Pharmacological and Pharmaceutical Sciences Research Retreat

Friday, August 18, 2023

8:00AM – 3:45PM

The Graduate Hotel
Iowa City, IA

Hosted by:

The Pharmacological Sciences Training Program
Department of Neuroscience & Pharmacology
Department of Pharmaceutical Sciences and Experimental Therapeutics

Welcome to the **Pharmacological and Pharmaceutical Sciences Research Retreat.**

We look forward to gathering to celebrate research from multiple laboratories. Your involvement makes this retreat a success. Thank you for your continued efforts, flexibility, and dedication during these uncertain and challenging times.

Also, please save the date for next year's retreat:

- **Friday, August 9, 2024**

Executive Committee

Stefan Strack, PhD (Co-Director)
Dave Roman, PhD (Co-Director)
Michael G. Anderson, PhD
Rebecca Dodd, PhD
Ernesto Fuentes, PhD
Dawn Quelle, PhD
Florence Williams, PhD

Pharmacological and Pharmaceutical Sciences
2023 Research Retreat
Graduate Hotel

Schedule of Events
Friday, August 18th

8:00 - 8:50 AM	Registration and Poster setup Continental Breakfast	Lower level Wayne C
8:50 AM	Welcome and Opening Remarks: Stefan Strack, PhD, Co-Director of T32	Wayne A/B
9:00 - 10:00 AM	Short Talk Presentations by T32 Trainees *(8 minute talks + 2 minutes Q&A)*	Wayne A/B
9:00 - 9:10	Trevor Butler, Neuroscience Graduate Program Mentor: Jon Resch, PhD ***Leaving the Lamina Terminalis: Investigating Thirst Circuitry Downstream of the Median Preoptic Nucleus***	
9:10 - 9:20	Rachel M. Crawford, Human Toxicology Graduate Program Mentor: Ethan Anderson, PhD ***Mitochondrial toxicity of catecholamine metabolites in the heart***	
9:20 - 9:30	Logan Dawson, Biochemistry Graduate Program Mentor: Kris DeMali, PhD ***Mechanisms Coupling Actin Cytoskeletal Reinforcement and Metabolism***	
9:30 - 9:40	Short Break	
9:40 - 9:50	Alexis Ramos, Cancer Biology Graduate Program Mentor: Adam Mailloux, PhD ***Hypoxia regulates tumor antigen processing machinery***	
9:50 - 10:00	Nathan Mohar, Genetics Graduate Program Mentor: Lori Wallrath, PhD ***DNA sequence variation in SMAD7 enhances LMNA-associated muscular dystrophy in Drosophila models***	
10:00 - 10:20 AM	Break & Group Photo	Lower level
10:20 - 12:00 PM	Poster Session - Viewing/Judging	Lower level
10:20 - 11:10	- Odd numbered posters present	
11:10 - 12:00	- Even numbered posters present	

12:00 - 1:00 PM	Lunch	Wayne C
1:15 - 1:55 PM	Short Talk Presentations by T32 Trainees *(8 minute talks + 2 minutes Q&A)*	Wayne A/B
1:15 - 1:25	Angela Smith, Neuroscience Graduate Program Mentor: Kathleen Sluka, PT, PhD ***The role of spinal dopamine in the transition to chronic pain***	
1:25 - 1:35	Israel Wipf, Cell & Developmental Biology Graduate Program Mentor: Tina Tootle, PhD ***Defining the Role of Adipose Triglyceride Lipase in Drosophila Border Cell Migration***	
1:35 - 1:45	Josh Lingo, Cancer Biology Graduate Program Mentor: Dawn Quelle, PhD ***CDK4/6-MEK inhibition in MPNSTs causes plasma cell infiltration, sensitization to PD-L1 blockade, and tumor regression***	
1:45 - 1:55	Miriam McDonough, Molecular Medicine Graduate Program Mentor: Jon Resch, PhD ***Investigating the molecular mechanisms underlying aldosterone-mediated sodium appetite***	
2:00 - 2:45 PM	**Keynote Speaker** *(35-minute talk + 10 minute Q&A)* Pan Li, PhD, Assistant Professor Dept of Psychiatry and Behavioral Sciences Johns Hopkins University ***Molecular pathogenesis of spinocerebellar ataxia type 12***	Wayne A/B
3:00 - 3:30 PM	Faculty Short Talks *(12-minute talks + 3 minutes Q&A)*	Wayne A/B
3:00 - 3:15	Sarah Ferri, PhD Assistant Professor Department of Pediatrics ***The role of the basolateral amygdala in affiliative social behaviors***	
3:15 - 3:30	Kyle Flippo, PhD Assistant Professor Department of Internal Medicine ***Endocrine regulation of alcohol consumption and fear learning***	
3:30 - 3:45 PM	Awards Announcement and Closing Remarks	Wayne A/B

Keynote Speaker

Molecular pathogenesis of spinocerebellar ataxia type 12

Pan Li, PhD
Assistant Professor
Division of Neurobiology
Dept of Psychiatry and Behavioral Sciences
Johns Hopkins University School of Medicine

Dr. Pan Li is currently an Assistant Professor in Division of Neurobiology, Department of Psychiatry and Behavioral Sciences at Johns Hopkins University School of Medicine. Dr. Li obtained her Ph.D. degree in Neuroscience from the Hong Kong University of Science and Technology in 2010, and later received her postdoctoral training at Johns Hopkins Department of Psychiatry and Behavioral Sciences before she joined faculty there in 2018. The research in her lab focuses on the molecular pathogeneses of neurodegenerative disorders such as spinocerebellar ataxia types 2 and 12 (SCA2 and SCA12), and psychiatric disorders such as schizophrenia, with the goal of finding new therapeutic targets. Particular interest includes the molecular biology of disease loci containing expanded microsatellites, the contribution of antisense transcripts to pathogenesis, the role of RNA in neurotoxicity, exosome-based drug delivery, and functional characterization of schizophrenia rare variants.

Faculty Short Talks

Sarah Ferri, PhD
Assistant Professor
Pediatrics

The role of the basolateral amygdala in affiliative social behaviors

The Ferri lab is interested in the pathophysiology of neurodevelopmental and neuropsychiatric disorders. Specifically, a main area of focus is on the neural mechanisms underlying social behaviors. The ability to engage in and interpret species-specific social behaviors is integral to an organism's physical and psychological well-being. Social impairments are associated with neurodevelopmental and neuropsychiatric disorders such as Autism Spectrum Disorder (ASD) and schizophrenia, and these deficits can lead to decreased quality of life. Mouse models and multi-disciplinary approaches, including behavioral, molecular, and in vivo imaging techniques are used. Another main area of focus is sex differences in health and disease, with a focus on the role of steroid hormones during development in the increased prevalence of neurodevelopmental disorders in males compared to females.

Kyle Flippo, PhD
Assistant Professor
Internal Medicine

Endocrine regulation of alcohol consumption and fear learning

The Flippo lab is broadly interested in how the brain and endocrine system communicate to coordinate physiologic homeostasis and behavior and how these systems are disrupted in disease. In vivo approaches in mice to target specific neural-to-endocrine circuits and investigate their contribution to physiology, behavior, and disease are used.

Poster Presentations

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Abstracts

1 **Inhibition of HIF-1 signaling reduces visual impairment in a mouse model of Multiple Sclerosis-like optic neuritis**

Jeffrey J. Anders, BA[1,2], Benjamin W. Elwood, MSc[1,2], Randy H. Kardon, M.D., Ph.D.[1,2], Oliver W. Gramlich, Ph.D[1,2,3]

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[3]Department of Neuroscience and Pharmacology, University of Iowa, Iowa City, IA

Daily Acriflavine injections reduce motor-sensory impairments and increase visual system recovery in an EAE- optic neuritis model. Rescue of visual structure and function is related to the compounds immunomodulation and neuroprotection properties linked to HIF-1 inhibition and UPR blocking.

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2 **Deciphering Glucose Concentration Dynamics in Hippocampus with Novel Glucose Sensor**

[1]Tayfun Ates, MD, [1]Connor Laule, [1]Hyojin Kim, [1]Nilufer Sayar-Atasoy, Ph.D., [1]Deniz Atasoy, Ph.D.

Department of Neurosciences and Pharmacology, University of Iowa[1]

Energy homeostasis is governed by the co-operation of central nervous system (CNS) and peripheral counter regulatory hormonal systems. Since glucose is the main source of energy, dynamics of glucose utilization are one of the primary components of this harmony. In CNS, disruption of glucose exchange dynamics between cells and their environment might lead to undermined cellular functions bringing about dysregulated glucose sensing and response mechanisms. These alterations can cause metabolic irregularities resulting in many diseases such as obesity, Alzheimer's disease, and diabetes mellitus. To prevent the onset and exacerbation of prognosis of these diseases, exploring the role of glucose concentration dynamics are crucial.

Recently, significant number of studies showed that cells located in the hippocampus are involved in this imbalanced glucose sensing process. According to these findings, hippocampal cells' functioning mechanisms are dramatically affected by the impairments resulting from these diseases, which in turn results in cognitive decline and memory impairment. However, the exact mechanism of how glucose concentrations change in extracellular and intracellular conditions in these situations and consequences of these alterations have not been fully elucidated. In this study, using fluorescence lifetime sensors (iGlucoSnFR) which enable monitoring extracellular and intracellular glucose concentrations, we assessed glucose dynamics in hippocampal cells and their environment in response to various conditions, *ex vivo* and *in vivo*. These results can be used for further studies which aim to illuminate glucose dynamics in hippocampal cells in several diseases.

3 **Novel Analogs of the Natural Product Fraxinellone Protect Against Endogenous and Exogenous Toxicants**

Anna E. Bartman[1], *Mersad Raeisi*[2], *Clarence D. Peiris*[2], *Isabella E. Jacobsen*[2], *David B.C. Martin*[2,*], *Jonathan A. Doorn*[1,*]

[1]Department of Pharmaceutical Sciences & Experimental Therapeutics, College of Pharmacy, University of Iowa, Iowa City, Iowa; [2]Department of Chemistry, College of Liberal Arts & Sciences, University of Iowa, Iowa City, Iowa

Numerous insults, both endogenous (e.g., Glu) and exogenous (e.g., pesticides), compromise function of the nervous system and pose as risk factors for mediators of damage and/or later disease. For example, when extracellular levels of the excitatory neurotransmitter Glu rise due to pathological conditions (e.g., epilepsy, amyotrophic lateral sclerosis) or injury (e.g., stroke), aberrant synaptic signaling leads to excitotoxicity and subsequent oxidative stress, thought to contribute to neurodegeneration. Numerous pesticides, e.g., rotenone, are known to induce oxidative stress and cause injury to neurons. In previous reports, limonoids such as fraxinellone showed significant neuroprotective activity against Glu excitotoxicity and reactive oxygen species (ROS) production in vitro. Given these findings, a library of novel fraxinellone analogs was synthesized with the goal of identifying analogs exhibiting neuroprotection against endogenous and exogenous insults. One analog was found to be protective against Glu-mediated toxicity with a measured EC_{50} of 44 nM and 39 nM in in vitro assays using PC12 and SH-SY5Y cells, respectively. To probe the mechanism of action, a series of experiments were performed demonstrating antioxidant activity following treatment with the fraxinellone-derived analog and rapid activation of the Nrf2 pathway. Pre-treatment with the agent yielded rapid induction of antioxidant genes, namely, *GPX4*, *SOD1* and *NQO1*, as measured via qPCR. The analog mitigated Glu-mediated ROS as measured using two different fluorescent probes. Cytoprotection could be replicated using sulforaphane (SFN), a Nrf2 activator, and inhibited via ML-385, which binds Nrf2 and interferes with its binding to regulatory DNA sequences, thereby blocking downstream gene expression. Nrf2 DNA-binding activity of nuclear extracts was performed using a Nrf2 ELISA-based transcription factor assay kit. In addition, we found that pre-treatment with the thiol, N-acetyl Cys (NAC), completely mitigated SFN-mediated induction of antioxidant genes but had no effect on the activity of the fraxinellone-analog, suggesting thiol modification is not critical for its mechanism of action. In summary, our data demonstrate a fraxinellone analog to be a novel, potent, and rapid activator of the Nrf2-mediated antioxidant defense system, providing robust protection against endogenous and exogenous insults. Given that, further development of this class of analogs may yield promising therapeutic strategies to address pathogenic or neurodegenerative conditions, such as those involving Glu excitotoxicity and excessive production of ROS.

