Pharmacological and Pharmaceutical Sciences Research Retreat

November 9, 2019

Levitt Center for University Advancement

Hosted by:

The Pharmacological Sciences Training Program

Department of Neuroscience and Pharmacology

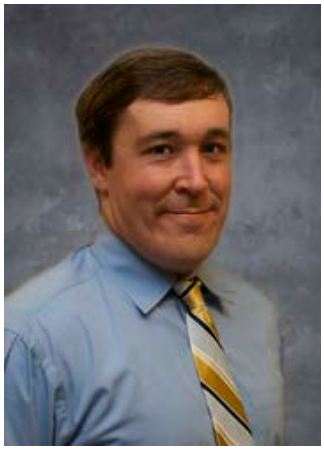
Department of Pharmaceutical Sciences and Experimental Therapeutics

The University of Iowa
2019 Pharmacological and Pharmaceutical Sciences Research Retreat
Levitt Center for University Advancement, Level 4

8:00 - 9:00 AM	Registration, Continental Breakfast and Poster Hanging (4th floor Assembly Halls)
9:00 AM	Welcome and Introduction: Stefan Strack, PhD (Morse Board Room)
9:05 - 10:20 AM	***Presentations by Trainees appointed to the TG*** *(Each speaker gets 12 minutes + 3 minutes Q&A)*
9:05 - 9:20	Rachel Crawford, MNPC/PSET Mentor: Jon Doorn, PhD ***A Reactive Dopamine Metabolite Inhibits Glutathione-S-Transferase but is Scavenged by L-carnosine and L-cysteine***
9:20 - 9:35	Alex Keyes, Neuroscience and Pharmacology Mentor: Yuriy Usachev, PhD ***Elucidating Presynaptic NMDA Receptors in Pain Sensitization***
9:35 - 9:50	Devon Moose, Cancer Biology Mentor: Michael Henry, PhD ***Cancer Cells Adapt to Hemodynamic Forces to Enhance Metastatic Behavior***
9:50 - 10:05	Maria Nunez-Hernandez, Biochemistry Mentor: Miles Pufall, PhD ***Mechanism of Selective Regulation of Genes Through Transcription Factor Phosphorylation***
10:05 - 10:20	Mackenzie Spicer, Molecular Medicine Mentor: Rory Fisher, PhD ***Defining the Role and Significance of RGS6 in Parkinson's Disease***
10:20 - 10:40 AM	Break
10:40 - 10:55 AM	Group Photo
11:00 - 12:00 PM	***Keynote Speaker*** (Morse Board Room) Kevin Tidgewell, PhD Associate Professor of Medicinal Chemistry Duquesne University Pittsburgh, PA ***Using Plant and Bacterial Secondary Metabolites to Target Membrane Receptors Involved in Disease***

12:00 - 12:30 PM	Lunch (Harding and Hawkinson Assembly Hall)
12:30 - 2:30 PM	Poster Session - Viewing/Judging (Green Assembly Hall)
12:30 - 1:30	Odd numbered posters
1:30 - 2:30	Even numbered posters
2:30 - 3:30 PM	***Faculty Presentations*** (Morse Board Room) *(Each speaker gets 25 minutes + 5 minutes Q&A)*
2:30 - 3:00	Thomas Nickl-Jockschat, MD, Associate Professor of Psychiatry ***Translational Research in Psychiatry: Focusing on Brain Structure and Function in Genetic Mouse Models***
3:00 - 3:30	Aislinn Williams, MD, PhD, Assistant Professor of Psychiatry ***L-type Voltage-gated Calcium Channels in Models of Neuropsychiatric Disease***
3:30 - 4:00 PM	*Award Ceremony and Closing*

Keynote Speaker



Kevin Tidgewell, PhD
Associate Professor of Medicinal Chemistry
Duquesne University
Pittsburgh, PA

Using Plant and bacterial Secondary Metabolites to Target Membrane Receptors Involved in Disease

The Tidgewell lab works in the fields of Medicinal and Natural Products Chemistry. Our work focuses on the discovery of novel ligands from marine cyanobacteria for GPCR targets. Projects in the lab range from extraction, isolation and structure elucidation of novel leads to semi-synthetic medicinal chemistry lead optimization. The major focus of our work is on pain and addiction with the broader scope being GPCRs involved in regulating CNS processes and other complex disease states.

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Abstracts

1 **Cancer Cells Adapt to Hemodynamic Forces to Survive Transit in Circulation and Enhance Metastatic Behavior**

Devon Moose, MS[1,2], Benjamin Krog, MS[1], Lei Zhao, PhD[1], Tae-Hyung Kim, PhD[8], Sophia Williams-Perez, BS[3], Gretchen Burke, BS[1], Lillian Rhodes, BSE[1], Marion Vanneste, PhD[1], Mohammed Milhem, MBBS[4,5], Christopher Stipp, PhD[1,5,6], Amy Rowat, PhD[8], and Michael Henry, PhD[1,2,5,7]

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During metastasis, circulating tumor cells (CTCs) are formed as obligate intermediates that function to seed distant sites. In the fluid microenvironment of the circulation, CTCs are exposed to hemodynamic forces, including fluid shear stress (FSS), which are potentially mechanically destructive. However, recent studies evaluating the effects of FSS on cancer cells have found that transformed epithelial cells exhibit elevated resistance to FSS compared to non-transformed epithelial cells from the same tissues. FSS resistance has been shown to depend on the nucleo- and cyto-skeleton. Here we show that cancer cells rapidly activate Rho GTPases after exposure to brief (millisecond) pulses of FSS. Moreover, we demonstrate that RhoA-myosin II axis prevents FSS-induced plasma membrane damage. To determine if the RhoA-myosin II axis is required for FSS resistance of CTCs in vivo, we utilized two different mouse models. In the first model, we pre-treated cells with either 20μM blebbistatin, a myosin II inhibitor, or a vehicle control and different fluorescent dyes before injecting the cells as a mixture with 15μm fluorescent microspheres into the tail vein of a mouse. We then collect blood and the lungs of the mice within 3 minutes of injection. From sections of the lungs we found that ~10% fewer blebbistatin treated cells are lodged in the lung microvasculature. By measuring the release of intracellular luciferase into the plasma to quantitate cell destruction we found that ~2 times more blebbistatin-treated cells are destroyed in circulation. In the second model, we treated mice bearing orthotopic prostate tumors with (2.5mg/kg) blebbistatin for 3 hours and found a 10-fold reduction in steady-state levels of CTCs. Since Rho GTPases have been shown to be important in the formation of metastases, we asked whether exposure to FSS “primes” cancer cells for metastatic colonization. Thus, we exposed PC-3 prostate cancer cells to FSS in vitro and evaluated metastatic colonization and survival following tail-vein injection. We observed a 30% reduction in median tumor-free survival compared to cells under static conditions. Taken together, our data shows that cancer cells rapidly adapt to exposure to FSS in the fluid microenvironment to promote cell survival and metastasis.

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2 **Adolescent alcohol use and behavior dysregulation in later life: Roles for serotonin and microglia**

Kanza M. Khan, Gabrielle Bierlein-De La Rosa, Catherine A. Marcinkiewcz

Department of Neuroscience and Pharmacology, University of Iowa

Episodic binge drinking during adolescence is a significant public health problem that has been associated with many long-lasting and deleterious effects on brain function and mental health. Emerging evidence suggests that chronic alcohol can exacerbate anxiety and pain, leading to repeated cycles of alcohol and other drug-seeking behaviors to alleviate these debilitating symptoms. The dorsal raphe nucleus (DRN) is a major source of serotonin (5-HT) neurons that project widely throughout the brain, playing a role in a variety of behaviors. Accumulating evidence suggests that 5-HT neurons in the DRN play a crucial role in aversive behavior, indicating that alcohol-induced neuroadaptations in 5-HT signaling may result in permanent behavioral changes. Using a model of intermittent access to alcohol drinking in adolescent C57BL/6J mice, we observed a substantial reduction in 5-HT neurons in the rostral DRN in adulthood, while 5-HT neurons in the caudal DRN and median raphe nucleus (MRN) remained intact. This was accompanied by an increase in cell body area of microglia in the rostral DRN, indicating that these microglia remain in an activated state. Alcohol drinkers also exhibited an increase in anxiety in the open field, as well as reduced pain thresholds in the Von Frey test relative to water drinkers. These results suggest that chronic intermittent alcohol exposure during adolescence causes persistent activation of microglia in the DRN, resulting in 5-HT neuronal loss in the rostral DRN which in turn induces anxiety and pain-related behaviors. Current studies will determine whether targeting microglia could rescue these physiological and behavioral phenotypes of adolescent alcohol use.

