinoid receptor agonism, likely through its intrinsic, negative regulatory activity at $G_{i/o}$-coupled GPCRs. Future work will determine the extent to which RGS12 deletion may also potentiate THC tolerance and withdrawal. Given that humans may express different RGS12 polymorphisms, these data suggest a potential physiological mechanism through which individual differences in THC effects occur.

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Poster Session B / No. 9

Generation and characterization of a novel HA epitope-tagged C1QL3 knock-in mouse line

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Synaptic adhesion molecules (SAMs) are a class of trans-synaptic proteins essential for the development, organization, and maintenance of synapses across the mammalian brain. C1QL3, a member of the C1q protein superfamily, is a presynaptically secreted SAM that may act as a molecular bridge to align various pre- and post- synaptic binding partners. Loss of C1QL3 leads to a selective reduction in excitatory synapses in several brain regions, including the basolateral amygdala (BLA) and suprachiasmatic nucleus (SCN), suggesting a critical role in excitatory circuit formation. However, there is currently no effective commercially available antibody to localize C1QL3 protein expression in the rodent brain. To investigate C1QL3 protein expression, we generated and utilized a novel C1ql3-HA epitope tag knock-in mouse line. To validate C1QL3 localization and expression in our novel mouse line, we crossed our novel C1ql3-HA mouse with a previously established genetic reporter line (C1ql3f/f-mVenus), enabling parallel visualization of protein and genetic reporter expression in known C1QL3-enriched areas. Whole-brain expression patterns of C1QL3 were visualized via optical clearing and light sheet microscopy in collaboration with LifeCanvas Technologies, revealing a widespread but regionally enriched distribution throughout the mouse brain. To identify specific neuronal populations expressing C1QL3, we performed double-immunohistochemistry for the HA-tag and various cell-type specific markers across multiple brain regions, revealing previously unreported C1QL3-expressing cell-types in the brain. Together, these approaches confirm the utility of the C1ql3-HA mouse as a powerful genetic tool for mapping C1QL3 expression and provide a foundation for future studies investigating its role in synapse regulation and circuit function in specific brain regions.

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Poster Session B / No. 10

Autism-associated ASPM variant links macrocephaly to social-cognitive deficits

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Autism spectrum disorder (ASD) is a genetic neurodevelopmental disorder with social-cognitive deficits and distinct comorbidities1. One such comorbidity is macrocephaly (“large head”), a head circumference exceeding the mean by >2 standard deviations, observed in approximately 20% of patients exhibiting severe ASD symptoms, including intellectual disability2-7. However, the role of macrocephaly in ASD pathogenesis remains unclear. The *ASPM (abnormal spindle-like microcephaly associated)* gene is a primary determinant of cerebral cortical size, with early-truncating loss-of-function (LOF) mutations being a leading cause of genetic microcephaly (“small head”)12, 13. Analysis of ASD and cancer gene databases[30, 31] reveals a few missense variants in *ASPM* that are associated with both conditions. Identifying such variants could clarify the specific role of macrocephaly in ASD.

Among these, we found that the A1877T mutation[32], a substitution of alanine with threonine at position 1877, significantly upregulates ASPM protein levels, suggesting a GOF effect. To investigate further, we generated knock-in (KI) mice carrying the orthologous mutation. We found that *Aspm* gain-of-function mutation induces macrocephaly with increased cortical volume and neuronal density, while maintaining comparable cortical thickness in mice. *Aspm* gain-of-function knock-in mice exhibit excessive embryonic neurogenesis with expanded outer radial glia, an increased excitatory-inhibitory (E-I) ratio, brain hyperconnectivity, and social-cognitive deficits with male specificity. Our results suggest that macrocephaly in ASD is not a proportional expansion of excitatory and inhibitory neurons, but a shift in the E-I ratio, independent of the expression patterns of the causative gene. Thus, macrocephaly alone can cause a subset of ASD-like symptoms. Our macrocephalic Aspm GOF mouse model will provide novel mechanistic insights into ASD and neurodevelopmental disorders in general, especially when studied in parallel with microcephalic Aspm LOF mice.

We propose that the method used to identify the A1877T GOF mutation—the discovery of genetic variants associated with both ASD and cancer through publicly available databases—can be applied to identify GOF mutations in other neurodevelopmental genes. It is likely that many genes and signaling pathways are shared between ASD and cancer, providing a basis for the identification of additional relevant genetic variants.