4 **Iron scavenging and suppression of collagen cross-linking underlie antifibrotic effects of carnosine in the heart with obesity**

Islam Berdaweel[1], T. Blake Monroe[1,5], Amany A. Alowaisi[1], Jolonda C. Mahoney[1], Kaitlyn A. Berns[1], Dylan Gao[1], Jared M. McLendon[2,3], Ethan J. Anderson[1,3,4]*

[1]Department of Pharmaceutical Sciences and Experimental Therapeutics, College of Pharmacy, [2]Department of Internal Medicine, [3]Abboud Cardiovascular Research Center, and [4]Fraternal Order of Eagles Diabetes Research Center, Carver College of Medicine, Univ of Iowa, Iowa City, IA
[5]Current address: Department of Biochemistry, University of Minnesota, Minneapolis, MN

Oral consumption of histidyl dipeptides such as l-carnosine has been suggested to promote cardiometabolic health, although therapeutic mechanisms remain incompletely understood. We recently reported that oral consumption of a carnosine analog suppressed markers of fibrosis in liver of obese mice, but whether antifibrotic effects of carnosine extend to the heart is not known, nor are the mechanisms by which carnosine is acting. Here, we investigated whether oral carnosine was able to mitigate the adverse cardiac remodeling associated with diet induced obesity in a mouse model of enhanced lipid peroxidation (i.e., glutathione peroxidase 4 deficient mice, $GPx4^{+/-}$), a model which mimics many of the pathophysiological aspects of metabolic syndrome and T2 diabetes in humans. Wild-type (WT) and $GPx4^{+/-}$ male mice were randomly fed a standard (CNTL) or high fat high sucrose diet (HFHS) for 16 weeks. Seven weeks after starting the diet, a subset of the HFHS mice received carnosine (80 mM) in their drinking water for duration of the study. Carnosine treatment led to a moderate improvement in glycemic control in WT and $GPx4^{+/-}$ mice on HFHS diet, although insulin sensitivity was largely unchanged. Interestingly, while our transcriptomic analysis revealed that carnosine therapy had no significant impact on global gene expression in the heart, carnosine substantially upregulated cardiac GPx4 expression in both WT and $GPx4^{+/-}$ mice on HFHS diet. Carnosine also significantly reduced protein carbonyls and iron levels in myocardial tissue from both genotypes on HFHS diet. Importantly, we observed a robust antifibrotic effect of carnosine therapy in hearts from mice on HFHS diet, which further *in vitro* experiments suggest is due to carnosine's ability to suppress collagen-cross-linking. Collectively, this study reveals antifibrotic potential of carnosine in the heart with obesity, and illustrates key mechanisms by which it may be acting. **Keywords:** Obesity, cardiac fibrosis, lipid peroxidation, carnosine, metal chelation, carbonyl stress, collagen

5 **Synchronized proinsulin trafficking reveals delayed Golgi export accompanies β-cell secretory dysfunction in rodent models of hyperglycemia**

Cierra K. Boyer [1,2], Casey J. Bauchle[2,3], Jianchao Zhang[4], Yanzhuang Wang[4,5] and Samuel B. Stephens[2,3,6]

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[2]Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa City, IA, USA
[3]Department of Internal Medicine, Division of Endocrinology and Metabolism, University of Iowa, Iowa City, IA, USA
[4]Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI, USA
[5]Department of Neurology, School of Medicine, University of Michigan, Ann Arbor, MI, USA

The pancreatic islet β-cell's preference for release of newly synthesized insulin requires careful coordination of insulin exocytosis with sufficient insulin granule production to ensure that insulin stores exceed peripheral demands for glucose homeostasis. Thus, the cellular mechanisms regulating insulin granule production are critical to maintaining β-cell function. In this report, we utilized the synchronous protein trafficking system, RUSH, in primary β-cells to evaluate proinsulin transit through the secretory pathway leading to insulin granule formation. We demonstrate that the trafficking, processing, and secretion of the proinsulin RUSH reporter, proCpepRUSH, are consistent with current models of insulin maturation and release. Using both a rodent dietary and genetic model of hyperglycemia and β-cell dysfunction, we show that proinsulin trafficking is impeded at the Golgi and coincides with the decreased appearance of nascent insulin granules at the plasma membrane. Ultrastructural analysis of β-cells from diabetic leptin receptor deficient mice revealed gross morphological changes in Golgi structure, including shortened and swollen cisternae, and partial Golgi vesiculation, which are consistent with defects in secretory protein export. Collectively, this work highlights the utility of the proCpepRUSH reporter in studying proinsulin trafficking dynamics and suggests that altered Golgi export function contributes to β-cell secretory defects in the pathogenesis of Type 2 diabetes.

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6 **Leaving the Lamina Terminalis: Investigating Thirst Circuitry Downstream of the Median Preoptic Nucleus**.

Trevor Butler, Yuxi Li, Alysia Berns, Jon Resch, PhD

Neuroscience and Pharmacology Department

Water loss occurs in a variety of physiological processes including respiration, sweating, and urine production. To counteract this loss, the brain responds to interoceptive signals of dehydration, such as reductions in peripheral blood pressure and increases in circulating angiotensin II or osmolyte concentrations by driving thirst, the motivation to seek out and ingest water. Thirst signals are generated in the lamina terminalis (LT) which consists of three interconnected brain regions, the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO), and the median preoptic nucleus (MnPO). The SFO and OVLT are circumventricular organs that lack a blood-brain barrier and can therefore directly monitor the bloodstream for circulating signals of dehydration. The SFO and OVLT then send excitatory projections to the MnPO to drive thirst. However, the efferent targets of MnPO thirst signals are poorly understood. Anterograde tracing of MnPO neurons has revealed the paraventricular hypothalamus (PVH), paraventricular thalamus (PVT), and lateral hypothalamus (LH), among other regions, as MnPO downstream targets. Optogenetic stimulation of glutamatergic MnPO terminals projecting to the PVH, PVT, and LH induce drinking behavior, suggesting neurons in these regions are involved in thirst circuitry. However, individual MnPO neurons send collateral projections between the PVH, PVT, and LH, therefore, it is unclear whether MnPO projections to some sites drive thirst through backpropagating action potentials driven by ChR2 terminal stimulation to collaterals. My research investigates whether MnPO projections to the PVH, PVT, and LH are necessary for thirst through optogenetic silencing of localized MnPO projection fields targeting the PVH, PVT, or LH. Additionally, my experiments combine chemogenetic tools with recombinase driver mice to directly and specifically stimulate neuronal subpopulations in either the PVH, PVT, or LH to investigate their sufficiency to drive thirst.

7 **Behavioral and biochemical characterization in a Mouse Model of Jordan's Syndrome**

Chunling Chen, BS[1], Chian Ju Jong, PhD[1], Ronald A Merrill, PhD[1], Marisol Lauffer, MS[1], Arminja Kettenbach, PhD[2], Gail Healey, BS[1], Yufang Kong, BS[1] and Stefan Strack, PhD[1]

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[2]Department of Biochemistry and Cell Biology, Geisel School of Medicine at Dartmouth, Lebanon, NH

PP2A is one of the major Ser and Thr phosphatases and constitutes up to 1% of total protein in mammalian cells. PP2A is a trimeric holoenzyme consisting of a scaffolding A subunit, a regulatory B subunit and a catalytic C subunit. A de novo single nucleotide mutation in PPP2R5D, which encodes a regulatory subunit of a PP2A, generates a heterozygous dominant missense variant resulting in a neurodevelopmental disorder characterized by intellectual disability, autism spectrum disorder, recurrent seizures, hypotonia, macrocephaly and other features. We used a CRISPR/Cas9-mediated gene editing to introduce the E198K/E200K/E420K mutation, in the murine Ppp2r5d locus to generate three knock-in mice model of this disease, which are three most common mutations in human. To evaluate if these mice model can recapitulate most of the common symptoms of patients, we used standard behavior batteries to assess developmental milestones and adult behaviors, as well as CT scan to capture early postnatal changes in brain development. Our data revealed that mice harboring the E198K, E420K and E200K recapitulate cardinal symptoms and severity of Jordan Syndrome and may be a good model for studying the effects of the disease cause alleles. To evaluate the biochemical phenotype in mice, we screened for differentially phosphorylated proteins by immunoblotting sub dissected brain regions with phospho-specific antibodies. Our results revealed that mice harboring mice harboring the E198K, E420K mutation has an overall less phosphorylation of PKA substrates. To investigate the pathologic mechanism, we use western blot and immunochemistry to look at the neurogenesis of hippocampus, our results revealed that our mice have a dysregulation of neogenesis.

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8 **Dopamine receptor signaling pathway as a pharmaceutical target for extending micropore closure timeframes across diverse skin types**

Valeria Cota[1]*, Martine Dunnwald[2], Nicole K. Brogden[1]. [1]University of Iowa College of Pharmacy, Department of Pharmaceutical Sciences and Experimental Therapeutics, Iowa City, IA. [2]University of Iowa Carver College of Medicine, Department of Anatomy and Cell Biology, Iowa City, IA

PSET, Human Toxicology

Microneedles are micron scale projections which form transient micropores in the skin, allowing for improved drug delivery by bypassing the strong stratum corneum layer. As drug delivery stops following micropore closure, it is critical that these micropores or "microwounds" remain open for consistent drug delivery. Dopamine is involved in cutaneous wound healing and is secreted upon barrier disruption. Dopamine accelerates or inhibits wound healing and barrier function, depending on the class of receptor it acts on. Dopamine D2-like receptor (DRD2) agonists accelerate epidermal wound healing while D1-like receptors (DRD1) delay it. Due to delayed micropore closure in skin of color *in-vivo*, we compared dopamine levels across skin types by using both tristimulus colorimetry and a melanin platereader assay to classify tissue pigmentation. Utilizing high pressure liquid chromatography with electrochemical detection (HPLC-ECD) we investigated dopamine levels in whole tissue and keratinocyte extracts. Our data show that dopamine levels are higher in samples from darker vs lighter donors. Notably, whole tissue dopamine levels in darker skin were higher than lighter skin: 24.98 ± 11.65 ρmol/mg (L* <40) and 4.47 ± 4.55 ρmol/mg (L* >50). To determine the significance of the dopamine pathway to keratinocyte migration, we used *in vitro* scratch wound assay. Using foreskin-derived keratinocytes from diverse donors, we showed reduced scratch closure (% wound closure) at 12 hrs ($p < 0.05$) and 24 hrs ($p < 0.01$) following incubation with DRD1 agonist SKF-38393. Pretreatment with DRD1 antagonist SCH-23390 blocked the response, resulting in closure similar to DMSO vehicle control. DRD2 agonists show an opposite, yet non-significant, trend with DRD2 antagonist L-741,626 delaying micropore closure at 12 and 24 hrs ($p > 0.05$). While previous studies show dopamine receptor signaling may accelerate wound healing, our data show that intentional delay in epidermal wound closure could extend dermal drug delivery.

9 **Monoamine oxidase disrupts mitochondrial fatty acid oxidation in the heart**

Rachel Crawford B.S.[1], Ran Huo B.S.[2], Ethan Anderson PhD[2,3]

[1]Interdisciplinary Graduate Program in Human Toxicology, University of Iowa
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Abstract: Catecholamines are important signaling molecules in the heart for regulating heart rate and contractility, but their intracellular metabolism by monoamine oxidase (MAO) has recently emerged as a source of cardiotoxicity. Oxidative deamination of catecholamines by MAO generates reactive oxygen species and catecholaldehydes which have consequential affects on mitochondrial metabolism and redox balance. Studies show that inhibition of MAO in heart leads to beneficial effects in cardiac injury and diseases including ischemia/reperfusion, arrythmogenesis, and diabetic cardiomyopathy. However, the mechanisms underlying these benefits are not clear. MAO is located on the outer mitochondrial membrane and as such, its activity and oxidative products are likely to react within the mitochondria and could disrupt oxidative phosphorylation and substrate oxidation. To better understand the effects of MAO-mediated catecholamine metabolism on mitochondria, we measured the effects of norepinephrine (NE) on mitochondrial respiration and ATP production in permeabilized left ventricular myofibers and isolated mitochondria from healthy young adult (8-15 weeks) wild-type (WT) and cardiomyocyte-specific MAO-A knockout (cMAO-A$^{-/-}$) mice. Pyruvate (carbohydrate) and palmitoyl carnitine (fatty acid) were substrates provided to support respiration, and increasing amounts of NE were added to the respiring mitochondria to activate MAO metabolism. Maximal palmitoyl-carnitine supported respiration was increased by 1.6 fold in permeabilized left ventricular myofibers from cMAO-A$^{-/-}$ mice compared with WT, while in isolated mitochondria the differences were not as robust. No differences in pyruvate supported respiration were observed between WT and cMAO-A$^{-/-}$. Interestingly, adding NE appears to blunt palmitoyl carnitine supported respiration (but not pyruvate) in isolated mitochondria from WT (but not cMAO-A$^{-/-}$) mice, although more work is needed to confirm significance of these findings. Further investigation of the mechanisms by which MAO activity and catecholamine metabolism may impact fatty acid oxidation and in turn, how this may be involved in the pathological role of MAO in heart is ongoing.