3 **cAMP MEDIATES THE IMPACT OF SLEEP DEPRIVATION ON MEMORY AND SYNAPTIC PLASTICITY**

Emily Walsh, Mahesh Shetty, Ted Abel

Department of Neuroscience and Pharmacology, Iowa Neuroscience Institute, University of Iowa, Iowa City, IA

We spend nearly one-third of our life asleep, yet the biological reasons for this state have not been identified. Sleep facilitates memory storage, and sleep loss leads to impairments in memory. Memory for tasks that involve the hippocampus, a brain region that mediates memory for facts and events, is particularly sensitive to sleep loss. Our lab has found that within the hippocampus, sleep loss leads to deficits in synaptic plasticity, decreased dendritic spine density, and decreased protein synthesis. Previous work in our lab has also found that sleep deprivation reduces levels of cyclic AMP (cAMP) in the hippocampus, and that the reduction of cAMP mediates the memory loss that accompanies sleep deprivation. Our hypothesis is that cAMP not only acts as a mediator for these cognitive outcomes of sleep deprivation, but also for the observed deficits in hippocampal plasticity. By expressing the *Drosophila Melanogaster* G-protein coupled octopamine receptor (OAR) in excitatory hippocampal neurons, we were able to selectively increase cAMP levels in those neurons during a five-consecutive hour sleep deprivation period. Immediately following sleep deprivation, hippocampal slices were prepared for electrophysiological recordings. We found that cAMP rescue during the five-hour deprivation period prevents deficits in 4-train long-term potentiation (LTP), a long lasting (late) form of LTP. This finding demonstrates that cAMP mediates not only the hippocampal-memory effects of sleep deprivation, but also some of the changes in hippocampal synaptic plasticity. Future work will focus on the effect of cAMP rescue on other forms of LTP, and on the effects of cAMP rescue on dendritic spine density in the hippocampus.

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4 **Exploring the phospho-proteome of Jordan's Syndrome, an autosomal dominant neurodevelopmental disorders caused by *de novo* mutations in a protein phosphatase 2A regulatory subunit**

Chian Ju Jong, Ronald A Merrill, Yu Fang Kong, Stefan Strack
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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 would enhance cellular signaling cascades that promote growth and proliferation. To identify cellular and biochemical phenotypes of JS causing ID, we generated stable HEK293 cell lines inducibly expressing either wild-type or mutant B'δ using a PP2A reduction model. We characterized the most common B'δ mutation in JS, E198K. The PP2A reduction cell lines were induced with doxycycline or vehicle for at least three days followed by assessment of PP2A subunit expression, cellular growth, and substrate dephosphorylation by unbiased phosphoproteomics. In our reduction model, wild-type and mutant B'δ were overexpressed by about 3-fold, concomitant with a 2- to 3-fold loss of endogenous PP2A regulatory subunits. Quantitative global phosphoproteomic analyses of reduction and overexpression models shows that the reduction model, but not the overexpression model, defines consensus dephosphorylation motifs for B'δ-containing PP2A holoenzymes. In the reduction model, wild-type B'δ expression caused growth arrest within 4 days, while the E198K mutation attenuated the growth inhibitory effect of B'δ. The proliferative effects of wild-type and mutant B'δ are likely associated with a change in substrates specificity. Indeed, our global phosphoproteomic analyses suggest that wild-type B'δ preferentially dephosphorylates substrates containing positively charged residues, while E198K-mutant B'δ favors substrates containing negatively charged residues adjacent to the phosphorylation site. Our data also suggest that the E198K mutation confers high basal activity to the PP2A holoenzyme, while wild-type PP2A/B'δ requires phosphorylation by PKA for full activity. In conclusion, using the PP2A reduction model, we provide preliminary evidence that *de novo* mutations in PPP2R5D blunt the growth inhibitory effect of B'δ likely by a change in PP2A substrates specificity. We speculate that altered PP2A activity in JS deregulates signaling pathways mediating cell proliferation, differentiation, and morphogenesis, which in turn leads to abnormal brain development.

5 **Targeting RABL6A-RB1 Signaling Suppresses MPNST Pathogenesis**

Jordan L Kohlmeyer[1,2], Courtney A Kaemmer[2], Casey Pulliam[3], Chandra K Maharjan[2], Allison Moreno Samayoa[4], Heather Major[10], Kendall Cornick[10], Vickie Knepper-Adrian[5], Rajesh Khanna[7], Jessica C Sieren[6], Mariah R. Leidinger[8], David K. Meyerholz[8], Gideon Zamba[9], Jill M Weimer[11], Rebecca D Dodd[1,5], Benjamin W Darbro[10], Munir R Tanas[8], and Dawn E Quelle[1,2,8,#]

Molecular Medicine Graduate Program[1], The Department of Neuroscience and Pharmacology[2], Human Toxicology Graduate Program[3], Post Baccalaureate Research Education Program[4], and Departments of Internal Medicine[5], Radiology[6], Pathology[8], Biostatistics[9], Pediatrics[10], in the Colleges of Medicine or Public Health, Holden Comprehensive Cancer Center, University of Iowa, Iowa. Department of Pharmacology[7], University of Arizona, Tucson, AZ. Pediatrics and Rare Diseases Group[11], Sanford Research, Sioux Falls, SD

Background: Malignant peripheral nerve sheath tumors (MPNSTs) are deadly sarcomas that lack effective therapies. Greater insight into MPNST pathogenesis is needed to develop new, more targeted treatments. In most MPNSTs, the retinoblastoma (RB1) tumor suppressor is disabled by hyperactivation of cyclin dependent kinases (CDKs), commonly through loss of CDK inhibitors such as p27(Kip1). RABL6A is an oncogenic GTPase and newly recognized inhibitor of the RB1 pathway whose role in MPNST biology has not been studied. We hypothesized the RABL6A-RB1 pathway is an important, new therapeutic target and critical driver of MPNST pathogenesis.

Methods: RNA-Seq and immunohistochemical analyses of tissue microarrays (TMAs) containing human patient-matched PNFs and MPNSTs were conducted. RABL6A-RB1 pathway silencing was performed in multiple MPNST cell lines. Analyses included: western blotting of RABL6A-RB1 pathway, proliferation and viability, and drug response assays. Orthotopic xenografts were generated using MPNST cell lines in immunocompromised mice. Mice were treated with CDK inhibitors (alone or in combination) and tumor growth was monitored. Western blotting and immunohistochemical analyses were conducted on tumor samples.

Results: We discovered dramatic upregulation of *RABL6A* mRNA and protein in human MPNSTs compared to precursor neurofibromas. Remarkable overlap existed between the RABL6A gene expression signature and that found in the patient MPNSTs. Tumor expression of p27 and RABL6A proteins was inversely correlated. Silencing RABL6A in MPNST cell lines caused cellular death and G1 phase arrest that coincided with p27 upregulation, CDK downregulation, and RB1 activation. The growth suppressive effects of RABL6A loss, and its regulation of RB1, were largely rescued by p27 depletion. These findings establish a critical role for RABL6A-p27-RB1 signaling in MPNST pathogenesis. Indeed, reactivation of RB1 using a CDK4/6 inhibitor (palbociclib) killed MPNST cells *in vitro* in a RABL6A-dependent manner and suppressed MPNST growth *in vivo*. Resistance to CDK4/6 inhibitor monotherapy became apparent towards the end of treatment, which has been previously attributed to compensation via CDK2. Combination of palbociclib plus dinaciclib, a CDK2 inhibitor, showed that dual CDK targeted therapy prevented tumor growth more effectively than single agent treatment.

Conclusions: This work identifies RABL6A as a powerful driver of MPNST proliferation and survival that acts, in part, through p27-RB1 inactivation. Our findings suggest that RB1 reactivation in tumors using multiple CDK inhibitors may reduce drug resistance and effectively treat MPNSTs.