Our research raises two key questions. First, given that macrocephaly caused by the ASPM GOF mutation is associated with an increased E-I ratio, is microcephaly caused by the ASPM LOF mutation associated with a decreased E-I ratio? Second, although macrocephaly is observed in both male and female GOF mice, brain hyperconnectivity, social novelty deficits, and anxiety are significantly more pronounced in males. What mechanisms underlie the differential functional consequences of macrocephaly? Future studies are needed to address these questions.

Limitations. It is unknown whether the seven ASD patients carrying the A1877T heterozygous mutation exhibit macrocephaly. Although genetic studies have identified at least 23 individuals homozygous for this mutation53, no clinical data is available. Detailed information on specific genetic variants is often lacking unless they significantly impact the carrier's quality of life and are documented by physicians. In this context, human organoid studies could provide valuable additional insights.

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Poster Session B / No. 11

Characterization of *CTNNB1* Variants Associated with Autism and Cancer

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Abnormal proliferation of cortical progenitor cells underlies neurodevelopmental disorders such as autism spectrum disorder (ASD), intellectual disability (ID) and attention deficit hyperactivity disorder (ADHD). Patients with ASD often exhibit macrocephaly (“large cortex”) from 6 months into adulthood as well as microcephaly (“small cortex”), although the latter is typically present in patients with ADHD. Although excessive progenitor proliferation results in macrocephaly and insufficient proliferation results in microcephaly, the complex relationship between abnormal progenitor proliferation and neurodevelopmental disorders remains largely unknown. *CTNNB1* encodes beta-catenin, which plays an essential role in health and disease, and disruptions to this gene have been linked to ASD and cancer, a disease of excessive cell proliferation. Knock-out of *Ctnnb1* leads to microcephaly where its overexpression leads to macrocephaly, both resulting in ASD- and intellectual disability-like phenotypes in mice. At least six potential *CTNNB1* gain of function (GOF) mutations have been identified as they are associated with both ASD and cancer as well as 2 potential loss of function (LOF) mutations associated with ASD only. Using molecular cloning strategies, I generated mammalian expression vectors containing FLAG-tagged human *CTNNB1* with each point mutation. Each vector was transfected into HEK293T/17 cells and were used in a luciferase assay to measure relative gene expression levels between variants. Relative luciferase levels were calculated by taking the ratio of luciferase/renilla and relative protein levels were measured by conducting a western blot with a FLAG antibody. Of the point mutations, R376H and T551M have been previously identified as GOF and LOF, respectively, where R376H has been reported in 5 cancer cases, suggesting it is the strongest GOF mutation. In our luciferase assay and western blot, we identified R376H and T551M having increased and decreased luciferase and protein levels compared to WT 293T cells, respectively. This suggests that R376H and T551M *CTNNB1* variants may play a significant role in ASD and other neurodevelopmental disorders by resulting in abnormal progenitor proliferation through altering the overall expression of *CTNNB1*. Future studies in knock-in human cortical organoids and mice will elucidate their developmental impact.

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Poster Session 2 / No. 12

Evidence of sustained monocytosis during chronic stroke recovery Authors and Affiliations