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10 **Mechanisms that Couple Actin Cytoskeletal Reinforcement and Metabolism**

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Cells interact with their environment by sensing internal and external forces via surface adhesion receptors, triggering actin cytoskeletal changes and growth of the adhesion complex, a process called actin cytoskeletal reinforcement. Given the ATP-intensive nature of this process, we hypothesized the existence of mechanisms that coordinate force transduction with energy production. Our lab recently identified a role for AMP-activated protein kinase (AMPK) in this process. In response to force, AMPK is activated at the E-cadherin adhesion complex, promoting glucose uptake, ATP production, and actomyosin contractility. This suggests an intimate link between mechanical force and cellular metabolism. In this study, we focus on phosphofructokinase-1 (PFK-1), a major enzyme in glycolytic regulation. We propose that force can trigger the release of PFK-1 from the actin cytoskeleton, thus enhancing its enzymatic activity and promoting energy production for actin reinforcement. Our project focuses on two aspects: (1) to investigate the functional and localization changes in PFK-1 upon force-induced liberation from the actin cytoskeleton, and (2) to unravel the precise mechanism of PFK-1 release. This work will provide insights into the regulation of cellular metabolism in response to mechanical forces and may uncover new therapeutic targets for conditions involving disrupted cytoskeletal reinforcement and metabolism.

11 **Characterization of *PRKAR1B* mutations associated with neurodegenerative and neurodevelopmental disorders**

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While neurodevelopmental disorders (**NDDs**) result from improper development of neurons in the central nervous system, our understanding of the mechanisms underlying these disorders is limited. NDDs stem from *de novo* mutations, and patients with NDDs typically have mutations in proteins such as kinases and transcription regulators. Thus, there is a ***critical need*** to understand the role *de novo* mutations play in NDDs to develop effective treatments for these disorders. Recent studies have identified a novel NDD called Marbach-Schaaf Neurodevelopmental Syndrome (**MASNS**) associated with the *de novo* mutations Q167L, E196K, and R335W in *PRKAR1B*. *PRKAR1B* encodes the regulatory R1β subunit of protein kinase A (**PKA**). Children with MASNS demonstrate global developmental delay, speech delay, and motor skill deficits. PKA R1β has also been associated with a neurodegenerative disorder, where the L50R mutation was found in individuals with dementia and parkinsonism. These findings suggest that PKA plays a crucial role in the pathogenesis of both neurodevelopmental and neurodegenerative disorders. Using HeLa cells, we found that L50R prevents R1β from colocalizing with both AKAP1 (a protein that targets R1β to mitochondria) and smAKAP (a protein that targets R1β to the plasma membrane). Using HEK293T R1α knockout (**KO**) cells, we found that, following stimulation with either 8cpt-cAMP (a non-hydrolysable cAMP analog) or isoproterenol (a β-adrenergic receptor agonist), R335W severely reduces PKA-mediated transcriptional activity. Recent data using plasmids encoding both short hairpin RNA (**shRNA**) to knockdown endogenous R1β and RNA interference (**RNAi**)-resistant R1β showed that the L50R and MASNS mutations reduce PKA-mediated transcriptional activity following isoproterenol stimulation in HEK293T R1α KO cells. These findings reveal that L50R promotes neurodegeneration by impairing both PKA-mediated transcription and PKA localization. On the other hand, the MASNS mutants hinder neurodevelopment solely by impairing PKA-mediated transcription. This suggests that PKA-mediated neurodegeneration and neurodevelopment proceed via distinct mechanisms.

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12 **Effects of neonatal gonadal hormones of Autism Relevant Phenotypes**

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Social behavior is seen across almost all living species and when these behaviors are disrupted it can result in decreased chances of survival, reproduction, and quality of life. Social deficits are exhibited in neuropsychiatric and neurodevelopmental disorders, and are a core symptom of autism spectrum disorder (ASD). ASD affects 1 in 36 children and 4 times as many males as females. Within an ASD diagnosis, males are more likely to exhibit social impairments. The mechanism of this robust sex bias is not well understood. Here, we used neonatal injections of gonadal hormones as a novel experimental system to disrupt sex-specific developmental pathways in mice to determine their effects on behaviors relevant to ASD. We found that testosterone administration on the day of birth, which is equivalent to late gestation in humans, induces male-specific deficits in social approach and fear memory. These deficits were only present when the injection was given on the day of birth and not at postnatal day 18. Furthermore, while testosterone injected on the day of birth did cause social and contextual fear conditioning deficits, it did not affect anxiety-like behavior on an elevated zero maze or body weight over development. Administration of D-cycloserine, a NMDAR partial agonist, which has been shown to ameliorate social deficits preclinically, alleviated the testosterone-induced social and fear deficits. Surprisingly, estradiol given on the day of birth led to female specific social deficits. Currently we are investigating the mechanisms of these sex specific vulnerabilities to social and fear deficits. These findings will aid in advancing the current understanding of how the brain is susceptible to social impairments and help identify novel treatment targets.

13 **Targeting adenylyl cyclase type 8, Orai1, and stromal interaction molecule 1 in triple-negative breast cancer**

Moana E. Hala'ufia, and David L. Roman

Triple-negative breast cancer (TNBC) is one of the most aggressive forms of breast cancer that hormonal therapies cannot treat. Current standards of care include surgical removal of the tumor and aggressive chemotherapy. Consequently, there is an urgent need for new, molecular-based therapies to treat these patients effectively[1,2]. On a molecular level, two proteins facilitate finely tuned calcium entry: Orai1, a pore-forming calcium channel, and stromal interaction molecule 1 (STIM1), a calcium sensor embedded in the ER membrane. Upon ER calcium store depletion, STIM1 undergoes a conformational change to bind to Orai1, which triggers extracellular calcium entry to achieve homeostasis[3]. Recently, it has been discovered that a third protein is implicated in this process: adenylyl cyclase type 8 (AC8)[4]. The N-terminus of AC8 can bind to the N-terminus of Orai1 to prolong calcium influx, which ceases upon Orai1 phosphorylation[4]. In normal breast epithelial cells, AC8 is expressed at a basal level and does not interfere with Orai1-STIM1 calcium entry. However, in TNBC cells, AC8 is grossly overexpressed so that all Orai1 channels become bound[4,5]. As AC8 binds to Orai1 on its phosphorylation site, the Orai1 channel cannot be inactivated, leading to excess calcium influx and adverse cellular effects such as proliferation and migration.

We hypothesize that if we inhibit the interaction between AC8 and Orai1 with a therapeutic small molecule, we will observe a decrease in proliferation in TNBC cells. We also expect that inhibiting the interaction between Orai1 and STIM1 will produce similar effects. Ultimately, we aim to screen a library of small molecules against our protein-protein interactions to find therapeutic inhibitors. We will use the NanoBiT system to study each different protein-protein interaction. Briefly, the NanoBiT system tags each protein with a split luciferase, allowing for the generation of a luminescent signal upon protein binding. Using this method, **we have found** that the interaction between STIM1 and Orai1 is strictly governed by calcium, whereas the interaction between AC8 and Orai1 is more constitutive. Interestingly, we found that forskolin, an AC8 activator, prevents AC8 binding to Orai1, as evident through loss of luminescence. This suggests that AC8 must be inactive to interact with Orai1 and that phosphorylated Orai1 cannot bind to AC8. These findings have allowed us to conduct Z-Score studies to prime for initial screening, which fall within a 0.6-0.8 range for each protein-protein interaction. To date, we have accomplished screening two libraries of small molecule compounds; NCI Diversity Set VI and NCI Approved Oncology Set X, which will be extended further to other diversity libraries. In addition, we plan to test any inhibitors in different TNBC cell lines (MDA-MB-231 and MDA-MB-157) to observe effects on cancer cell migration. **In conclusion**, understanding the unique interplay between AC8, Orai1, and STIM1 is critical to understanding how to prevent TNBC cell proliferation and migration.

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14 **Cerebellar nuclei neurons projecting to the lateral parabrachial nucleus modulate classical fear conditioning**

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Multiple brain regions are engaged in classical fear conditioning. Despite evidence for cerebellar involvement in fear conditioning, the mechanisms by which cerebellar outputs modulate fear learning and memory remain unclear. We identify a population of deep cerebellar nuclei (DCN) neurons with monosynaptic glutamatergic projections to the lateral parabrachial nucleus (lPBN) ($DCN^{\rightarrow lPBN}$ neurons) in mice. While optogenetic suppression of $DCN^{\rightarrow lPBN}$ neurons impairs auditory fear memory, activation of $DCN^{\rightarrow lPBN}$ neurons elicits freezing behavior only after auditory fear conditioning. Moreover, auditory fear conditioning potentiates DCN-lPBN synapses and subsequently, auditory cue activates the DCN-lPBN pathway after fear conditioning. These findings demonstrate that cerebellar nuclei modulate auditory fear conditioning via transmitting conditioned stimuli signals to the lPBN. Collectively, our findings suggest that the DCN-lPBN circuit is a part of neuronal substrates within interconnected brain regions underscoring auditory fear memory. **Keywords:** cerebellum, cerebellar non-motor function, parabrachial nucleus, auditory fear conditioning, synaptic plasticity, fear memory network

15 **Developing a Novel Mouse Model for "Secretome" Exploration**

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In the Potthoff Lab we are interested in discovering novel endocrine factors. Typical approaches to do this include shotgun proteomics of cell culture media or serum in response to treatment of choice, or RNA-sequencing and bioinformatic analysis for peptides encoded by transcripts that are predicted to be secreted. The technical challenges of mass spectrometry analysis of serum are many: not knowing which organs secreted the protein of interest, if the secreted proteins in cell culture media are representative of how an in vivo system operates, and analysis of whole serum is itself a large task for even the most skilled proteomics cores. RNA-sequencing for peptides that include a secretory signal sequence is also limited by the post-transcriptionally regulated parts of protein assembly and will always need further validation after parsing through genes that may encode secreted proteins.

We have recently begun validation with a novel in-vivo proximity labeling system that tags proteins traveling through the secretory pathway at the endoplasmic reticulum with a biotin molecule. This system can be targeted to a specific cell type or organ. After the system has been induced via viral transduction of our ER-targeted biotin enzyme (TurboID) – we can pulldown proteins that have been labeled with streptavidin binding and send the purified proteins for LC-MS analysis at our proteomics core. We are generating a Cre dependent mouse model to utilize this system in hopes of discovering peptides that are enabling metabolic crosstalk between various organs (brain, fat, liver, muscle) under nutrient sensing or stress-induced conditions.

16 **The role of hypothalamic astrocytes on appetite regulation**

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While astrocytes are widely distributed throughout the brain regions implicated in energy homeostasis, their role is not fully understood. In this study, we sought to understand the role of hypothalamic astrocytes in appetite. We hypothesized that hypothalamic astrocytes modulate AgRP neurons to regulate feeding behavior. To assess the role of hypothalamic astrocytes on AgRP neurons and appetite, we performed fiber photometry in combination with chemogenetics for GFAP expressing astrocytes. We found that acute manipulation of GFAP expressing hypothalamic astrocytes rapidly alters AgRP neuron activity and feeding behavior in different metabolic states. Moreover, we found that hypothalamic astrocytes have calcium activity that is responsive to various food related cues. Our findings suggest that hypothalamic astrocytes may affect appetite and AgRP neuron activity.

17 **Differentially methylated regions in *ARHGEF38* in individuals with bipolar disorder and history of suicide attempt**

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Bipolar disorder (BD) is characterized by episodic mood swings ranging from depression to mania. Approximately 2.6% of the US adult population is diagnosed with BD, with about half attempting suicide in their lifetime. As one out of every five individuals with BD die by suicide, it is imperative that suicidal behavior within BD be studied. Previous work investigated DNA methylation (DNAm) changes associated with suicide in BD. A differentially methylated region (DMR) within the Rho guanine nucleotide exchange factor 38 (*ARHGEF38*) gene was found when the epigenome from brain samples of 50 BD individuals was interrogated, with 23 suicide decedents and 27 who died due to other causes. When comparing BDS to non-psychiatric controls (CON), a differentially methylated region with four CpG sites in *ARHGEF38* was significantly hypomethylated by 23.4% in suicide decedents (Gaine et al, 2019). This finding was then validated with pyrosequencing. Rho guanine nucleotide exchange factors such as *ARHGEF38* accelerate Rho-GTPase activity, which has been associated with suicidal attempt and a decrease in *ARHGEF38* DNAm in the brain may alter suicidal behavior in BD.

In this study, we investigated the *ARHGEF38* gene in whole blood samples from BD individuals with and without a history of suicide attempt (BDNS). Using DNAm arrays, we quantified DNAm in both coding and non-coding regions (BDS, n=48; BDNS, n=47). Pyrosequencing was used to quantify DNAm at the *ARHGEF38* CpG sites previously identified in Gaine et al. in both BD (BDS and BDNS) and CON groups (n=46). This expands prior work by investigating other types of suicidality, specifically suicide attempt, and the comparability between brain and blood samples. Replicating this finding in independent suicide cohorts, will further establish the association with suicide, and move towards the goal of identifying individuals at risk for suicidality.