6 PVN RGS2 is Critical for the Regulation of Energy Homeostasis

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Obesity has become a major public health concern due to its high risk of developing life-threatening chronic diseases, such as hypertension and diabetes. While there is a general consensus that the central nervous system (CNS) plays a crucial role in the regulation of energy balance, the underlying neural substrates mediating this effect remain incompletely understood. Accumulating evidence implicate the paraventricular nucleus of hypothalamus (PVN) as a key intersection point that regulates energy homeostasis. However, the detailed regulatory components within the PVN that are critical for these physiological processes have not been thoroughly identified. Regulator of G protein signaling (RGS) proteins function as endogenous negative regulators of GPCR signaling. RGS2 belongs to the B/R4 family of RGS proteins and *RGS2* knockout mice exhibit resistance to age-related weight gain. Since our RNAscope analyses revealed that RGS2 is highly expressed in PVN neurons – some of which express melanocortin 4 receptor (MC4R) that is essential for energy homeostasis, here we hypothesized that PVN RGS2 plays an important in the regulation of energy balance. To test this hypothesis, we selectively manipulated RGS2 expression in the PVN of adult mice by stereotaxic microinjection of AAV-Cre-GFP or AAV-GFP into the PVN of *RGS2^{Flox}* mice. In striking contrast to decreased body weight in whole-body RGS2 knockout mice, specific deletion of RGS2 in the PVN (*RGS2^{Flox}* mice received AAV-Cre-GFP injection into the PVN) resulted in significantly increased body weight compared to their control littermates (*RGS2^{Flox}* mice received AAV-GFP injection into the PVN) without significant changes in food intake or resting metabolic rate. Body composition analysis by nuclear magnetic resonance (NMR) indicates that both fat mass and lean mass are increased, suggesting potential role of PVN RGS2 in neuroendocrine regulation of growth. These results identify a unique role for PVN RGS2 in the homeostatic regulation of body weight and provide first evidence that there might be an opposing role of central versus peripheral RGS2 in terms of energy balance.

7 Defining Pre- and Post-synaptic Ca^{2+} Signaling in the Spinal Cord in Pain Sensitization

Alex L. Keyes[1], Charles A. Warwick PhD[1], Leonid P. Shutov PhD[1], Volodymyr V. Krotov PhD[2], Pavel V. Belan PhD[2], and Yuriy M. Usachev PhD[1]

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Chronic pain affects approximately 100 million Americans and only a minority of patients experience satisfactory relief of their pain with currently available pharmaceutics. One type of chronic pain caused by direct injury to the nerve is called neuropathic pain, and it affects ~10% of the overall population. Despite the prevalence, the underlying mechanisms of neuropathic pain are not well-defined, and better understanding of the mechanisms that promote central sensitization after injury could lead to better treatment of this condition. Among various mechanisms, central sensitization, or the enhancement of synaptic transmission in the spinal cord, is thought to be especially important in the pathogenesis of neuropathic pain. Primary afferents, responsible for transducing painful stimuli, terminate in the spinal cord at what is called the first sensory synapse. This synapse is a key regulator of pain signaling, and aberrant processes at this synapse can lead to an amplification of pain. As most researchers have only examined the post-synaptic signaling of this synapse via patch-clamp recordings, the critical questions regarding the role of presynaptic changes in Ca^{2+} signaling in central sensitization have not been addressed. Furthermore, there are many questions remaining regarding the relationship between pre- and post-synaptic changes in Ca^{2+} signaling and Ca^{2+} signaling in microglia in central sensitization. We are now able to examine Ca^{2+} signaling in the spinal cord with high spatial (~0.5 µm) and temporal (~100 ms) resolution while retaining neuronal networks using an *ex vivo* intact spinal cord preparation and multiphoton microscopy to image Ca^{2+} signaling in mouse lines that express GCaMP3 in pre-synaptic neurons, RGECO in post-synaptic neurons, as well as GCaMP5G-TdTomato in microglia.

8 **Investigating the role of a *Prkar1b* mutation previously associated with a rare neurodegenerative disorder**

Marie E. Gaine[1,2], Michela Argue[1,2], Ronit Ilouz[3], and Ted Abel[1,2]

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[2]Iowa Neuroscience Institute, University of Iowa, Iowa City, IA
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The Protein Kinase CAMP-Dependent Type I Regulatory Subunit Beta (*Prkar1b*) gene encodes a regulatory subunit of cyclic AMP-dependent protein kinase A (PKA). Within the first coding exon of this gene, a heterozygous missense mutation was identified in a family with FUS-negative neuronal intermediate filament inclusion disease. The affected family members showed behavioral changes including motor deficits, increased anxiety and memory loss and, therefore, we wanted to study the impact of this mutation in different behavioral paradigms. To do this, we replicated the mutation in a mouse model using CRISPR/Cas9 technology and performed both behavioral and molecular testing. We chose to initially study the potential motor deficits in this mouse model using Rotarod testing. Because the human phenotype was seen at age 45-64, we tested a wide range of ages (2months -19 months old). There was no overall difference between the groups (Wildtype N=45, *Prkar1b*$^{+/-}$ N=40; mixed sex cohort). Notably, when we focused only on aged mice (15-19 months old) we found a significant decrease in latency to fall in *Prkar1b* mice (N=16) compared to wildtypes (N=12) on the second and third day of testing ($P<0.05$). On the molecular level, the presence of the mutation has been predicted to be damaging (PolyPhen-2) and to alter splice sites (MutationTaster). Therefore, we used Q-PCR to quantify *Prkar1b* gene expression and confirm the functional relevance. In the cerebellum, hippocampus, and striatum, *Prkar1b* expression was not differentially changed in *Prkar1b* mice. However, using immunohistochemistry we found an increase of Prkar1b protein in the hippocampus. Further work has begun to investigate anxiety behavior and memory deficits in our mouse model. Complete characterization will further our understanding of this gene and its role in neurodegenerative disorders.

Keywords: Prkar1b, PKA, Aging

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9 **Development of a Broadly Applicable Method for Identification and Analysis of Polychlorinated Biphenyl Sulfates in Human Serum**

Duo Zhang[1,2], Panithi Saktrakulkla[1,3], Kris Tuttle[2], Rachel F. Marek[3], Hans-Joachim Lehmler[1,4], Keri C. Hornbuckle[1,3], and Michael W. Duffel[1,2]

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Polychlorinated biphenyls (PCBs) are a class of toxic persistent chemicals with both legacy sources (e.g. Aroclors) and new current sources (e.g., unintentionally produced contaminants in some pigments and varnishes). PCB sulfates are metabolic products derived from hydroxylated metabolites of PCBs (OH-PCBs). Both OH-PCBs and PCB sulfates exert multiple toxicological effects on human health such as disrupting thyroid hormone transport and inhibiting steroid sulfotransferases (SULT1E1 and SULT2A1). Although PCB 11 sulfate has been previously detected in human serum samples, the lack of a generally applicable method for a broad range of PCB sulfates in human serum has limited our understanding of their prevalence and importance. We propose a method that employs acetonitrile extraction of the PCB sulfates from serum followed by differential analysis with, and without, hydrolysis to OH-PCBs catalyzed by a purified sulfatase from *Helix pomatia*. After purification of the sulfatase by affinity chromatography, its broad specificity for PCB sulfates indicated the feasibility of its use for their quantitative hydrolysis to OH-PCBs. These OH-PCBs were quantitated by GC/MS/MS after derivatization to their corresponding methoxy PCBs. Using ^{13}C-OH-PCB 29 as surrogate standard, we were able to recover 76 ± 7 % of 10 ng of 4-PCB 11 sulfate added to 2 g of pooled human serum. An initial evaluation of this method in individual human serum samples has led to observation of PCB 25 sulfate, a metabolite of the commonly occurring PCB 28. Further analyses of serum samples for PCB sulfates are in progress. [Supported by NIH: P42 ES013661]

10 **β-klotho in Glutamatergic Cells is Necessary for FGF21-mediated Weight Loss**

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Fibroblast growth factor 21 (FGF21) is an endocrine hormone that controls energy homeostasis. Critically, the metabolic effects of FGF21 on energy expenditure occur through its action in the central nervous system. *In silico* reanalysis of data obtained from single cell RNA sequencing demonstrated that FGF21 co-receptors, FGFR1 and β-klotho (KLB), are both expressed on glutamatergic (Vglut2+) cells within the hypothalamus. Thus, we hypothesize that FGF21 signals to glutamatergic cells within the hypothalamus to control energy expenditure and overall body weight. To determine whether β-klotho is required in glutamatergic (Vglut2+) expressing cells to mediate the metabolic actions of FGF21, we generated a novel mouse model which lacks β-klotho in Vglut2-expressing cells ($KLB^{Vglut2\text{-}KO}$ mice). Intriguingly, diet-induced obese (DIO) $KLB^{Vglut2\text{-}KO}$ mice do not respond to FGF21 administration with any increase in energy expenditure or decrease in body weight suggesting that FGF21 signaling to glutamatergic cells is absolutely required for FGF21's effects to regulate body weight. To determine whether overexpression of β-klotho can protect mice from DIO, we generated a novel mouse model which overexpresses β-klotho specifically in Vglut2-expressing cells ($KLB^{Vglut2\text{-}TG}$ mice). Despite significant overexpression of β-klotho within the hypothalamus, $KLB^{Vglut2\text{-}TG}$ mice still exhibit significant increases in body weight after high fat diet consumption. Taken together, these data suggest that FGF21 signaling to glutamatergic cells is required for pharmacological, but not physiological, regulation of body weight by FGF21.