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An acute ischemic stroke (AIS) triggers rapid mobilization of immune cells from bone marrow, leading to the infiltration of circulating immune cells into the brain. Among these immune cells, monocytes, which are pivotal in both adaptive and innate immune responses, play a crucial role in stroke injury and recovery in a temporal manner. Previous research indicates that tissue injury such as AIS causes acute monocytosis. However, it remains unclear if this upregulation persists during the recovery phase. Given the diverse roles of monocytes during post-stroke recovery, we hypothesize that their numbers remain elevated during chronic recovery. In this study, we will analyze the effect of stroke duration on monocytosis and the factors affecting this process after AIS. We recruited consecutive AIS patients (58-90 years) undergoing endovascular clot retrieval and age-matched healthy control subjects of both sexes, with informed consent obtained from all participants. Flow cytometric analysis of whole blood-derived immune cells, including monocytes, was performed at 0–2 days (acute, n=20), 3–7 days (subacute, n=20), and 65±20 days (chronic, n=14) after stroke onset. These were compared with healthy subjects (n=20 per age group). A pure monocyte population was isolated using a side and forward scatter plot with cell-surface markers, such as HLA-DR, CD45, CD11b, CD14, and CD16, along with a negative gating strategy. Plasma samples from these subjects were analyzed for various secreted cytokine levels using the Millipore multiplex array. Single-cell RNA sequencing (scRNA-seq) analysis was employed to support the evidence of monocytosis using total immune cells from these subjects. We found that the total monocyte count (% of total immune cells) progressively increased during recovery in AIS patients ($P<0.05$), which was corroborated by unbiased scRNA-seq analysis data. Cytokine analysis indicated that the levels of M-CSF and G-CSF also progressively increased, further supporting the evidence of monocytosis after stroke. Subclassification of monocyte clusters from RNA-seq data suggested that specific monocyte subsets are the major contributors to monocytosis. In summary, our data supports the evidence of continued monocytosis after ischemic stroke, indicating diverse roles for monocytes beyond the acute inflammatory response. Detailed molecular characterization of monocytes, their subpopulations, and their generic composition over time may provide novel insights into their pathophysiological roles during stroke recovery.

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Poster Session B / No. 13

Neuroanatomical mapping of tachykinin-3 receptor (*Tacr3*)-expressing neurons in the mouse brain using a novel knock-in line

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Tachykinins are a family of neuropeptides that act as both neuromodulators and neurotransmitters, regulating a wide range of physiological processes, including cardiovascular function, inflammation, and the modulation of mood and stress. Tachykinin receptor 3 (*Tacr3*) encodes NK3R, a G-protein-coupled receptor preferentially bound to by its endogenous ligand neurokinin B (NKB), which is encoded by the *Tac2* gene in rodents. Our previous work has defined a *Tac2* circuit within the central amygdala, in which the bed nucleus of the stria terminalis (BNST) and central extended amygdala (CeA) project in parallel to multiple downstream targets, including the lateral hypothalamus (LHA), ventral tegmental area (VTA), periaqueductal gray (PAG), and parabrachial nucleus (PBN). However, the anatomical distribution of *Tacr3* expressing neurons and its functional relevance within these regions remains largely unexplored. To address this, we utilized a novel *Tacr3*-Flpo;tdT knock-in mouse line to visualize *Tacr3*-expressing populations. Validation of this tool was achieved using fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) to confirm specificity and recombinase fidelity. Whole-brain *Tacr3* expression was mapped using optical clearing and light sheet microscopy in collaboration with LifeCanvas Technologies, revealing a broad yet regionally enriched distribution across the mouse brain. Subsequent IHC analysis identified cellular markers of previously unappreciated *Tacr3*-enriched neuronal populations throughout the rodent brain. Additionally, we validated BNST and CeA *Tac2* projections using retrograde viral tracing. Collectively, these approaches establish the utility of the *Tacr3*-Flpo;tdT mouse as a genetic tool for investigating *Tacr3* expression and lays the groundwork for future studies into its circuit-specific roles.

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Poster Session B / No. 14

Parallel genetic strategies to access transcriptionally distinct melanin-concentrating hormone neuron subpopulations in the lateral hypothalamus

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The lateral hypothalamic area (LHA) coordinates crucial innate behaviors through heterogenous, yet poorly understood neuronal populations. Melanin-concentrating hormone (MCH; encoded by *Pmch*)-expressing neurons, are uniquely expressed in the LHA and are key contributors to this complex physiological control. In rodents, excitation of MCH neurons leads to increases in REM sleep and increases in wake-related behaviors, such as feeding, exploration, and learning. Given its paradoxical role in promoting wake and sleep related behavioral states, it is hypothesized that MCH neurons are functionally and molecularly heterogenous. Building upon foundational anatomical and developmental studies, our single cell transcriptomic analysis of the LHA described a large set of discriminatory markers that identify MCH neuron subpopulations. These subpopulations are defined by the presence (MCH^{Tacr3+}) or the absence (MCH^{Tacr3-}) of the *Tacr3* gene, which encodes for the neurokinin-3 receptor of the tachykinin family (NK3R). We hypothesize that MCH subpopulations operate through functionally distinct, parallel subcircuits that exhibit unique neuroanatomical projections and synaptic properties, and that *Tacr3* expression may be leveraged to dissociate these subcircuits. Our project has defined a multi-pronged approach to gain genetic access to MCH^{Tacr3+} and MCH^{Tacr3-} subpopulations and their neuroanatomical projection targets through use of novel mouse lines and intersectional viruses. We first demonstrated the cell-type specificity in the proposed mouse lines and viruses through fluorescence in situ hybridization and immunohistochemistry in male and female mice. After validation of these novel genetic tools, we identified brain subregions with distinct enrichment of MCH^{Tacr3+} or MCH^{Tacr3-} axonal projections. Finally, we used slice electrophysiology and optogenetics to interrogate MCH subcircuits in vitro and to understand MCH synaptic properties in identified subregions. Ultimately, we aim to characterize the unique anatomical properties of MCH subcircuits to further our understanding of circuit-level synaptic and behavioral mechanisms mediated by transcriptionally distinct MCH subpopulations.