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18 **Modulation of Stress Through an Ascending Norepinephrine-Melanocortin Circuit**

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Stress caused by physiological or psychological factors imparts tremendous health burdens. Despite these health risks, neural underpinnings of stress are not fully resolved. Catecholaminergic tyrosine hydroxylase (TH) expressing neurons in the nucleus tractus solitarius (NTS) are critically involved in stress, however their downstream mediators are poorly characterized. A recent study from our lab showed that NTS^{TH} predominantly inhibit melanocortin-4 receptor (MC4R) expressing neurons in the paraventricular hypothalamus (PVH) and is important for hypoglycemic hunger, a form of physiological stress. Here, we hypothesized that adrenergic $NTS^{TH}\rightarrow PVH^{MC4R}$ connection also contributes to behavioral response to stress. To test this, we performed a series of *in vivo* imaging studies involving NTS^{TH}, PVH^{MC4R}, and norepinephrine dynamics in PVH in response to various stressors. Using fluorescent reporters, Axo-GCaMP and NE2h, we found that stress evokes robust activation of NTS^{TH} axons in the PVH which was mirrored by rapid norepinephrine release onto PVH^{MC4R} neurons. Moreover, GCaMP recordings suggest that stress suppresses PVH^{MC4R} activity, consistent with a stress sensitive inhibitory $NTS^{TH}\rightarrow PVH^{MC4R}$ circuit. Lastly, we employed chemogenetic PVH^{MC4R} activation to assess their functional role in LiCl-induced aversion. Our results suggest that conditioned place aversion caused by LiCl requires PVH^{MC4R} silencing. Collectively, these findings suggest an important role for the $NTS^{TH}\rightarrow PVH^{MC4R}$ circuit in stress response. Given the well-characterized role of PVH^{MC4R} neurons on satiety, future studies are warranted to elucidate the role of this pathway in stress-induced feeding disorders.

19 **CDK4/6-MEK inhibition in MPNSTs causes plasma cell infiltration, sensitization to PD-L1 blockade, and tumor regression**

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Malignant peripheral nerve sheath tumors (MPNSTs) are lethal, Ras-driven sarcomas that lack effective therapies. Ras downstream effectors, CDK4/6 and MEK, were identified as actionable targets for combination therapy from patient MPNST analyses and preclinical drug studies. In MPNST cells, low-dose combinations of CDK4/6 and MEK inhibitors synergistically reactivated the retinoblastoma (RB1) tumor suppressor, induced cell death, and decreased clonogenic survival. In immune-deficient mice, dual CDK4/6-MEK inhibition slowed growth but did not shrink MPNSTs in 4 of 5 patient-derived xenografts (PDXs). By comparison, combination therapy of de novo MPNSTs in immunocompetent mice caused tumors to regress, delayed resistant tumor outgrowth, and improved survival relative to monotherapies. While drug-resistant tumors adopted an immunosuppressive microenvironment enriched with MHC II-low macrophages, drug-sensitive tumors that regressed contained plasma cells and increased T cell clustering. In other cancers, intratumoral plasma cellsare associated with better response to immune checkpoint blockade (ICB). Impressively, CDK4/6-MEK inhibition sensitized MPNSTs to anti-PD-L1 ICB therapy with some mice displaying complete tumor regression. These results reveal a novel plasma cell-associated immune response and extended antitumor activity of combined CDK4/6-MEK inhibition in MPNSTs, which dramatically enhanced the efficacy of ICB therapy. This work highlights a promising, potentially curative treatment option for MPNSTs

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20 **Investigating the molecular mechanisms underlying aldosterone-mediated sodium appetite**

Miriam McDonough and Jon Resch

Sodium facilitates vital processes in the body including growth, development, and extracellular fluid volume regulation, making it critical for survival. However, excess sodium intake is associated with an increased risk of hypertension and cardiovascular disorders and, despite efforts by the World Health Organization, average daily salt intake worldwide is approximately twice the recommended level. Due to sodium's ability to regulate the extracellular fluid volume, and thus impact cardiovascular function, its excretion and intake are tightly regulated with the steroid hormone aldosterone playing a major role. Aldosterone binds to the mineralocorticoid receptor (MR), forming a complex that acts as a transcription factor to promote sodium reabsorption in the kidney and sodium appetite in the brain. A single population of neurons within the adult mouse brain express the required genes for aldosterone signaling: MR and 11β-hydroxysteroid dehydrogenase type 2 (HSD2), the latter of which prevents glucocorticoid stimulation of MR. These "HSD2 neurons" are required for aldosterone-induced sodium appetite and their activation using chemo- or optogenetic tool drives sodium intake. Furthermore, chronic treatment with aldosterone increases the intrinsic firing rate of HSD2 neurons; though the molecular mechanism by which aldosterone facilitates this increase in cell-autonomous activity is unknown. Given that aldosterone acts as a transcription factor when complexed with the MR and that sodium appetite takes ~1-3 days to manifest after the start of aldosterone treatment, we hypothesize that aldosterone signaling coordinates a transcriptional program that functions to promote NTS^{HSD2} neurons activity to drive sodium appetite. To test this, we used CRISPR-Cas9 methodologies to knock out MR expression within HSD2 neurons. These mice display decreased sodium appetite in response to sodium deficiency and aldosterone treatment. Further, we created knockouts of a previously identified gene candidate, *Scn5a*/Nav1.5, within HSD2 neurons and show that its expression is important for deficiency-mediated sodium appetite.

21 **DNA sequence variation in *SMAD7* enhances *LMNA*-associated muscular dystrophy in Drosophila models**

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Mutations in *LMNA* cause a collection of diseases known as laminopathies, which include multiple types of muscular dystrophy (*LMNA*-MD). *LMNA*-MD is sensitive to genetic background, as individuals with the same *LMNA* mutation (including siblings) can have clinically distinct diagnoses and/or variable disease severity. Here, we describe a family in which four siblings with the same *LMNA* mutation exhibit highly variable muscle disease severity. Using whole genome sequencing, we identified a variant in the *SMAD7* gene, encoding a negative regulator of the TGFβ/SMAD signaling pathway, that segregates with severe disease. To functionally test the *SMAD7* variant, we generated a Drosophila model containing both the *LMNA* mutation and *SMAD7* variant. We demonstrate that the *SMAD7* variant has minimal effects on muscle function alone but enhances defects caused by the *LMNA* mutation. Furthermore, we show that the *SMAD7* variant increases TGFβ/SMAD signaling in muscle, suggesting a mechanism for the enhanced muscle disease and representing a therapeutic target. To broaden our analysis, sequenced the *SMAD7* gene in a cohort of 45 *LMNA*-MD and identified six additional variants. Taken together, our findings support *SMAD7* as a modifier gene for *LMNA*-MD and identifies the TGFβ/SMAD signaling pathway as a therapeutic target.

22 **Contribution of ASICs to Cardiac Remodeling and Dysautonomia Associated with Heart Failure**

Karley Monaghan, Chad Ward, David Gibbons, Anne Harding, Rasna Sabharwal, Maram El-Geneidy, Harald Stauss, Chris Benson

INTRODUCTION: The American Heart Association projects that by 2030 more than 8 million American adults will have heart failure (HF). HF is a condition in which the heart muscle is unable to sufficiently pump blood to meet the needs of the body. Our lab and others have shown that the Acid Sensing Ion Channel subunit 3 (ASIC3) is highly expressed in the sensory nerves innervating the heart. Recent studies showed that chemically ablating these nerves attenuates cardiac remodeling after myocardial infarction (MI). **RESULTS:** We have demonstrated that ASIC3 plays a role in the development of disadvantageous remodeling after MI in mice. Two days after MI surgery both WT and ASIC3$^{-/-}$ mice show signs of remodeling. However, three weeks after MI the ASIC3-/- mice, despite having significantly larger MI's they tended to remodel less than their WT counterparts. When separated into groups of small MI's and large MI's the ASIC3$^{-/-}$ mice with small MI's had significantly reduced remodeling compared to WT mice with small MI's. Preliminary studies found no differences between WT and ASIC3$^{-/-}$. Interestingly, the baseline heart rates of both MI groups was lower than both Sham groups, indicating that mice don't undergo the same changes in autonomic tone that we see in other animals such as humans or rats. There were also no statistically significant differences in contractile reserve via isoproterenol infusion, despite less remodeling. **CONCLUSION:** Our echo data shows that ASIC3 contributes, at least in part, to the remodeling that occurs after MI. Our data also suggests that for large injuries other factors may lead to disadvantageous remodeling. Autonomic studies indicate that the dysregulation of the autonomic nervous system in mice is different than what is expected from studies done in humans and rats.

23 The Role of Mitochondrial Calcium Uniporter (MCU) and MCUb in Stroke

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Stroke is the leading cause of death and long-term disability in the United States and worldwide. Current treatments for stroke have a narrow therapeutic window and currently there are no effective treatments to promote recovery following a stroke, suggesting the need for new effective therapeutic approaches. Following a stroke, mitochondrial dysfunction has been shown to play a critical role in pathogenesis and, mitochondrial Ca^{2+} overload is implicated in neurotoxic processes including Ca^{2+} dependent excitotoxicity and neurodegeneration and can induce mitochondrial dysregulation. The mitochondrial calcium uniporter (MCU) is a highly conserved pore-forming subunit of the MCU complex that mediates Ca^{2+} uptake into the mitochondrial matrix. We found that deletion of MCU resulted in impaired Ca^{2+} uptake into mitochondria in neurons, inhibited mitochondrial Ca^{2+} dysregulation and excitotoxicity in brain mitochondria. MCUb is MCU paralog and is thought to function as a negative regulator of the MCU complex. We found that MCUb deletion increased mitochondria Ca^{2+} uptake in neurons. In vitro glutamate stimulation on cultured hippocampal neurons revealed that MCUb deletion increased glutamate-induced Ca^{2+} deregulation and excitotoxicity. To further determine the role of MCUb in ischemic stroke, we performed the Middle Cerebral Artery Occlusion (MCAO) in MCUb knockout (KO) mice. Interestingly, MCUb deletion increased stroke damage in male but not female mice. These results show that MCU and MCUb impose counteracting effects on neuronal Ca^{2+} and excitotoxicity and provides evidence of a critical role of mitochondria Ca^{2+} transport in stroke, which may lead to the development of new therapeutics that target MCU for treating this severe cerebrovascular disease.

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24 Regulation of Alcohol Consumption By FGF21

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Alcohol use disorder (AUD) is a pattern of alcohol consumption accompanied by a clinically significant impairment. AUD is a major public health problem worldwide, yet effective therapies to treat individuals to reduce alcohol consumption or facilitating alcohol abstinence are lacking. Further, the underlying physiology of AUD is still poorly understood. Fibroblast growth factor 21 (FGF21) is an endocrine hormone produced by the liver that regulates nutrient and energy homeostasis. Genome-wide association studies have identified multiple single nucleotide polymorphisms in the gene β-klotho (KLB), which is the obligate co-receptor for FGF21 signaling, as being associated with increased alcohol consumption in humans. Previous work from our lab has shown that pharmacologic administration of FGF21, or a long-acting FGF21 analog (PF-05231023), in rodents and non-human primates reduces alcohol consumption. Our findings demonstrate therapeutic potential of an FGF21 analog in suppressing alcohol consumption. We previously identified that FGF21 signals to $KLB^{BLA \rightarrow NAc}$-projecting neurons to suppress alcohol consumption. Here we show that FGF21 signals to KLB^{+}/$Vglut1^{+}$ and KLB^{+}/$Vglut2^{+}$ positive neurons to reduce alcohol intake. Importantly, we reveal that this circuit is distinct from FGF21 signaling to KLB^{+}/$Vglut2^{+}$ double positive neurons to reduce sucrose intake. In addition, FGF21 uniquely impacts feeding behavior in response to sucrose versus alcohol. These results provide additional insight into effective pharmacological treatments and new avenues of therapy for AUD.

25 **X-ray crystal structure of the first selective allosteric inhibitor of caspase-7**

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Caspase-7 (C7), a cysteine protease involved in apoptosis, is a valuable drug target for its role in human diseases (e.g. Parkinson's, Alzheimer's, sepsis). The C7 active site is a challenging drug target due to involvement of i) protein-protein interactions, ii) preference for negatively charged substrates, and iii) overlapping substrate specificity with other caspases—especially caspase-3. An allosteric site at the dimer interface shows greater promise for drug development, although precious few allosteric ligands have been discovered. Here we present the first selective, drug-like inhibitor of C7 discovered through optimization of a previous fragment hit. Using X-ray crystallography, we obtained a structure of this novel allosteric inhibitor complexed with C7. By interrogating the impact on structural features and dynamics, we provide a rational basis for the impact of allosteric binding on C7 inhibition.

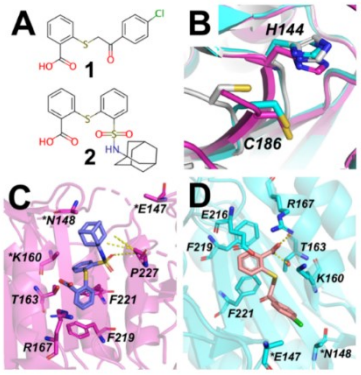

Figure 2. C7 structures bound to inhibitor 1 and 2 show several hallmarks of allosteric inhibition A) Chemical structures of 1 and 2 identified from in silico and in vitro screening. B) 1-C7 (PDB ID: 5V6Z, cyan) and 2-C7 (PDB ID: 8DJ3, magenta) show displacement of the catalytic cysteine (C186) compared to apo C7 (PDB ID: 4FDL, white). C) 2-C7 (8DJ3, protein in magenta and 2 in purple) and D) 1-C7 (5V6Z, protein in cyan and 1 in pink) allosteric site residues shown as sticks. Bonds between the inhibitor and residues are shown in yellow. Truncated residues are denoted by asterisks.