11 **Disruption of Endothelial BBSome Causes Vascular Dysfunction and Hepatosteatosis**

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Almost every single cell in the human body possesses primary cilium. The primary cilium function as a sensor of blood flow and sheer stress and contribute to both calcium and nitric oxide signaling in the endothelium. The BBS1 protein is an important member of the BBSome complex which mediates protein trafficking to the cilia membrane and other cellular compartments. To investigate the role of the BBSome in endothelial cells, we generated mice lacking the *Bbs1* gene specifically in endothelial cells by crossing the $Bbs1^{fl/fl}$ mice with the endothelial–specific $Tie2^{cre}$ mice. The $Bbs1^{fl/fl}/Tie2^{Cre}$ mice developed normally compared to littermate controls. We assessed vascular function *ex vivo* using aortic rings and resistance-sized mesenteric arteries. Interestingly, loss of the *Bbs1* gene in endothelial cells caused endothelial dysfunction with a more severe effect in female mice as indicated by the impaired acetylcholine (ACh)-induced. Of note, the relaxation responses evoked by sodium nitroprusside in both the aorta and mesenteric artery were not different in male and female $Bbs1^{fl/fl}/Tie2^{Cre}$ mice, indicative of endothelial but not smooth muscle dysfunction in the endothelium-specific Bbs1 null mice. Blood pressure tended to be elevated in $Bbs1^{fl/fl}/Tie2^{Cre}$ mice particularly females. Strikingly, endothelial *Bbs1* gene deletion caused hepatosteatosis as indicated by the significant lipid deposition in the liver of $Bbs1^{fl/fl}/Tie2^{Cre}$ mice by oil-red-O staining. Lipid extraction revealed increase in triglycerides and cholesterol in the liver of $Bbs1^{fl/fl}/Tie2^{Cre}$ mice. Consistent with this, $Bbs1^{fl/fl}/Tie2^{Cre}$ mice exhibited reduction in the expression of liver fatty acid transport and binding protein (Fatp2 and Fabp1). Thus, we conclude that the endothelial BBSome is required for the control of vascular endothelial function and hepatic lipid metabolism.

12 **A reactive dopamine metabolite inhibits Glutathione-S-Transferase but is scavenged by L-carnosine and L-cysteine**

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Parkinson's disease (PD) is a neurodegenerative disorder that is characterized by the death of dopaminergic cells in the substantia nigra. Motor dysfunction results from this neuronal loss. While the etiopathogenesis of PD is not well understood, it is accepted that dopamine dyshomeostasis plays an important role and represents a mechanistic link between exposure to environmental agents, such as organochlorines, and neurodegeneration. Dopamine is metabolized to 3,4-dihydroxyphenylacetaldehyde (DOPAL) by monoamine oxidase before it is further detoxified by aldehyde dehydrogenase to 3,4-dihydroxyphenylacetic acid (DOPAC). It is established in literature that DOPAL, a highly reactive electrophile, can cause aggregation of alpha-synuclein, a phenomenon observed in PD. The speculation that aberrant levels of DOPAL can result in protein aggregation, oxidative stress, cell death, and potentially disease is called the catecholaldehyde hypothesis. The goal of this study is to identify mechanistic targets of DOPAL relevant to disease. This is valuable because it may yield elucidation of biomarkers for earlier diagnosis or mechanistic targets for drug discovery.

A previous study identified Glutathione-S-Transferase (GST) as modified by DOPAL *in vitro* via a proteomic scan. GST is an important enzyme involved in the detoxification of xenobiotics and endogenous toxins. Inhibition of GST activity could lead to a hostile cell environment and cause cell death and disease. This project utilizes GST isolated from dopaminergic cells to examine the effects of dopamine and its metabolites on GST function. Dopamine, DOPAL, and DOPAC inhibit GST activity in a time and concentration dependent manner.

Furthermore, this study has identified and quantified conjugation of dopamine metabolites with the scavengers L-carnosine and L-cysteine. Nitroblue tetrazolium, a stain that identifies the presence of quinones, was used to quantify DOPAL-protein adducts. Staining was significantly reduced with the addition of L-cysteine or L-carnosine. Analytical methods such as HPLC-ECD (electrochemical detection) and LC-MS were used to identify DOPAL conjugates. DOPAL-cysteine and DOPAL-carnosine conjugates have been identified. Activity of GST incubated with DOPAL is restored when the solution also contains millimolar concentrations of L-carnosine, L-cysteine, or glutathione. These compounds prevent cell death when dopaminergic cells are assaulted with rotenone, a pesticide implicated in PD.

13 **The translin/trax microRNA-degrading complex mediates translation-dependent synaptic plasticity in the hippocampus**

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Enduring forms of synaptic plasticity require protein synthesis which involves local translation of mRNAs that have been pre-positioned near the synapses. As the microRNA system suppresses translation, the ability of plasticity-inducing stimuli to trigger rapid degradation of microRNAs could play a key role in driving *de novo* translation underlying persistent forms of synaptic plasticity. As recent studies have identified the translin/trax (TN/TX) complex as a microRNA-degrading enzyme enriched in brain, we are investigating the hypothesis that the TN/TX complex mediates translation-dependent forms of synaptic plasticity. In support of this, we have found that translin KO mice display defects in synaptic tagging and spatial object location memory (Park et al., 2017). However, since translin deletion also causes loss of trax protein, which has been shown to exert translin-independent cellular effects, it is unclear if the defects in synaptic plasticity or memory displayed by translin KO mice are due to loss of the TN/TX microRNA-degrading enzyme.
To address this, we have generated mice containing a point mutation in trax, E126A, that abolishes TN/TX RNase activity and are assessing if this mutation is sufficient to phenocopy the defects in synaptic plasticity and memory displayed by translin KO mice. In preliminary studies, we have confirmed that this mutation does not alter the expression of translin or trax proteins in the brain, nor their ability to co-precipitate. As a first step in assessing the impact of the TraxE126A mutation on hippocampal synaptic plasticity, we have evaluated its effect on two stimulation paradigms that induce early-LTP and late-LTP which differ in their requirement for translation. We have found that TraxE126A mice show deficits in late-LTP but not early-LTP. Thus, these findings strongly support the hypothesis that synaptic activation of the TN/TX microRNA-degrading enzyme is involved in *de novo* translation critical for persistent LTP.

14 **CDK-targeted combination therapies of pancreatic neuroendocrine tumors (PNETs) guided by RABL6A-dependent regulation of the PNET kinome and phosphoproteome**

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Background: More effective therapeutics are needed to improve survival of advanced PNET patients. RABL6A is a novel oncoprotein that drives PNET proliferation and survival through multiple pathways, including suppression of the retinoblastoma (RB1) tumor suppressor via cyclin-dependent kinase (CDK) activation. In recent PNET clinical trials, overall patient survival has not been improved by CDK4/6 monotherapy, warranting the development of combination targeted therapies. Here, we examined the global PNET kinome and phosphoproteome to identify other kinases regulated by RABL6A whose inhibition may synergize with CDK4/6 inhibitor therapy.

Methods: Quantitative proteomics (kinome and phosphoproteome analyses) of PNET cells that express or lack RABL6A was performed. Effects of altered RABL6A expression on cellular protein (immunoblotting), proliferation & survival (trypan blue exclusion, cell counting, colony formation), drug sensitivity (AlamarBlue), and tumor growth / drug response in vivo (mouse xenografts) were measured.

Results: RABL6A depletion in PNET cells caused significant downregulation of >1,100 cellular phosphoproteins and reprogramming of the global kinome. Expression of RABL6A was required for activity of many cell cycle, mitotic and tumor-promoting kinases, including CDKs 1/2/6, Aurora kinases A and B, Polo-like kinase 1 and select tyrosine directed and MAP kinases. Cellular analyses verified PNET cell sensitivity to CDK4/6 inhibitors (e.g., palbociclib) was dependent on RABL6A expression. The combination of drugs targeting CDK4/6 and CDK1/2, which more fully activates RB1 tumor suppressive activity, was more effective against PNET growth than either inhibitor alone. Ongoing studies are examining the efficacy of other innovative, rational therapies that combine inhibitors of CDKs with drugs targeting other RABL6A regulated kinases.

Conclusion: Quantitative kinome and phosphoproteomic analyses, which have not been performed before in NETs, identified a global kinase signature associated with PNET proliferation / survival and RABL6A signaling. This approach allows rational design of novel kinase-targeted combination therapies for NET patients.