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Poster Session B / No. 15

Targeting Non-Canonical HSF1 Functions to Treat Early Alzheimer's Disease

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Alzheimer's disease (AD) begins with the accumulation of misfolded intracellular amyloid-beta (Aβ) oligomers well before the formation of extracellular Aβ plaques and cognitive decline. This early phase is marked by subtle but progressive cellular stress, including impaired proteostasis (the system for regulating protein folding, degradation, and clearance), mitochondrial dysfunction, and shifts in metabolic gene expression, yet current therapeutic interventions exclusively target later pathology, after irreversible damage has occurred. Identifying endogenous protective mechanisms that could act during this early phase will be critical for developing preventative therapies for AD. One candidate is Heat Shock Factor 1 (HSF1), a master regulator of transcriptional responses to cellular stress. While classically known for its role as a transcription factor, in vitro studies suggest HSF1 may also act outside the nucleus to promote cell survival, potentially through the modulation of proteostasis and other cytoplasmic functions. These non-transcriptional functions have not been tested in vivo. To explore this, we developed a novel mouse model, *Hsf1*$^{\Delta HRab}$, which contains a targeted deletion of HSF1's oligomerization domains, preventing trimerization and DNA binding while preserving domains thought to mediate cytoplasmic activity. We have experimentally confirmed that HSF1$^{\Delta HRab}$ protein is expressed in cortex and hippocampus but does not form trimers. We plan to cross *Hsf1*$^{\Delta HRab}$ mice with 5xFAD transgenic AD mice to assess whether HSF1$^{\Delta HRab}$ mitigates Aβ in vivo. Cognitive function, plaque burden, and neuronal survival will be assessed. We hypothesize that HSF1$^{\Delta HRab}$ will retain its cytoplasmic neuroprotective functions to alleviate Aβ toxicity. However, we also propose a second hypothesis: that the beneficial effects of cytoplasmic HSF1 activity may be insufficient under chronic stress, reflecting the known decline in HSF1 transcriptional activity in AD brains. Supporting this, we observe increased expression of HSF1 isoforms previously shown to localize preferentially to the cytoplasm in the hippocampi of *Hsf1*$^{\Delta HRab}$ mice, but not in the cortex. This region-specific isoform shift is accompanied by reduced expression of the anti-apoptotic gene *Bcl2* and increased expression of the pro-apoptotic gene *Cidea* in the brains of *Hsf1*$^{\Delta HRab}$ mice, but not littermate controls. In contrast, the expression of *Pgc1a*, a pro-survival metabolic gene downstream of HSF1 also known to be reduced in AD, was increased in the cortex of *Hsf1*$^{\Delta HRab}$ mice compared to the cortex of control mice, but not in hippocampus. These findings suggest that the hippocampus is especially vulnerable to transcriptionally-insufficient HSF1 over time, potentially highlighting HSF1 polymorphisms as predictive markers for chronic cell stress. Together, this work aims to define both the therapeutic potential and limits of HSF1's cytoplasmic functions in AD, particularly their role in regulating intracellular Aβ oligomers. Understanding this balance will allow us to determine whether modulating HSF1 is a viable strategy for early intervention in AD.

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THANK YOU for attending this year's retreat.

Special thanks to all trainees, judges and vendors for their time and contributions to this event.

We hope you enjoyed yourselves!

WE LOOK FORWARD TO SEEING YOU NEXT YEAR!

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