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26 **Tumor Hypoxia Regulates Antigen Processing Machinery**

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Antigen presentation is critical for adaptive immune response to cancer but is often subverted to evade immune response. While hypoxia is a pervasive feature of solid tumors, it's role in tumor antigen processing remains understudied. To assess the effects of reduced oxygen on antigen processing machinery, we used interferon (IFN)-γ to induce the expression of the immunoproteasome (IP), a proteasome isoform critical for the tumor antigen processing, in A549 human lung cancer cells cultured under 20% O2, or 2% O2. Treatment with IFN-γ induced protein expression of IP subunits β1i, β2i, and β5i in a dose-dependent manner under 20% O2 as assessed by western blotting. In contrast, IFN-γ failed to induce protein expression of these subunits under hypoxic conditions. We observed similar blockades of other antigen processing pathway components in response to IFN-γ under hypoxic conditions, as well as secondary mediators of IFN-γ-stimulated genes including several members of the interferon regulatory factor (IRF) family. Surprisingly, under identical conditions, induction of IP subunit mRNAs appear intact under hypoxia as assessed by RT-PCR, suggesting hypoxic IP blockade occurs post-transcriptionally. To test if this phenomenon is regulated by epigenic means, we treated cells with 5-Azacytidine (5-aza), a cystine analog nucleoside, and observed a re-establishment of IP subunit protein expression under hypoxia in response to IFN-γ. Given that 5-aza preferentially integrates RNA over DNA, and that IP subunit mRNA levels appear unaffected by hypoxia, suggests that hypoxia induced IP blockade may be due to hypoxia-specific cytosine modifications within mRNA transcripts. Current studies include efforts to map mRNA methylation patterns and interrogate mRNA methylation transferases under hypoxic conditions. These studies introduce a novel link between hypoxia and reduced cancer cell antigen processing machinery and may help identify druggable epigenetic targets that can be used to enhance the immunogenicity of hypoxic tumors.

27 **Defining the mechanisms of FGF21-mediated reversal of nonalcoholic steatohepatitis (NASH)**

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Non-alcoholic fatty liver disease (NAFLD) is defined as fat accumulation in the liver and is often associated with metabolic diseases including obesity and type 2 diabetes. NAFLD can subsequently progress to non-alcoholic steatohepatitis (NASH), which greatly increases the risk for life-threatening conditions such as cirrhosis, and hepatocellular carcinoma. The endocrine hormone fibroblast growth factor 21 (FGF21) has emerged as a potential therapeutic target for the treatment of NASH. Clinical and pre-clinical studies have demonstrated that FGF21 and FGF21 analogs reduce hepatic lipid accumulation and show great promise as therapeutic options to reverse NASH. However, the mechanisms that underlie FGF21's effects in the liver are poorly understood. Here, we present evidence that FGF21 acts both in the brain and directly on the liver to exert its full range of beneficial effects on NASH. We first demonstrate that FGF21 potently reduces hepatic lipid content and collagen deposition in a physiologically relevant rodent dietary NASH model. We next generated mouse lines lacking β-Klotho (KLB), the obligate co-receptor for FGF21, in several FGF21 target tissues including Vglut2^{+} neurons, hepatocytes, and adipose tissue. We found that KLB expression in both Vglut2^{+} and hepatocytes are required for the full beneficial effects of FGF21 on NASH. Finally, using a tissue-specific KLB expression mouse model, we demonstrate that FGF21 signaling directly to hepatocytes is sufficient FGF21 to restore liver cholesterol homeostasis. These results open interesting avenues for further research including investigating the brain regions and peripheral nervous system pathways that carry out the effects of FGF21 and the distinct hepatic metabolic pathways that are activated by FGF21 signaling to the brain and liver.

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28 **The role of descending dopaminergic pain modulation in the transition to chronic pain**

Angela F. Smith, Ashley N. Plumb, Kathleen A. Sluka

Chronic pain is a significant health burden, and the mechanisms underlying the transition to chronic pain are still poorly understood. Following an acute peripheral insult, 10-50% of people develop chronic pain, and currently available therapies aiming to prevent this transition have had limited success. Changes in descending pain modulation pathways may underly the transition to chronic pain. Our current understanding of these changes is insufficient to adequately treat this population, therefore further studies into the mechanisms underlying the transition to chronic pain are necessary.

The descending dopaminergic pain modulation pathway originates in the A11 nucleus of the hypothalamus and releases dopamine into the spinal cord. Lesioning A11 or blocking dopamine D1 like receptors in the spinal cord can prevent or delay the transition to chronic pain in inflammatory models. We hypothesized that A11 neurons become active following peripheral insult to facilitate the transition to chronic pain via D1 like receptors.

Adult male and female C57BL6/J mice were used in this study. We used a model of chronic widespread non-inflammatory pain that is induced by two spaced muscle insults. To assess hyperalgesia, muscle withdrawal threshold and paw sensitivity was measured before and after induction of the model. This model is ideal for studying the transition to chronic pain because the first insult leaves the animal vulnerable to transition to chronic pain at the time of the second insult. By applying drugs at the time of the second insult, we can probe mechanisms of the transition to chronic pain. We inhibited the descending dopaminergic pain modulation pathway during the second insult to test if this pathway is required for the transition to chronic pain. We found that blockade of the dopamine pathway delayed the development of hyperalgesia in our model. Thus, spinal dopamine appears to mediate the transition to chronic pain.

29 **Obesity Associated SNP rs1421085 Alters IRX3 Expression and Hypothalamic Function**

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Obesity and metabolic dysfunction represent a significant health concern and economic burden. Risk for developing obesity is dependent on a complex relationship between genetic and environmental factors. Supporting a strong role for genetics in contributing to obesity, common single nucleotide polymorphism (SNPs) in the fat mass and obesity associated (FTO) gene have the strongest association with obesity of the entire human genome. These risk alleles are not only strongly associated with obesity but are also associated with increased energy intake. Interestingly, SNPs in FTO have been reported not to influence FTO expression or function but instead impact the expression of the gene Iroquois homeobox 3 (IRX3). Specifically, the region of FTO containing obesity associated SNPs forms functional long-distance connections with the promoter of IRX3 and the presence of risk-alleles for these SNPs is associated with increased expression of IRX3. However, despite the strong association between SNPs in FTO and obesity in humans, the brain regions that drive the association between the FTO SNPs, IRX3, and obesity are unknown. We will address this gap in knowledge through investigating how the FTO SNP most strongly associated with obesity (rs1421085) influences IRX3 expression in specific brain regions and how this altered IRX3 expression impacts bodyweight homeostasis. Our preliminary data suggests that 1) mice containing rs1421085 are susceptible to diet-induced obesity 2) mice containing rs1421085 have a dose-dependent increase in IRX3 expression in the brain 3) increased IRX3 expression in IRX3(+) neurons of the posterior hypothalamus leads to increased food intake and body weight. Based on our data, my hypothesis is that increased IRX3 in the posterior hypothalamus is a driving factor in the association between rs1421085 and obesity.

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30 **Spatial transcriptomics reveals unique gene expression changes in different brain regions after sleep deprivation**

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Sleep deprivation has extensive effects on both the brain and behavior, impacting memory, attention, and metabolism. Previous studies have primarily investigated changes in gene expression within individual brain regions. However, the uniformity or heterogeneity of sleep loss's effects on the brain remains unclear. In this study, we employ spatial transcriptomics to assess the impact of short-term sleep deprivation on the entire brain of male mice.

Our findings reveal substantial differences in gene expression across the brain because of sleep deprivation, with the most significant alterations observed in the hippocampus, neocortex, hypothalamus, and thalamus. Using a rank-sum test like Kruskal-Wallis and stringent thresholds (FDR < 0.001 ; fold-change > |1.2|), differentially expressed genes and their regulatory direction exhibited significant variation among the different regions. Notably, the hippocampal region exhibited the highest sensitivity, characterized by a substantial decrease in gene expression, especially in RNA processing-related molecular functions. Interestingly, the neocortex demonstrated the second highest sensitivity, displaying significant and robust upregulation of gene expression, primarily associated with transcription factor binding, ubiquitin ligase activity, and protein kinase activity. Additionally, we conducted deconvolution analysis using reference scRNA-seq datasets to investigate the gene expression profiles in individual cortical layers (L2-3, L4, L5, and L6) and specific hippocampal subregions (CA1, CA2, CA3, Dentate Gyrus, stratum radiatum, and stratum oriens).

Importantly, we have developed bioinformatic tools enabling the registration of tissue sections and gene expression data into a unified anatomical space, facilitating a comprehensive comparison of gene expression patterns across the entire brain. Our results suggest that distinct molecular mechanisms acting in discrete brain regions underlie the biological effects of sleep deprivation.

31 **Developing RAIDAR: A tool for examining neurons through their connections.**

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The current standard for neurocircuitry experiments in vivo is typically limited to utilizing a tracing virus alongside a genetic driver, a system which confers one-sided specificity to either the projecting or the receiving population of neurons. RAIDAR is a prospective tool for in vivo tracing and construct expression, aiming to expand specificity to both projecting and receiving neurons, which is not currently possible with modern in vivo techniques. RAIDAR is designed to express a Dre recombinase only in cells of a target neuron population which project to another specific downstream neuron population. To do this, RAIDAR utilizes an intein-split Dre recombinase, composed of a stationary and a traveling half which can join together to form an active Dre recombinase when the halves meet in the target neuron population. The production of each half is gated behind the activity of Cre or FLP recombinase, which allows the use of RAIDAR to study any connected set of neuron populations with distinct genetic markers. RAIDAR has been tested and is fully functional, and as such, it can be used as a tool to garner a vast amount of previously unobtainable data.

32 **GPCRome Screening Reveals Specific Interactions of Contaminants with Melatonin and Sphingosine Receptors**

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From intentional production and unintended byproducts of industrialization, large amounts of anthropogenic chemicals have been produced and amassed in the environment. Despite longstanding knowledge that environmental contaminants have adverse effects on human health, critical gaps remain in understanding the specific actions of many pollutants – warranting more comprehensive, mechanism-focused approaches for assessing biological activity. Past literature and emerging data show that specific environmental contaminants can activate particular GPCRs. However, studies focus on contaminant-receptor pairs in detail, leaving the vast majority of the GPCR landscape unexplored. As GPCRs act as signaling nodes in many physiological processes, we hypothesized that interrogating the activity of a contaminant against the GPCRome could be used to assess biological impact while simultaneously providing insight into potential mechanisms of pathology. Utilizing the open-source platform (PRESTO-Tango), we developed a screening methodology capable of investigating the activity of contaminants against 314 non-olfactory GPCRs amendable to high-throughput. From our initial screen of eight compounds, we identified several interactions between polychlorinated biphenyls (PCBs) and GPCRs. Most interesting are the relationships uncovered between specific PCB congeners and receptor isoforms involved in sphingosine-1-phosphate and melatonin signaling pathways (S1PR4 and MTb). To our knowledge, this is the first report of these interactions, highlighting the utility of our approach to quickly assess the biological impact and pathology of a contaminant at the receptor level. Additionally, while detailed pharmacological experiments are underway, these findings could support the involvement of sphingosine and melatonin signaling interplay in pathologies beyond the contaminant(s) alone.

33 **Defining the Role of Adipose Triglyceride Lipase in Drosophila Border Cell Migration**

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Department: Anatomy & Cell Biology

Abstract: Lipid droplets (LDs) are dynamic cellular organelles responsible for mediating key steps in lipid metabolism and signaling. LD dysregulation is thought to promote cancer invasion and metastasis, but the precise role of LDs during collective cell migration is unknown. One possibility is that LDs regulate cell migration by modulating cytoskeletal dynamics. Previously, we identified the conserved LD lipase, Adipose Triglyceride Lipase (ATGL), as a novel regulator of actin remodeling during Drosophila oogenesis. To test whether ATGL has a role in cell migration, we used border cell migration during Stage 9 of Drosophila oogenesis, an ideal in vivo model of collective cell migration. We analyzed the migration of border cells in wildtype vs ATGL null flies and found that loss of ATGL results in both delayed migration and failed epithelial detachment (aka delamination). Furthermore, loss of ATGL results in larger LDs within the migrating cells, suggesting that ATGL-mediated lipolysis is normally active within these cells during their migration. These findings suggest a novel role for both ATGL and LDs in facilitating on-time border cell migration, which is necessary to produce fertilization-competent oocytes. As the fatty acids released by lipolysis can serve as building blocks for membrane lipids and signaling molecules or as substrates for energy production, future work will aim to determine precisely how ATGL-mediated lipolysis promotes collective cell migration. Finally, given that border cell migration is an in vivo model of collective, invasive cell migration, these findings have relevance beyond development in processes such as wound healing and cancer metastasis. New insights into the diverse functions of LDs and their associated proteins are likely to lead to improved understanding of and new therapeutics for a variety of diseases.