15 **MECHANISMS THAT UNDERLIE THE ANTICONVULSANT EFFECTS OF MITOCHONDRIAL Ca^{2+} UNIPORTER DELETION**

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Epilepsy affects approximately 70 million people worldwide and approximately 30% of those people develop refractory epilepsy, which cannot be controlled by the current available pharmaceutics. Refractory epilepsy increases a person's risk for sudden unexpected death in epilepsy (SUDEP), which is the leading cause of death in epilepsy patients. Epilepsy is characterized by increased neuronal network excitability and an important aspect of neuronal network regulation are cytosolic Ca^{2+} levels. Neuronal cytosolic Ca^{2+} concentrations are regulated by mitochondria which efficiently buffer cytosolic Ca^{2+} and shape Ca^{2+} signals. Mitochondrial Ca^{2+} transport is controlled by a complex of proteins though the molecule known as the mitochondrial Ca^{2+} uniporter (MCU) mediates mitochondrial Ca^{2+} uptake by forming a Ca^{2+} permeable channel. Deletion of MCU results in impaired Ca^{2+} uptake into mitochondria in neurons, as would be expected. Interestingly, we also found that global MCU deletion resulted in a profound anticonvulsant effect in mice in a maximal electroshock seizure threshold (MES) paradigm. Electrophysiological experiments revealed increased GABAergic synaptic activity in MCU-KO mice, possibly explaining the anticonvulsant phenotype. To further determine if the anticonvulsant effect is driven by increased GABAergic signaling, we developed MCU floxed mice and crossed them with pan neuronal specific Nestin-Cre, excitatory CaMKII-Cre, and inhibitory VGAT-Cre mice. We determined that pan neuronal and inhibitory neuron specific deletion of MCU resulted in increased MES thresholds, but excitatory specific deletion did not. Collectively, these experiments elucidate the mechanisms that underlie the robust anticonvulsant effects of MCU deletion. The results of these studies begin to provide insights into the role that mitochondrial Ca^{2+} transport in epilepsy and could guide the development of new therapeutic approaches targeting MCU and other mitochondrial Ca^{2+} transporters for treatment of epilepsy.

16 **Transcriptomic Changes in the Arcuate Nucleus of the Hypothalamus in Response to Prolonged High Fat Diet: Insights from Single-Nucleus RNA-Sequencing**

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Selective leptin resistance (SLR) describes a pathological state caused by prolonged high fat diet (HFD) feeding in which cardiovascular responses to leptin are preserved, but metabolic effects of leptin are attenuated. SLR is therefore hypothesized to contribute to obesity-associated hypertension, but the mechanisms causing SLR remain unclear. Neurons expressing agouti-related peptide (Agrp) within the arcuate nucleus (ARC) represent a major target for leptin signaling and may play a role in SLR. Previous studies reported that a 10-week HFD feeding is sufficient to cause SLR in mice despite elevated plasma leptin concentration and that short term HFD feeding suppressed Agrp expression. To examine the transcriptomic changes induced by prolonged HFD exposure in Agrp neurons, we performed single-nucleus RNA-sequencing on nuclei isolated from ARC of ad libitum fed male C57BL/6J mice fed standard chow diet (7013; CD, n=5) or 45% HFD (D12451; n=6) from 8 to 18 weeks of age. As expected, HFD caused increased food intake (10 wk of HFD: +13.1±1.4 vs +7.2±0.3 kcal/d, $p<0.01$) and fat mass gains (10 wk of HFD: +10.1±1.4 vs +3.2±0.4g, $p<0.01$). After pooling biological replicates and filtering out nuclei with low quality reads, a total of 6,621 (CD) and 14,396 (HFD) nuclei remained. Unbiased clustering (using Seurat R package) identified 22 cell-type clusters, including a unique cluster with high expression of Agrp (n=136 CD, 453 HFD cells). HFD caused differential expression of 73 genes in the Agrp cluster, including genes previously implicated in feeding and energy balance such as Grm5 (68% of CD, Bonferroni $p<0.01$), Fth1 (81%, $p<0.01$), though surprisingly, Agrp itself was not suppressed (126%, $p=0.11$). Ingenuity Pathway Analysis revealed gene expression perturbations in canonical pathways implicated in the pathogenesis of obesity, including "Leptin Signaling in Obesity" and "Protein Kinase A Signaling." Taken together, these results underscore the role for dysregulated Agrp expression in the development of SLR.

17 **Targeting mitochondrial fission for neuroprotection in peripheral diabetic neuropathy**

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Diabetes has become a fully-blown global epidemic requiring global attention. Presenting with loss of sensation and chronic pain, peripheral diabetic neuropathy (PDN) is a debilitating co-morbidity affecting at least 50% of the diabetic patients. With palliative care being the only option, there is an urgent need of innovative therapies for PDN. Emerging evidence recently indicated compromised mitochondria structure and function in diabetes. Mitochondria forms highly dynamic networks that constantly undergoing the process of fission and fusion, governed by the dynamin family of large GTPases. Balanced fission and fusion are required for proper function of mitochondria. Interestingly, excessive mitochondrial fission was implicated in many neurodegenerative disorders including Alzheimer's and Huntington's diseases. Therefore, we investigated whether targeting mitochondrial fission can be a potential therapeutic strategy for PDN. Dynamin-related protein 1 (Drp1) is an essential mitochondrial fission enzyme. Drp1 is activated by dephosphorylation via two phosphatases including calcineurin and a neuron-specific, mitochondria localized isoform of protein phosphatase 2A containing the Bβ2 regulatory sub-unit (PP2A/Bβ2). We generated the Bβ2 knock-out (KO) mouse in which we observed elongated mitochondria in neurons as well as reduced high-glucose induced superoxide production in their DRG neurons. Bβ2 KO mice also showed increased mitochondrial axonal localization, while preventing axonal mitochondrial fission and depletion in sciatic nerve in the STZ model of type-1 diabetes. These animals were shown to be protected from thermal hypoalgesia. Moreover, KO of Bβ2 also showed protection from both thermal and mechanical hypoalgesia as well as impaired nerve conductivity in db/db mice modeling type-2 diabetes. Taken together, our preliminary data showed that Bβ2 KO animals are resistant to PDN in both type-1 and type-2 diabetes models. Hence, targeting mitochondrial fission through PP2A/Bβ2 shed light onto developing an innovative treatment for PDN.

18 **NEK2 Mediated Epigenetic Reprogramming in Multiple Myeloma Progression**

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Epigenetics is the collective heritable changes in phenotype through mechanisms that are independent of alterations in the primary DNA sequence [1]. Epigenetic changes have been observed in early stages of tumor development and described as contributing to cancer initiation and progression. Histone modifications (post-translational modifications to the N-terminal tails of histone proteins) and DNA methylation are two common modifications observed in many cancers including breast cancer [3], colon cancer [4], lung cancer [5], and multiple myeloma [6]. Multiple Myeloma (MM) is a presently uncured malignant neoplasm of plasma cells in the bone marrow. MM is characterized by the presence of overproduction of monoclonal (M)-protein, anemia, calcium dysregulation, and bone damage and is clinically preceded by two asymptomatic plasma cell dyscrasias, smoldering multiple myeloma (SMM) and monoclonal gammopathy of undetermined significance (MGUS) [7]. Recent efforts by our group have identified a number of critical functions for NIMA-related kinase 2 (NEK2), a cell-cycle serine/threonine kinase upregulated in MM patients and correlated with poor patient prognosis [8-15]. CDK/cyclin complexes, and other cell cycle regulators, are known to mediate the maintenance and propagation of epigenetic modifications, such as DNA (DMNT1) and histone methylation (EZH2) [16]. However, little is known about how these mechanisms interact and regulate each other. SETDB1 is a trimethyl transferase known to methylate N-terminal tails of histone 3 proteins at the ninth lysine (H3K9) resulting in subsequent transcriptional repression. SETDB1 expression is associated with progression from MGUS to MM and is a poor prognostic marker. In MM patients, high SETDB1 expression is positively correlated with NEK2 expression. Using a transgenic mouse model, we observed that in the absence of *Nek2*, *Setdb1* expression is diminished prior to tumor formation. Additionally, NEK2 expression is decreased when SETDB1 is knocked down in MM cells *in vitro*. These data suggest that NEK2 mediates key epigenetic reprogramming mechanisms that are crucial to progression from MGUS to MM and MM tumorigenesis. Further understanding how NEK2 regulates epigenetic reprogramming will lead to a better understanding of the mechanism involved in cell cycle as well as improved detection methods and novel therapies for MM patients.

19 **The role of RABL6A in PNET development and progression *in vivo***

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Background: Pancreatic neuroendocrine tumors (PNETs) are rare, indolent malignancies that have risen 4-fold in incidence. Current PNET therapies fail to improve overall patient survival. There is an urgent need to identify meaningful prognostic biomarkers and therapeutic targets, which requires a better understanding of molecular mechanisms governing PNET pathogenesis. RABL6A (a RAB-like GTPase) is a new oncogenic driver of PNET cell survival and proliferation. It is highly expressed in patient NETs and predicted to induce PNET development and angiogenesis from our *in vitro* studies. This study employs genetically altered mouse models to investigate the *in vivo* role of RABL6A in PNET development and progression.
Methods: RIP/Tag2 (RT2) mice express oncogenic SV40 large T-antigen (Tag) under the rat insulin promoter (RIP) resulting in islet β cell transformation to develop hyperplastic islets, angiogenic islets, and insulinomas in a time-dependent fashion. We crossed RT2 mice with RABL6A knockout (KO) mice to generate four cohorts: a) WT, b) RT2, c) RABL6A KO, and d) RT2-RABL6A KO. Pancreata of euthanized mice were either perfused with collagenase to isolate islets for molecular analyses or fixed in paraformaldehyde for histopathological studies at specific time points.
Results: RT2 mice exhibit sex differences in their survival and PNET burden: RT2 males have higher tumor burden but survive longer than the females. RT2 mice develop highly angiogenic PNETs and their plasma insulin levels correlate to tumor burden. Our preliminary findings in the RT2-RABL6A KO cohort suggest that loss of RABL6A improves survival of female RT2 mice, and reduces tumor burden, pancreatic endocrine area and angiogenic islets, and number of mitoses within their islets.
Conclusions: RT2 mice form high-grade angiogenic PNETs and show sex differences in tumor progression. Studies are still ongoing; however, early data are promising and indicate that RABL6A promotes early events in PNET development and angiogenic switch.

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20 **Genetic and environmental modifiers of the neurological phenotypes in a fly voltage-gated sodium channel mutant**

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The advent of new technologies for genomic research has made it possible to identify causative gene mutations of inherited neurological disorders. However, the exact outcomes of the primary genetic defects are often difficult to predict, because variants at other genomic loci and various environmental factors significantly impact the severity of their symptoms. It is critically important to identify these phenotypic modifiers and elucidate their mechanisms of action, as such knowledge is necessary to fully understand the biological processes underlying the etiology and pathogenesis of congenital neurological disorders. To study gene-gene and gene-environment interactions important for the manifestation of neurological mutant phenotypes, our lab uses *para^{Shu}*, a gain-of-function allele of the *Drosophila* voltage-gated sodium (Na_V) channel gene, *paralytic* (*para*). Adult *para^{Shu}* mutants exhibit severe behavioral phenotypes including spontaneous tremors and heat-induced seizure due to neuronal hyperexcitability. Our serendipitous observations and an unbiased forward genetic screen have revealed that *para^{Shu}* phenotypes are significantly suppressed by diet supplemented with milk lipids or reduced function of *Glutathione S-transferase S1* (*GstS1*). Interestingly, suppression of adult *para^{Shu}* phenotypes requires milk lipid feeding or *GstS1* knockdown during larval/pupal stages, suggesting that both phenotypic modifiers exert effects on *para^{Shu}* by altering the development of the nervous system. Considering that *GstS1* is a putative fly ortholog of mammalian hematopoietic prostaglandin D synthase, an enzyme responsible for the synthesis of biologically active lipids, we are currently pursuing the possibility that the underlying mechanisms of these genetic and environmental modifiers may partially overlap in lipid-mediated pathways.

21 **Central Regulation of Energy Expenditure by FGF21**

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Fibroblast growth factor 21 (FGF21) is an endocrine hormone that controls energy homeostasis by signaling to the central nervous system. Importantly, the metabolic effects of FGF21 on energy expenditure appear to require the adipokine leptin, as mice deficient in leptin or the leptin receptor exhibit significantly attenuated weight loss in response to FGF21 treatment. Moreover, co-administration of FGF21 with a leptin agonist enhances body weight loss in diet induced obese (DIO) mice compared to administration of either alone. Thus, we hypothesize that FGF21 and leptin signaling interact to control energy expenditure and overall body weight. To determine whether leptin-sensitive cells express the FGF21 co-receptor, β-klotho, which confers specificity for FGF21 action and is absolutely required for FGF21 signaling, we administered leptin to β-klotho-Cre mice which conditionally express tdTomato in the presence of Cre recombinase. Leptin-mediated activation of phosphorylation of STAT3 (pSTAT3) co-localized with β-klotho-positive cells in the arcuate nucleus (ARC) and ventromedial hypothalamus (VMH), regions of the brain critically involved in the control of energy expenditure. To determine whether β-klotho is required in leptin receptor-expressing cells to mediate the metabolic actions of FGF21, we generated a novel mouse model which lacks β-klotho in leptin-sensitive cells ($KLB^{LepR\text{-}KO}$ mice). Intriguingly, DIO $KLB^{LepR\text{-}KO}$ mice have markedly impaired decreases in body weight in response to extended FGF21 administration. However, in contrast to body weight, FGF21-mediated improvements in glucose homeostasis and insulin sensitivity were retained in DIO $KLB^{LepR\text{-}KO}$ mice compared to wildtype littermates as determined by glucose tolerance and insulin tolerance tests, respectively. Taken together, these data suggest that FGF21 signaling to leptin-sensitive cells is critical for its full effects on body weight reduction.

22 **Behavioral deficits and rescue in the Protocadherin 10 (Pcdh10) mouse model relevant to autism**

Sarah L Ferri, Edward S Brodkin, Ted Abel

Protocadherin10 (Pcdh10) is a member of the cadherin superfamily and a nonclustered protocadherin. The activity-dependent cell adhesion molecule is highly expressed in the amygdala, striatum, and cerebellum, and is involved in the formation and elimination of dendritic spines and the guidance of thalamocortical projections. It has been linked to Autism Spectrum Disorder (ASD), a neurodevelopmental disorder that causes a number of symptoms, including impairments in social behavior and cognition. ASD affects four times as many males as females. Our group has previously reported male-specific deficits in social behavior and fear memory in mice heterozygous for a deletion of *Pcdh10* ($Pcdh10^{+/-}$), as well as increased spine density and decreased expression of NMDAR in the basolateral amygdala (BLA). Additionally, social behavior was rescued by systemic administration of the NMDAR partial agonist d-cycloserine. Currently, we are using a new transgenic mouse line with the Pcdh10 gene flanked by *loxP* sites in combination with a fast-expressing helper-dependent Cre-expressing virus to determine the effects of ablation of Pcdh10 in specific cell types and brain areas. In addition, preliminary data suggests that male $Pcdh10^{+/-}$ mice have decreased expression of the estrogen receptors alpha and beta, which may contribute to the sex differences observed. Future studies will focus on functional activity in the BLA and mechanisms underlying sex-specificity and behavioral rescue.

23 MCH Neuron Activity Is Sufficient for Reward and Reinforces Feeding

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Melanin-concentrating hormone (MCH) expressing neurons have been implicated in regulation of energy homeostasis and reward, yet the role of their electrical activity in short-term appetite and reward modulation has not been fully understood. We investigated short-term behavioral and physiological effects of MCH neuron activity manipulations. We used optogenetic and chemogenetic approaches in *Pmch-cre* transgenic mice to acutely stimulate/inhibit MCH neuronal activity while probing feeding, locomotor activity, anxiety-like behaviors, glucose homeostasis, and reward. MCH neuron activity is neither required nor sufficient for short-term appetite unless stimulation is temporally paired with consumption. MCH neuronal activation does not affect short-term locomotor activity, but inhibition improves glucose tolerance and is mildly anxiolytic. Finally, using two different operant tasks, we showed that activation of MCH neurons alone is sufficient to induce reward. Our results confirm diverse behavioral/physiological functions of MCH neurons and suggest a direct role in reward function.
* Equal contribution

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24 The Functional Roles of Protein Aggregation: Establishing Nucleolar Architecture

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Protein aggregation is the hallmark of diseases such as Huntington's and Parkinson's diseases, but recent research has established protein aggregation as essential to many cellular processes as it aids in cellular organization and spatial control of protein distribution. Many of the proteins involved in ribosome biogenesis form aggregates. Ribosome biogenesis can be described by three general, sequential processes: synthesis of components, rRNA modification, and ribosome assembly. Our objective is to map the distribution of aggregating proteins involved in ribosome biogenesis to the domains of the nucleolus to understand how aggregation may aid the sequential process of ribosome biogenesis. We are also interested in the state of the aggregation of these proteins under normal conditions, and how they change in response to stress. We hypothesize that 1) ribosome biogenesis proteins involved in similar steps of the process will aggregate together, sharing nucleolar domains, and 2) the equilibrium of the aggregation phase will be altered when subjected to cellular stresses. We are testing our hypothesis by making fluorescent protein fusions to observe the aggregation in *Xenopus laevis* embryos and oocytes, and by using reagents such as thioflavin T and hexanediol to determine the state of aggregation. Recent studies suggest that the nucleolus is a site where both useful and pathological protein aggregation is regulated, which makes these studies instructive for multiple aggregate based diseases.