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34 **Alternative Polyadenylation of *SCN5A* Generates a Novel N-terminal NaV1.5 Microprotein**

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SCN5A encodes the cardiac voltage-gated Na+ channel, NaV1.5, that initiates action potentials. SCN5A gene variants cause arrhythmias and increased heart failure risk, and mechanisms controlling NaV1.5 expression and activity are not fully understood. We recently discovered a well-conserved alternative polyadenylation (APA) signal downstream of the first SCN5A coding exon. This yields a short SCN5A transcript expressed across several species (human, pig, and cat; rodents lack the upstream APA). Analysis of genome-wide cardiac APA data revealed a ~30% decrease in upstream APA signal usage in humans with dilated cardiomyopathy. Reanalysis of human cardiac RNA-seq data indicated that short/full-length SCN5A mRNA ratios are reduced ~25% in failing hearts. Notably, the short transcript could also produce a novel microprotein (SCN5A-NT), composed of an N-terminus identical to NaV1.5 and a C-terminus derived from “intronic” sequence. Western blot of human heart tissues using an N-terminal NaV1.5 antibody shows bands migrating identically to transgene-derived SCN5A-NT. SCN5A-NT is predicted to contain a mitochondrial targeting sequence, and immunostaining of SCN5A-NT expressed in neonatal rat cardiomyocytes colocalizes with mitochondria. These findings were corroborated in mice treated with AAV9 encoding SCN5A-NT, which showed clear myocardial SCN5A-NT expression by western blot and immunostaining colocalized to cardiomyocyte mitochondria. We will soon test if SCN5A-NT alters mitochondrial functions and/or NaV1.5 activity (perhaps indirectly via mitochondria ROS or NAD+/NADH). We have also generated knock-in mice harboring the human APA signal to investigate how upstream APA influences NaV1.5 expression/activity and whether upstream APA usage is dynamically regulated during cardiac stress.

35 **First Proofs for the Dual Intervention Point Model of Body Weight Regulation**

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Obesity is a complex, multifactorial, and costly chronic disease. More than half of the US population are either obese or overweight. Although dieting, exercise, and obesity therapeutics can lead to weight loss, weight regain remains the most substantial problem in obesity therapeutics. Moreover, force feeding humans, monkeys, or mice leads to the loss of the gained weight after the conclusion of the overfeeding which is accompanied by severe hypophagia for the duration of the weight loss. Based on these observations, there are two prominent models to explain the principles of body weight regulation, “set point” model and “Dual intervention point model”, none of which has been proven yet.

Our data reveals that diet-induced obese (DIO) mice in thermoneutral conditions experience the weight loss and hypophagia after switching from high-fat diet (HFD) to chow diet. This hypophagia is independent of the duration of HFD feeding. overfeeding the mice with gastric infusion of a low-fat diet, leads to the same hypophagic response following the stop of the overfeeding. This suggests that hypophagia is not related to the palatability of the HFD, but its due to active regulation of the body weight. Our results also suggest that HFD impairs this physiological ability to regulate body weight, the mechanisms of which remain unclear.

Moreover, calorie restricting mice on chow diet until they reach 80% of their original body weight and then giving them ad-libitum access to food leads for body weight rebound until reaching the lower intervention boundary of their body weight. All these suggests that the dual-intervention point model is the better way to explain how the body weight is being regulated. While our results are the first to show proofs for the dual intervention point system, the exact molecular mechanisms of how the upper and lower intervention points are defined are completely unknown.

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36 **Neuronal mechanisms of neurofibromin-mediated modulation of metabolic homeostasis**

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Neurofibromatosis type 1 (NF1) is a multisystemic, monogenetic disorder arising from loss-of-function mutations in the *NF1* gene. *NF1* encodes a large protein called neurofibromin that contains a central GAP-related domain (GRD). Neurofibromin modulates various cellular and molecular mechanisms, including gene transcription, cell proliferation and differentiation, and metabolic regulatory pathways. The clinical manifestations of NF1 are diverse and encompass peripheral nerve-associated tumors, brain tumors, skeletal and vascular abnormalities, and neurocognitive and behavioral deficits. Although evidence suggests that Nf1 is involved in regulating cellular and organismal metabolism, the precise mechanisms regulating this process remain largely unknown.

Leveraging the *Drosophila* model of NF1 and its highly conserved signaling pathways, we investigated the potential link between Nf1 and metabolism. Using *in vivo* genetic analysis, we established that the loss of Nf1 disrupts metabolic homeostasis, leading to increased metabolic rate and feeding, altered lipid storage and turnover kinetics, and decreased susceptibility to starvation. The Nf1-mediated metabolic effects mapped to a distinct set of interneurons in the nervous system of *Drosophila*. Acute stimulation of these neurons increased metabolic rate, suggesting that they dynamically modulate metabolism. Further exploration revealed that Nf1 regulates metabolic rate via neuronal mechanisms, with cell-autonomous contributions from muscle cells.

Ras GAP activity of Nf1 is mediated by its central GRD. We found that Ras signaling is critical for mediating Nf1 effects on metabolism, and further explored its downstream signaling with a series of gain- and loss-of-function genetic approaches. Expression of a constitutively-active ERK partially phenocopied the Nf1 metabolic phenotype. Additionally, independent *in vivo* genetic experiments targeting the necessity of the Raf/MEK/ERK and PI3K/AKT/mTOR pathways revealed that Nf1 mediates metabolism via coordinated actions of both pathways. These data reveal a novel interaction between Nf1 and metabolism – identifying neural circuits and signaling mechanisms underlying metabolic regulation by Nf1.

37 **Dissecting the Role of Dopaminergic Neuron Subsets on Reward Learning**

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While the dopamine neurons that drive approach and avoidance learned behaviors in drosophila are well established, there are other dopaminergic neurons that innervate the learning and memory centers (called the mushroom bodies) of the fly brain. One such cluster, the PPL2's, have recently been shown to enhance aversive learning (and therefore avoidance behaviors) without seeming to encode any valance. We will seek to further characterize these neurons by examining what role, if any, that the PPL2's play in appetitive memory. This will be done via optogenetic manipulation of the PPL2's during appetitive learning conditions. Additionally, we will also explore the possibility that the PPL2's lower the stimulus response threshold in the mushroom bodies, allowing the flies to make associations that they would not normally make. In the same vein, we will measure whether or not PPL2 activity alters arousal thresholds in the flies. If this is the case, then perhaps the PPL2's play a role in sleep or in attention. Perhaps PPL2 activation increases attention in the flies, allowing them to make associations at stimuli levels that they wouldn't normally be able to. This may or may not involve changes in mushroom body activity, a possibility we will explore using 3D-shot live imaging microscopy of acetylcholine release in the mushroom bodies while simultaneously activating the PPL2's. We hypothesize that the PPL2's alter activity in the mushroom bodies such that the intrinsic mushroom body Kenyon cells respond to normally sub-threshold and therefore allowing the fly to learn in sub-optimal conditions.

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38 **Novel insight into the function and mechanisms of CTLH E3 ubiquitin ligase complex in breast cancer development**

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The CTLH (carboxy-terminal to LisH domain) E3 ubiquitin ligase complex, a mammalian counterpart to the yeast GID (glucose-induced-degradation-deficient) complex, comprises several subunits, including E3 ubiquitin ligases RMND5a and MAEA, and the scaffold/adaptor protein WDR26. Although the CTLH complex has been implicated in tumorigenesis, its precise role in breast tumor development has remained largely unknown. In this study, we demonstrated that reducing the levels of WDR26, RMND5a, and MAEA led to a significant suppression of tumor cell proliferation and migration in vitro, as well as inhibited tumor growth in vivo. Furthermore, targeted deletion of the WDR26 gene in mammary cells did not impact normal mammary gland development but remarkably suppressed tumor growth and metastasis in two genetic mouse models of breast cancer (induced by PyMT and Neu). Our investigations also unveiled that the CTLH complex may govern breast cancer development by controlling the ubiquitination and proteasomal degradation of SNF5, a crucial epigenetic tumor suppressor. Interestingly, WDR26 appeared to recruit SNF5 to the CTLH complex through specific domains distinct from those involved in interactions with other CTLH components. In conclusion, our findings shed new light on the poorly understood CTLH E3 ubiquitin ligase complex's function and mechanisms in breast cancer development and identify SNF5 as a novel substrate of the complex.

39 **Anatomical Determinants of Leptin and Melanocortins Regulation of Cardiovascular and Metabolic Autonomic Neurocircuitries**

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The central nervous system (CNS) autonomic network plays an important role in regulation of cardiovascular and metabolic functions. Leptin, a hormone secreted by adipose tissue, acts in the brain to regulate several physiological processes. Melanocortin-4 receptor (MC-4R) containing neurons are key mediators of CNS leptin action. However, the anatomical substrates underlying cardiovascular and metabolic control by the leptin receptor (LepR) and MC-4R is unknown. We hypothesized that due to the physiological relationship between leptin and the melanocortin system that an anatomical relationship exists between the two. To test this, we assessed the presence of PRV-GFP injected into kidneys, or interscapular brown adipose tissue (iBAT) in td-Tomato labeled LepR or MC-4R neurons. At 5-6 days after kidney or iBAT inoculation, we observed about 10% of LepR neurons that express PRV-GFP in the preoptic nuclei and arcuate nucleus. Approximately 5% of LepR neurons in the lateral hypothalamic area, septal nucleus, ventral tegmental area, and nucleus tractus solitarius (nTS) were linked to kidney or iBAT. In contrast, about 10% of MC-4R neurons co-expressed PRV-GFP in the periventricular nucleus of the hypothalamus and nTS. Interestingly, after injection into the kidneys, but not iBAT, PRV-GFP/MC-4R co-localization was also detected in the agranular insular (AI) cortex and the amygdala (AMY). Many other common nuclei between the organ associated regions and either LepR or MC-4R neurons did not appear to demonstrate any co-localization. Therefore, although LepR and MC-4R neurons are widely distributed throughout the brain those involved in regulation of cardiovascular or metabolic function appear to be localized to only a few specific areas. Our results suggest that LepR and MC-4R neurons may regulate cardiovascular and metabolic functions through a small number of distinct nuclei in the CNS autonomic network.

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40 **Motor pattern alterations in a model of neurofibromatosis type 1**

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Neurofibromatosis type 1 is a genetic disorder caused by the loss of the neurofibromin protein (Nf1). Neurofibromatosis has a wide range of physical and cognitive manifestations including autism spectrum disorder and attention deficit hyperactivity disorder. Loss of Nf1 function may alter circuit activity, leading to behavior changes. We have found that loss of Nf1 in *Drosophila* alters spontaneous motor behaviors, including increasing grooming frequency. Whether Nf1 deficiency alters the frequency and pattern of sensory-evoked behaviors is unknown. If flies are covered with dust, they engage in vigorous grooming to remove the dust. Here we test the effects of dusting wild-type and *nf1* mutant flies. The data suggest that dusting increases grooming frequency in both genotypes, and that the grooming pattern in *nf1* mutants is altered: the normal sequence/prioritization is scrambled. This suggests that Nf1 loss both increases spontaneous grooming frequency and changes the pattern of this temporally-sequenced motor behavior.

41 **Loss of ciliary adenylyl cyclase 3 in the ventromedial hypothalamus resulted in a sex-dependent obese phenotype**

G. DENG, K. SAITO, U. SINGH, Y. DENG, J. DICKEY, J. JIANG, H. CUI

Obesity, a metabolic disorder characterized by an excessive accumulation of body fat, continues to be a major economic and health burden to the global populace, as it is the most significant risk factor for heart disease, diabetes, and hypertension. Genome-wide association studies have identified several genes involved in obesity development, including *ADCY3*, a gene encoding for the enzyme adenylyl cyclase 3 (AC3). AC3 is a critical mediator of the cyclic adenosine monophosphate (cAMP) signaling pathway and is enriched in the primary cilium, a solitary immotile organelle protruding from most mammalian cells, including neurons. The primary cilia act as the cellular antenna to sense and relay changes in the extracellular microenvironment and mounting evidence support a role for the primary cilia as a critical regulator of the energy homeostasis. Patients carrying loss-of-function *ADCY3* variants are obese and are at increased risk of developing type II diabetes mellitus (T2DM). Consistently, whole-body *Adcy3* knockout mice also develop obesity; however, the underlying mechanisms by which AC3 affects metabolic homeostasis remain unclear. Here, we examined the impact of AC3 loss within the ventromedial nucleus of hypothalamus (VMH), a brain region critical for coordinated control of energy and glucose homeostasis, on energy metabolism. We generated mice with VMH-specific *Adcy3* loss (Adcy3SF-1KO mice) by crossing SF-1 Cre+ mice with Adcy3F/F mice and subjected them to metabolic phenotyping to assess energy balance and glucose homeostasis. We observed a sexually dimorphic effect on body weight under standard chow diet; male Adcy3SF-1KO mice displayed normal body weight gain comparable to control littermates, whereas female Adcy3SF-1KO mice gained a significant more weight compared to control littermates (Con 24.34g v KO 30.61g, $p < 0.0001$). Strikingly, however, both sexes of Adcy3SF-1KOmice exhibited improved glucose tolerance (AUC: M Con 17,826 v KO 15,165, $p = 0.02$; F Con 15,263 v KO 13,238, $p = 0.03$) without affecting fed and overnight fasting blood glucose levels. Taken together, these results underscore the importance of AC3-mediated ciliary cAMP signaling in VMH neurons for the regulation of energy balance and glucose homeostasis in a sex-dependent manner.

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42 **Mitochondrial AKAP1 Protein Is Required for Diet-Induced Obesity and Glucose Dysregulation**

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Mitochondria are best known as the powerhouse of the cell playing a critical role in energy metabolism with important implications in the development of obesity, a major cause of type 2 diabetes. A-kinase anchoring protein 1 (AKAP1) is a mitochondrial scaffold protein that promote protein kinase A (PKA)-mediated phosphorylation of Drp1(Ser637) by increasing the local concentration of PKA at the outer mitochondrial membrane. However, the role of AKAP1 in the regulation of body weight and glucose homeostasis is not known. We used AKAP1 deficient mice to understand the physiological significance of this protein. Male and female AKAP1$^{-/-}$ and AKAP1$^{+/-}$ mice fed normal chow exhibit lower body weight relative to littermate controls at age of 16 weeks (male: 31.1$\pm$1.6g vs and 35.3$\pm$1.1g; female: 22.7$\pm$0.8g vs 24.8$\pm$1.2g). AKAP1$^{-/-}$ and AKAP1$^{+/-}$ mice fed high fat high/sucrose diet (HFHSD) also display attenuated weight gain compared to controls (male: 39.5$\pm$1.7 and 42.5$\pm$1.6 vs 47.3$\pm$2.3g, and female: 29.7$\pm$1.3 and 29.2$\pm$1.8 vs 32.5$\pm$1.5g). This was associated with significant decreased in fat mass in AKAP1$^{-/-}$ (male:16.2$\pm$0.9g and female: 8.7$\pm$1.1g) and AKAP1$^{+/-}$ (male:15.0$\pm$2.5g and female: 9.2$\pm$1.0g) mice compared to controls (male: 21.2$\pm$1.7g and female: 13.9$\pm$1.6g) whereas lean mass was not different between the three groups. Glucose tolerance test revealed that female AKAP1$^{-/-}$ mice have improved glucose handling, and insulin tolerance test showed that insulin sensitivity is better in male AKAP1$^{-/-}$ mice than controls. Leptin signaling was significantly improved in AKAP1$^{-/-}$ mice compared with control littermates fed HFHS diet as pSTAT3 activity was significantly higher in the Accurate Nucleus of hypothalamus in mice treated with 2 mg/g leptin of body weight via intraperitoneal administration for 1 hr. These findings demonstrated the importance of AKAP1 in the development of obesity and associated diabetes. Our data also point to mitochondria function as a potential therapeutic target for treatment of common obesity and related diseases.

43 **ALTERED PURKINJE CELL LOCALIZATION AND CEREBELLUM-DEPENDENT LEARNING IN 16P11.2 MICRODUPLICATION MICE**

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The 16p11.2 microduplication (16p11.2dup) is associated with several neuropsychiatric disorders including schizophrenia, autism spectrum disorder, and attention deficit/hyperactivity disorder (ADHD). Cerebellar abnormalities have been increasingly implicated in these neuropsychiatric disorders, and the cerebellum appears to be a site of transcriptional dysregulation in 16p11.2dup mouse models. However, the effect of 16p11.2dup on the morphology, location, and function of cells in the cerebellum has not been investigated. We histologically labeled Purkinje cells in a mouse model of 16p11.2dup. A nested t-test revealed a significant increase in the number of calbindin+ ectopic Purkinje cells in the granule layer of cerebellar lobule VI in adult heterozygous (HET) 16p11.2dup mice compared to wild-type (WT) mice. Preliminary observations suggest these ectopic Purkinje cells also occur in other cerebellar lobules, and they are smaller, have less dendritic branching, and express less calbindin/parvalbumin than typically located Purkinje cells. Cerebellar lobule VI is associated with motor learning and eyeblink conditioning, and behaviorally, we observed that adult HET 16p11.2dup mice are impaired in cerebellum-dependent associative learning in delay eyeblink conditioning. Specifically, adult HET 16p11.2dup mice showed deficits in both conditioned response (CR) percentage and CR onset latency relative to WT mice. These results suggest that altered Purkinje cell morphology and localization in adult HET 16p11.2dup mice could not only impair cerebellar learning, but also impair adaptive timing of cerebellar-driven responses when they occur. Thus, we have identified novel structural and functional alterations in the cerebellum that are associated with 16p11.2dup. Importantly, patients with schizophrenia and ADHD also show deficits in CR acquisition in delay eyeblink conditioning, suggesting that the behavioral impairments in the 16p11.2dup mouse model resemble impairments seen in neuropsychiatric disorders linked to 16p11.2dup in humans. Further investigation of the cerebellar cortex neurons in 16p11.2dup mice may provide insights into the pathogenesis of neuropsychiatric disorders linked to this CNV.

44 **Pharmacological rescue of cognitive deficits using PDE4 inhibitors in mice harboring Jordan's Syndrome, an autosomal dominant neurodevelopmental disorders caused by *de novo* mutations in a protein phosphatase 2A regulatory subunit**

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Jordan's Syndrome (JS) or autosomal-dominant mental retardation 35 (MRD35) is caused by *de novo* germline mutations in the protein Ser/Thr phosphatase 2A (PP2A) regulatory subunit B'δ (PPP2R5D). Relatively common among monogenic causes of neurodevelopmental disorders, JS typically presents with intellectual disability, human overgrowth, and autism. The most common mutations switch a negatively charged to a positively charged residue (glutamate to lysine) in the highly conserved acidic loop that extends from B'δ towards the active site of the PP2A catalytic subunit. We hypothesize that JS-associated mutations change PP2A substrate specificity (e.g. from positively to negatively charged phospho-peptides), which could affect cellular signaling cascades that promote growth and proliferation and/or to inhibit those that mediate differentiation and morphogenesis. In our mouse model of Jordan's Syndrome harboring the constitutive E198K knock-in mutation, which is the most common and the most severe mutation, the mice recapitulate most of the common features of Jordan's Syndrome which includes growth retardation, craniofacial anomalies, seizure occurrences and cognitive deficits. Further examination of the E198K brains reveal a hypo-phosphorylation state. Because our biochemical data show JS mutations do not affect PP2A holoenzyme assembly but enhance PP2A activity, we hypothesize that the JS mutations activate the PP2A/B'd holoenzyme, hence a gain-of-function mechanism. Boosting the cAMP-mediated PKA activity therefore could potentially reverse the hypo-phosphorylation state in the E198K brains. Indeed, our preliminary data show rescue of cognitive deficits and reversal of hypo-phosphorylation state in the E198K mice when treated with PDE4 inhibitors (such as rolipram and zaltomilast). PDE4 inhibitors inhibit the cAMP degradation thereby elevating the cAMP-mediated PKA activity. In conclusion, we provide preliminary evidence on the use of PDE4 inhibitors to rescue cognitive deficits in our mouse model of Jordan's Syndrome.

45 **A novel assay for tactile hypersensitivity in neurofibromatosis type 1**

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Neurofibromatosis 1 is an inherited monogenetic disorder caused by the mutation and subsequent dysfunction of the neurofibromin 1 gene (*NF1*), a known tumor suppressor that encodes the neurofibromin protein (Nf1). In the well-established *Drosophila* model of the disease, increases in overall grooming behavior and metabolic rate are observed. Interestingly, the grooming phenotype relies on proper Nf1 function during the pupal stage of *Drosophila* development; knockdown of Nf1 only in the adult stage does not drive excessive grooming. Additionally, the reduction of functioning Nf1 is shown to induce neuronal hyperexcitability and tactile hypersensitivity in *Drosophila* larvae. To identify the neuronal circuitry and cellular mechanisms underlying the developmental necessity of Nf1, we are utilizing *Drosophila* larvae as a model. Preliminary data from our lab suggests an increase in stereotyped escape behavior in *nf1* mutant larvae in response to global mechanical stimulation. The class IV multi-dendritic neurons (MD-IVs) are known to be sufficient and necessary for larval escape behavior. By enlisting the sophisticated genetic toolbox available for *Drosophila* manipulation, our aim is to determine whether the knockdown of Nf1 specifically in class IV multi-dendritic neurons (MD-IVs) is responsible for this behavioral phenotype.

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46 **AgRP Neurons Encode Circadian Feeding Time**

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Food intake follows a predictable daily pattern and synchronizes metabolic rhythms. AgRP neurons read-out physiological energetic state and elicit feeding, but the regulation of these neurons across daily timescales is poorly understood. Using a combination of neuron dynamics measurements and timed optogenetic activation, we found that daily variations in AgRP neuron activity was not fully consistent with homeostatic regulation. Instead of operating as deprivation counter, both food dependent and independent AgRP neuron activities primarily followed circadian rest-activity cycle through a process that required intact SCN and can be resynchronized by light. Imposing novel feeding patterns through time-restricted food access or periodic AgRP neuron stimulation was sufficient to resynchronize daily AgRP neuron activity rhythm and drive anticipatory-like behavior through a process that required DMH^{PDYN} neurons. Collectively our results reveal that AgRP neurons integrate time-of-day information of past feeding experience with current metabolic needs to predict circadian feeding time.

47 **THE ROLE OF LATERAL HYPOTHALAMIC LEPTIN CIRCUITRY IN AUTONOMIC CONTROL OF CARDIOVASCULAR FUNCTION**

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Obesity is commonly associated with sympathetic overactivity and elevated blood pressure, and accumulating evidence suggests that an adipocyte-derived metabolic hormone, leptin, may plays an important role in these pathological processes, likely through a mechanism of so-called 'selective leptin resistance'. However, underlying neural circuits through which leptin causes sympathoexcitation and increases blood pressure remain incompletely understood. Here we hypothesized that leptin may acts on a subset of lateral hypothalamic area (LHA) neurons to affect cardiovascular functions. We show that stereotaxic microinfusion of leptin into the LHA dose-dependently increases renal sympathetic nerve activity (RSNA) (% changes from baseline at 4th hour: vehicle -25.03 ± 7.09 % vs leptin 100.23 ± 26.94 %, $p<0.001$) and selective chemogenetic activation of LHA LepR-expressing neurons increase arterial pressure compared to control mice (19.43 ± 0.87 mm of Hg, $p<0.05$). Consistent with these functional observations, viral-mediated cell type-specific anterograde tracing revealed that LHA LepR-expressing neurons, which are distinct from well-known orexin and MCH neurons, broadly innervate brain regions known for autonomic regulation, including but not limited to paraventricular nucleus, nucleus of solitary tractus, locus coeruleus, and ventrolateral medulla. These findings identify the LHA as a novel brain site critical for leptin to regulate RSNA and arterial pressure.

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48 **Serotonergic receptors drive graded presynaptic plasticity of acetylcholine release in Kenyon cells following associative learning**

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Associative learning involves the temporally specific neuromodulation of multiple monoaminergic neurotransmitters including serotonin (5-HT). These neuromodulators act in an axon compartment-specific manner to drive potentiation/depression of presynaptic acetylcholine (ACh) release from Kenyon cells, a major site of sensory integration and plasticity underlying associative learning in *Drosophila*. To investigate the role of 5-HT in modulating this synaptic plasticity, we have imaged ACh release from KCs during appetitive conditioning. The role of 5-HT receptors was tested by knocking them down conditionally knockdown in the KCs. Conditional knockdown of two different 5-HT receptors in adult mushroom body Kenyon cells altered the release of odor-evoked ACh across KC axon compartments following appetitive classical conditioning, systematically shifting baseline odor-evoked ACh release and abolishing the plasticity of ACh release across compartments. Further, adult-specific knockdown of these receptors in KCs impaired behavioral reward learning, demonstrating their importance for learning in a behavioral context. Together with previous data on the role of dopamine, these data suggest that layered signaling via monoaminergic pathways modulates baseline ACh release and drives plasticity underlying reward learning.