25 Novel Mouse Models of Pancreatic Neuroendocrine Tumor Metastasis

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Background: Pancreatic neuroendocrine tumors (PNETs) are rare, slow growing cancers that lack effective treatments once they become metastatic. Unfortunately, 60% of PNET patients have distant metastatic disease (mainly in the liver) at diagnosis and current therapies fail to improve overall survival. Pre-clinical models of PNET metastasis are greatly needed to advance our understanding of mechanisms driving NET metastasis and to develop/test novel therapeutic interventions.
Methods: PNET cell lines stably expressing luciferase (BON1.luc and Qgp1.luc) were generated and transwell assays performed to measure in vitro migration. Bioluminescent cells were introduced into NSG immunodeficient mice by intravenous (IV, tail vein) or intracardiac (IC) injection. Tumor growth was monitored longitudinally on a weekly basis by non-invasive bioluminescence imaging (BLI). Animals with tumor burden exceeding 10^9 photons/sec or low body conditioning scores were euthanized, and tumor bearing tissues subjected to ex vivo BLI, histopathology and genetic analyses.
Results: One hundred percent tumor incidence was achieved for both IV and IC metastasis models. Qgp1.luc cells preferentially metastasized to the liver regardless of delivery route, mimicking the predominant site of PNET metastasis observed in patients. By comparison, BON1.luc cells always formed tumors in the lung regardless of administration route and colonized a wider variety of tissues compared to Qgp1.luc, including liver but also adrenal glands, kidney and ovaries with high frequency. Pre-clinical studies evaluating drugs with predicted anti-metastatic activities are ongoing.
Conclusions: We successfully developed new bioluminescent mouse tumor models of PNET metastasis. Qgp1.luc cells preferentially formed tumors in the liver while BON1.luc cells displayed a broader metastatic distribution. This system represents a rapid and relatively inexpensive platform for testing candidate metastasis genes and novel PNET therapies.

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26 Li^+ differentially affects mitochondrial Ca^{2+} efflux in central and peripheral neurons

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During neuronal activity, Ca^{2+} entering the cell is buffered by mitochondria and subsequently released back to the cytosol, primarily via mitochondrial Na^+/Ca^{2+} exchanger (mtNCX). This process contributes to shaping Ca^{2+} responses and regulating many neuronal processes including synaptic transmission, gene expression and neuronal survival. It is thought that $Na^+/Ca^{2+}/Li^+$ exchanger (NCLX) is the major mediator of mitochondrial Ca^{2+} efflux. However, some reports suggest that the Na^+/Ca^{2+} exchanger family members NCX1-3 are also involved in this transport in neurons. The hallmark of NCLX not shared by NCX1-3, is its ability to use Li^+ to effectively drive transmembrane Ca^{2+} transport in the absence of Na^+. To better understand the molecular and pharmacological properties of mtNCX in neurons, we exploited this unique property of NCLX and examined how Li^+ affects mitochondrial Ca^{2+} transport in central and peripheral neurons, represented by mouse hippocampal and dorsal root ganglia (DRG) neurons, respectively. By simultaneously monitoring depolarization-induced changes in mitochondrial and cytosolic Ca^{2+} substitution with Li^+ dramatically slowed mitochondrial Ca^{2+} efflux in hippocampal, but not DRG, neurons. The mtNCX inhibitor CGP37157 blocked mitochondrial Ca^{2+} efflux in both types of neurons. Quantitative RT-PCR showed similar NCLX expression in adult DRG, hippocampus, cortex, and neonatal hippocampal cultures. NCLX knockdown using shRNA did not significantly change the rate of mitochondrial Ca^{2+} efflux in either DRG or hippocampal neurons. Notably, Li^+-induced Ca^{2+} efflux from isolated brain mitochondria was significantly slower than that induced by Na^+. A similar effect was found in isolated liver and heart mitochondria. Collectively, our findings suggest that the properties of mitochondrial Ca^{2+} efflux differ between central and peripheral neurons, and that besides NCLX, other proteins may contribute to mitochondrial Ca^{2+} efflux in the brain.
Keywords: Mitochondria, Ca^{2+}, NCLX

27 Approaches to Targeting Adenylyl Cyclase 1 for Novel Pain Therapeutics

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Adenylyl cyclases (AC) catalyze the formation of cyclic AMP (cAMP) from ATP and are involved in a number of disease states, making them attractive potential drug targets. Recent preclinical studies have identified neuronal adenylyl cyclase type 1 (AC1) as a novel target for treating chronic pain. AC1 is highly expressed in neuronal tissues associated with pain processing and neuronal plasticity, and studies using AC1 knockout mice provide direct evidence linking AC1 to chronic inflammatory pain conditions. Furthermore, AC1 inhibitors would lack the side effects associated with other agents (e.g. opioids) used to treat chronic inflammatory pain. By designing our studies to target NOT the conserved P-site or forskolin-binding site, but rather a novel approach, targeting the unique protein-protein interaction of AC1 and calmodulin (CAM). AC1 and AC8 are both activated by CaM, however, the CaM binding domains are unique in structure and location providing an unprecedented opportunity to achieve AC1 selectivity. We hypothesize that developing a small molecule inhibitor of AC1 will allow us to mimic the AC1 knockout phenotype and provide a new avenue for the treatment of chronic inflammatory pain. Through the development and implementation of a novel biochemical high-throughput-screening paradigm we will interrogate a library of 100,000 compounds to identify inhibitors of the AC1/CaM protein-protein interaction. Hit compounds will be validated and chemically optimize lead molecules using cellular assays focused on selectivity and potency to guide medicinal chemistry efforts. To date, we have completed initial studies to develop the novel screening assay, established a subset of the necessary assays, and cemented the collaboration between the University of Iowa and Purdue University for the successful completion of our aims. We anticipate the identification of selective AC1 inhibitors that ultimately be improved and applied in models of chronic inflammatory pain.

28 Sex-specific involvement of indirect-pathway medium spiny neurons in behavioral alteration of 16p11.2 hemi-deletion mouse model

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One of most common genetic variations found in autism spectrum disorder (ASD) is 16p11.2 deletion syndrome. Patients with this deletion usually display developmental delay and intellectual disability, including at least some features of ASD. Moreover, Attention-Deficit/Hyperactivity Disorder (ADHD) is commonly reported in the patients. Because the 16p11.2 chromosomal region in humans is highly conserved in mice, 16p11.2 deletion mouse model is considered a faithful model. Using the mouse model, we previously showed both male and female 16p11.2 hemi-deletion mice display hyperactive behavior compared to wild type sex-matched control mice. Because the striatum is the input structure of the basal ganglia, the key neural substrates for motor control, the present study examined the role of medium spiny neurons (MSNs), the major type of neuron in the striatum, in hyperactivity in 16p11.2 mouse model. MSNs can be characterized by their projections as either direct-pathway or indirect-pathway. The direct-pathway MSNs express dopamine receptor 1 and project to the SNr/GPi directly. The indirect-pathway MSNs express dopamine receptor 2 as well as adenosine receptor 2a (A2A) and extend their projections primarily to the GPe. We hypothesized that the medium spiny neurons play an important role in behavioral alteration of 16p11.2 hemi-deletion mouse model. To identify the importance of indirect-pathway MSNs, we crossed 16p11.2 flox mice to the A2A-CRE mice to produce A2A-CREx16p11.2 flox mice that lack genes of the 16p11.2 region in MSNs expressing dopamine receptor 2. Interestingly, male A2A-CREx16p11.2 flox mice showed hyperactivity compared to sex matched control group, suggesting the importance of indirect-pathway MSN. However, female A2A-CREx16p11.2 flox mice did not show the hyperactivity. These results imply the possibility that two different types of MSNs are involved in a sex-dependent manner in behavioral alteration in the 16p11.2 mouse model. We are currently assessing the role of direct-pathway MSNs using a similar approach.