49 **The role of Pitx3-mediated transcriptional activation of *RGS6* promoter in Parkinson's disease**

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Parkinson's disease (PD) is a debilitating, neurological disorder that affects millions of people worldwide, making it the second most common neurodegenerative disease after Alzheimer's. The loss of midbrain dopaminergic (mDA) neurons in the substantia nigra compacta (SNc), resulting from the accumulation of α-synuclein protofibrils in Lewy bodies, is believed to be the underlying cause of sporadic PD. Pituitary homeobox 3 (Pitx3) is a bicoid homeodomain transcription factor crucial for the development and survival of mDA neurons. Mouse mutants of *Pitx3* exhibit selective loss of mDA neurons, and human *PITX3* variants have been linked with sporadic PD. We previously identified *Rgs6* (regulator of G-protein signaling 6) as the most downregulated transcript in the SNc of *Pitx3*$^{-/-}$ mice and found that *Rgs6*$^{-/-}$ mice exhibit loss of Pitx3-dependent gene expression in SNc DA neurons. We then demonstrated that mice with global or SNc-selective loss of RGS6 exhibit loss of SNc DA neurons, aberrant SNc α-synuclein accumulation, and hallmark motor deficits of PD. Finally, a recent proteomic study identified RGS6 as the most downregulated protein in the SNc of Lewy body-positive PD patients. Though these studies strongly suggest that RGS6 critically protects against PD neurodegeneration, nothing is known about how RGS6 expression is regulated or dysregulated in PD. We performed *in silico* analysis of ~1 kb of 5'-flanking region of the human *RGS6* gene and identified a bicoid homeodomain element that was the most highly conserved response element in mammals. Based on the aforementioned findings, we hypothesized that Pitx3 may act as a critical transactivator of the *RGS6* promoter. To investigate this hypothesis, we cloned mouse *RGS6* promoter into a mammalian luciferase plasmid and examined its activity in PC-6 cells following expression of Pitx3. Expression of Pitx3 significantly increased *RGS6* promoter activity, thus confirming its role as a transactivator of the *RGS6* promoter.

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50 **Characterizing a Novel Family of Alternative Open Reading Frame Encoded Microproteins**

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The SCN-family of genes encode voltage-gated sodium channels which control sodium ion influx in excitable cells during action potentials and display tissue specific expression patterns. Our lab recently discovered a microprotein encoded by the heart specific SCN5A gene, canonically encoding NaV1.5, which is generated by translation of an alternative open reading frame (altORF). In addition to generating the microprotein, the SCN5A altORF acts to regulate expression of full-length NaV1.5. Notably, this altORF is extremely highly conserved and are present in the remaining SCN-family genes. Here, we aim to expand our initial work on SCN5A to include the remaining SCN family genes which also contain altORFs. We used V5-tagged reporter constructs to demonstrate the altORFs are translated and to characterize how the altORF regulates translation of the sodium channel ORF. In addition, we used c-terminal GFP-tagged constructs to assess the altORF-derived microproteins' subcellular localization. 6 out of 8 localized to mitochondria, one localized to the plasma membrane, and one showed dual localization in mitochondria and the nucleus. Western blotting using custom antibodies we generated for 4 altORF microproteins demonstrates they are unstable in-vitro, however further in-vivo characterization is needed. Overall, this work describes a novel regulatory mechanism of sodium channel expression across various tissues which may also generate a family of undescribed microproteins.

51 **SerpinA3N in Leptin-Receptor Neurons is Required for Metabolic and Glucose Homeostasis**

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Obesity and diabetes which are associated with increased morbidity and mortality are among the most concerning health issues in the U.S. This highlights the importance of understanding the mechanisms underlying these diseases. In the central nervous system, leptin receptor signaling plays a pivotal role in the regulation of food intake and energy expenditure. High fat diet (HFD) induces inflammation within the hypothalamus, leading to leptin insensitivity which contribute to obesity. Previous studies have shown that a novel gene serpinA3N, a serine protease inhibitor, which is highly expressed in the arcuate nucleus of the hypothalamus (ARC), where the leptin receptor is also highly expressed, is upregulated by high fat diet (HFD) and leptin stimulation. However, the role of SerpinA3N in the leptin receptor expressing cells in the control of body weight and glucose homeostasis remains unknown. In the present study, we investigated whether specific serpinA3N deficiency in leptin receptor expressing neurons affects metabolic and glucose homeostasis. This was achieved by crossing serpinA3N$^{flox/flox}$ mice with mice expressing Cre recombinase under the control of the leptin receptor promoter.

Remarkably, female conditional knock-out (CKO) mice displayed significantly lower body weight after week 14 in mice fed normal chow diet or high fat high sucrose diet (HFHSD), whereas no body weight change was observed in male CKO fed normal chow diet or HFHSD. Lower body weight in female CKO mice was due to decreased fat mass, but not lean mass. No change in food intake was observed in female CKO mice relative to controls, indicating increased energy expenditure may contribute to lower body weight in female CKO mice. Interestingly, male CKO mice fed HFHSD had significantly improved glucose handling, but not change in insulin sensitivity. In addition, female CKO mice fed normal chow diet had enhanced insulin sensitivity compared to control animals. These results demonstrate that SerpinA3N in leptin receptor- expressing neurons is required for body weight regulation and glucose homeostasis in a sex- dependent manner.

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52 **Effects of the RGS6B Isoform on Motor Deficits and D_2R Signaling Pathways in Parkinson's Disease**

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Parkinson's disease (PD) is a neurodegenerative disorder resulting in progressive motor dysfunction due primarily to aging. PD is caused by α-synuclein accumulation and the degeneration of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Within the SNc, regulator of G protein signaling 6 (RGS6) is expressed specifically in DA neurons. RGS6 proteins exist predominantly as the RGS6L isoform, expressed throughout the body, and a brain-specific RGS6B isoform. Mice with global RGS6 deletion develop a late age-onset PD phenotype. Our studies revealed that RGS6L negatively regulates D_2 autoreceptor-$G_{i/o}$ signaling in SNc DA neurons, increasing cAMP signaling. This promotes dopaminergic transmission and protects against neurodegeneration and aberrant α-synuclein expression. The function of RGS6B is currently unknown, and this project attempts to characterize its role in SNc DA neurons. Remarkably, bilateral SNc-selective deletion of RGS6 from mice for 1mo recapitulated the PD phenotype observed in 12mo Rgs6-/- mice. AAV expression of RGS6L in the SNc of *Rgs6*$^{-/-}$ mice rescues dopaminergic degeneration and motor dysfunction. In contrast, expression of RGS6B failed to prevent neurodegeneration and motor dysfunction. However, RGS6B expression in wild type mice worsened motor performance, leading us to hypothesize that RGS6B might have a role opposite to that of RGS6L and exacerbate, rather than protect from SNc dopaminergic degeneration. To test this hypothesis, we performed unilateral Cre-mediated deletion of RGS6 from 12mo old *Rgs6*$^{fl/fl}$ mice and explored the ability of SNc expression of RGS6L and RGS6B to rescue dopaminergic degeneration and motor dysfunction. The D_2R agonist quinpirole was administered to mice with unilateral RGS6 SNc deletion to assess the ability of RGS6 isoforms to rescue hyperactive D_2R-$G_{i/o}$ signaling in SNc DA neurons by motor assays and immunofluorescent analysis of RGS6, α-synuclein, TH, and pS^{40}TH. The results will further clarify the role of RGS6B in signaling pathways linked to PD.

53 **Population Pharmacokinetic Model of Delta-9-Tetrahydrocannabinol (THC) and Metabolites in Healthy Adults Following Single Oral Dose**

Peizhi (Paige) Li, PharmD, Guohua An, MD, PhD

The use of recreational cannabis has been growing in recent years across the US after the expansion of marijuana legalization among multiple states. Of which, oral cannabis products have gained particular popularity over smoking/vaping due to the more substantial drug effects and more discreet administration. The primary psychoactive compound of cannabis is delta-9-tetrahydrocannabinol (THC). Studies found that THC undergoes first-pass metabolism following oral consumption to 11-OH-Δ9-tetrahydrocannabinol (11-OH-THC), which is more psychoactive than THC. Currently, most of THC population PK models focused on characterizing their disposition following i.v. or inhalation, which cannot be adapted directly for oral THC products due to the additional complexity introduced during absorption process. To fill this literature gap, we aimed to develop a population pharmacokinetic model of THC and its metabolites to quantitatively characterize THC disposition in human following oral THC administration.

The clinical plasma PK data for THC and its metabolites were obtained from two published studies in 35 healthy subjects using cannabis brownies containing 10, 25 or 50 mg of THC. All the THC and its metabolites PK data were analyzed simultaneously using the non-linear mixed effect modeling with NONMEM (version 7.4.1) interfaced with Pirana.

Among the models tested, the final model for THC and its metabolites consisted of one depot compartment, one compartment for THC, and one compartment each for 11-OH-THC and THC-COOH, with first-order elimination for all three compounds. A first-order metabolic rate constant (Kgut) was added from the depot compartment to the 11-OH-THC compartment to address the first-pass metabolism.

Our model successfully captured the THC, 11-OH-THC, and THC-COOH pharmacokinetics following oral THC. With this model set as a starting point, our next step is to build a comprehensive model using data from all different routes of administration as well as from various biomatrix such as urine, oral fluid, and human tissue.

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54 **Activity driven ribosomal tagging strategy to map translatome changes in mouse hippocampal neurons activated by acute sleep deprivation**

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The hippocampus plays critical roles in learning and memory storage which are facilitated by sleep. In previous studies, hippocampus-dependent memories are impaired by brief sleep deprivation (SD) accompanied with deficits in synaptic plasticity and neuronal connectivity related to new protein formation. Later studies reveal hundreds of differentially transcribed or translated genes with considerable differences between transcriptomic and translatome changes as well as suppressed mTOR-mediated protein synthesis after SD. However, immediate early genes, such as cFos, are upregulated at all transcription, translation, and protein levels indicating an elevation of hippocampal activity. Therefore, to understand the consequence of SD more specifically, we are interested in revealing translatome changes in hippocampal neurons that are activated by SD. We expressed the cFos-promoted Cre together with tamoxifen-dependent Cre-promoted HAtagged ribosomal protein Rpl22 (RiboTag) in mouse hippocampus to capture SD-activated neurons. 5h SD was performed to induce RiboTag expression, 7 days later, we repeated SD to induce neuron activation and gene expression changes again. Immediately after the 2nd SD, the hippocampus were collected for immunohistochemistry staining (IHC) against HAtag and cFos. Our results show that SD significantly induces RiboTag expression, and the cFos-RiboTag system successfully captures the activity induction in the CA1 band with about 25% of labeled neurons reactivated during the 2nd SD. In conclusion, we established a workable tagging system to capture active neurons which allows for translatome isolation. In our future experiments, we will focus on CA1, which mediates memory processing and storage but has not really been focused on in SD studies. Translating RNAs will be extracted from immunoprecipitated Rpl22 protein for comparison between SD and non-SD mice. For the longer-term direction, transcriptomic analysis in SD-induced neurons will be incorporated for a broader yet specific view of how deficits occur and identify targets of intervention.

55 **A Pharmacometrics Model to Characterize a New Type of Target-Mediated Drug Disposition (TMDD) – Nonlinear Pharmacokinetics of Small-Molecule PF-07059013 Mediated by Its High-capacity Pharmacological Target Hemoglobin with Positive Cooperative Binding**

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Objectives: PF-07059013, a noncovalent hemoglobin modulator with promising preclinical efficacy to treat sickle cell disease (SCD), showed complex nonlinear pharmacokinetics (PK) in mice with the fraction of unbound drug in blood decreased with an increase in PF-07059013 concentrations/doses due to the positive cooperative binding of PF-07059013 to hemoglobin. Since the nonlinear PK of PF-07059013 is mediated by its pharmacological target hemoglobin, this interesting phenomenon falls into the category of target-mediated drug disposition (TMDD). However, it represents a new type of TMDD as it is caused by a high-capacity target (i.e. hemoglobin) and a special binding manner (positive cooperativity). The aim of our work was to develop a pharmacometrics model to quantitatively characterize the nonlinear PK of PF-07059013 in mice to gain a better understanding of this new type of TMDD and facilitate appropriate dose regimen selection for PF-07059013 future experiments in animals or clinical trials.

Methods: The PF-07059013 data used for model development were from a study reported by Gopalsamy et al in 2021, which included 4 groups (10 mg/kg, 40 mg/kg, 220 mg/kg, and 490 mg/kg) and was conducted in male sickle Townes mice. NONMEM 7.4.3 with user-defined subroutine ADVAN13 and FOCEI interfaced with Pirana was used to develop the PF-07059013 PK model and to perform the related simulation.

Results: Among different models evaluated, the best one was a two-compartment semi-mechanistic model where only drug molecules not bound to hemoglobin were allowed for elimination, with the nonlinear PK being captured by incorporating cooperative binding for drug molecules bound to hemoglobin. The final model provided valuable insight on target binding-related parameters. The Hill coefficient γ is estimated to be 1.6, confirming that this is a positive cooperativity since the value is >1. The binding constant K_H was estimated to be 1450 μM. The amount of the total hemoglobin (i.e., R_{tot}) was predicted to be 2.13 μmol,. In addition, the model simulated PF-07059013 blood concentrations following 200 mg/kg BID doses of PF-07059013 in mice were in line with the observed data, which further indicated that the final model is robust.

Conclusions: A semi-mechanistic pharmacometrics model was successfully established to characterize a new type of TMDD, where the nonlinear PK of PF-07059013 is mediated by its high-capacity pharmacological target hemoglobin with positive cooperative binding. This model could facilitate rational dose regimen selection for future studies for PF-07059013 as well as other compounds whose nonlinear PK are caused by similar mechanisms.