29 **Metabolic regulation of sleep: The role of lateral hypothalamic circuits engaged by leptin**

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Adequate body energy store and sufficient sleep are essential for health, and any disruptions in these physiological processes can lead to serious health consequences including obesity, diabetes, and cardiovascular diseases. Literature support an intimate association between sleep and energy balance – short sleep duration and poor sleep quality are associated with weight gain and obesity, whereas obesity has been identified as an independent risk factor for poor sleep quality and excessive daytime sleepiness. Despite this bidirectional, feedforward, pernicious association between obesity and sleep disorders, the neural substrates underlying this association remain poorly understood. Adipocyte-derived metabolic hormone, leptin, exhibits diurnal rhythm in circulation and regulates energy balance and rhythmic expression of behaviors such as sleep and daily locomotor activity, yet the underlying neural circuits mediating these effects are unclear. Here we show that leptin acts on a subset of lateral hypothalamic area (LHA) GABAergic neurons to affect sleep and energy balance. Selective deletion of leptin receptor (LepR) in the LHA of adult mice resulted in increased body fat gain, decreased locomotor activity, frequent sleep onset and increased sleep time especially in subjective day, resembling excessive daytime sleepiness in human. Chemogenetic activation of this subpopulation of LHA GABAergic neurons completely disrupts sleep and dramatically increases exploratory locomotor activity. These findings identify the LHA as a critical site where circulating metabolic hormone leptin acts to affect sleep and provide a possible explanation why severely obese patients who are commonly associated with leptin resistance often experience poor sleep quality and excessive daytime sleepiness.

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30 **Effects of GR phosphorylation by Erk2 on GR DNA-binding**

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Glucocorticoids (GCs) are steroid hormones that have pleiotropic effects ranging from cell-death in lymphoid cells to increased glucose production in liver. GCs work by binding the glucocorticoid receptor (GR), a ligand-activated transcription factor, and directly regulating genes. The different effects of GCs in each tissue result, in part, from regulation of distinct sets of genes. In leukemia cells we have shown that some, but not all, GR regulated genes are attenuated by signaling through phosphorylation of GR by the PI3K/Ras/Erk2 pathway, including cell death genes. We hypothesize that phosphorylation affects the regulation of these genes by changing the DNA-binding of GR. In vitro phosphorylated GR by Erk2 results in multiple phosphorylated species. When phosphorylated at S226, GR becomes more compact, and DNA-binding affinity is reduced. However, phosphorylation at 4-6 sites abrogates this structural change, and much of the DNA binding inhibition. This indicates that patterns of phosphorylation have different effects on GR DNA-binding affinity. To test whether phosphorylation simply inhibits binding, or changes gene expression by altering DNA-binding specificity, we are pioneering a new technique, No Read Left Behind (NRLB). Here we show that NRLB generates an accurate affinity matrix describing GR DNA-binding specificity that captures low-affinity and different binding modes. Using this technique, we will model phosphorylation driven changes in DNA-binding specificity and link them to changes in downstream gene regulation.

31 The role of cerebellar CGRP in migraine animal models

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Calcitonin gene-related peptide (CGRP) is considered a major player in migraine. However, the location and mechanisms of CGRP in migraine are unclear. This project is to identify the brain regions regulated by CGRP to induce migraine-like behaviors (e.g. light aversion, impaired time perception). Previous studies have shown that intracerebroventricular injection of CGRP into mice induced light aversion, suggesting a role of central CGRP in migraine-like behaviors. One potential candidate site in the central nervous system (CNS) is the cerebellum. The cerebellum serves as a sensory and motor integrative center, is activated during migraine attacks, produces CGRP, and has the most CGRP binding sites in the CNS. Thus, we hypothesized that cerebellar CGRP induces migraine-like behaviors. We first measured CGRP expression in the cerebellum of mice. A CGRP sensitized mouse model (L7/hRAMP1 mice) was created to test the role of cerebellar CGRP in migraine. L7/hRAMP1 mice were injected with CGRP in the deep cerebellar nuclei (DCN) and tested in the light-dark assay to evaluate light aversion, open field test to evaluate anxiety and locomotion, and elevated zero maze to evaluate anxiety. Rats were injected with CGRP into the cerebellar cortex at crus I and tested in an interval estimation task. We detected RNAs for both CGRP and its receptor subunits in the cerebellum of mice. CGRP immunoreactivity was detected in Purkinje cells (PCs) and DCN. No EYFP was detected in PCs after injection of AAV-EF1a-DIO-EYFP into Calca-Cre mice. These results suggest that PCs do not produce CGRP but accumulate CGRP. Preliminary data showed that CGRP delivery to the fastigial nuclei (FN) of L7/hRAMP1 mice and control littermates both induced light aversion and impaired locomotion. Open field and elevated zero maze showed conflicting results with regards to CGRP injection into the FN inducing anxiety. In addition, CGRP injection in crus I showed a trend with impaired timing. Results suggest that cerebellar CGRP may contribute to light-aversive behaviors but further studies are needed to investigate its exact role. These studies will shed light on the underlying brain regions critical for migraine-like behaviors.

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32 Mutations of E3 Ubiquitin Ligase Adaptor KLHL15 in X-linked Intellectual Disability

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Intellectual disability (ID), which affects 1-2% of the general population, is a devastating neurodevelopmental disorder with the most lifetime costs of all diagnoses in the U.S. However, males are more susceptible to ID than females and are often found to have severe outcomes. Mutations in X-chromosomal genes are thought to account for this male-biased phenomenon. KLHL15 was recently identified as a novel XLID gene. It encodes Kelch-like protein 15 (KLHL15), a substrate adaptor of a Cullin-3 (Cul3)-based E3 ubiquitin ligase complex that targets proteins, including the brain-enriched B′β regulatory subunit of protein phosphatase 2A (PP2A), for degradation by the ubiquitin/proteasome system (UPS). Several KLHL15 mutations have been found in the poorly characterized BACK domain, which is a “hotspot” for many deleterious variants of the other KLHL family members resulting in either Mendelian diseases or human cancers. We identified both loss-of-function (ΔFY241, ::ACOT9) and gain-of-function (R249H) alleles, and we hypothesize that small deletions and point mutations in KLHL15’s BACK domain lead to structural rearrangement that change the alignment between bound substrates and the E3 ligase complex to either slow or accelerate substrate ubiquitination and degradation, causing dysregulated protein turnover of KLHL15-Cul3-targeted substrate(s) and eventually pathogenesis of ID. In addition, we applied bioinformatic approach coupled with mass spectrometry to identify other brain- and neuron-specific substrates also targeted by KLHL15 for polyubiquitination and proteaosomal degradation, which may uncover other molecular mechanisms underlying XLID and neurodevelopmental disorders (NDDs) in general.

33 **Persistent Organic Pollutants – Implications in the Disruption of Dopamine Homeostasis**

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Imbalances in dopamine (DA) homeostasis yields toxic intermediates via altered metabolism and trafficking. This imbalance may contribute to neurotoxicity, neurodevelopmental problems and neurodegenerative disease. Environmental organochlorine pollutants, such as polychlorinated biphenyls (PCBs) and the pesticide dieldrin are proposed risk factors for these neurological conditions. Recent evidence suggests these agents disrupt dopamine homeostasis in dopaminergic cells. While these organochlorine compounds have been phased out of use, they persist in the environment and bioaccumulate in humans. Altering dopamine homeostasis can yield elevated levels of the monoamine oxidase metabolite of dopamine, 3,4-dihydroxyphenylacetaldehyde (DOPAL), which is toxic and highly reactive towards proteins. Our goal here is determine if PCB-52 or its major human metabolite, 4-OH PCB-52, and dieldrin alter levels of dopamine and/or its metabolites including DOPAL, investigate oxidative stress induced by these agents, and finally to identify proteins modified by the reactive dopamine metabolite, DOPAL. Results show that 4-OH PCB 52 is toxic to dopaminergic PC12 and N27 cells at concentrations less than that of PCB-52 (less than 25µM) at 24hrs. Both PCB-52 and 4-OH PCB-52 were found to increase mitochondrial and whole cell reactive oxygen species in dopaminergic N27 cells. To support such findings, we will determine how organochlorines alter the amount of catechol-modified proteins. Using an aminophenylboronic acid agarose, we are able to isolate catechol modified proteins from N27 cells. This work aims to provide insight into the mechanism of toxicity of these organochlorine pollutants, and potential therapeutics to combat neurodegenerative and neurodevelopmental conditions.

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34 **Single-cell RNA sequencing reveals regulation of protein folding gene expression by the Nuclear Receptor Nr4A in hippocampal CA1 neurons after learning**

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New experiences are initially encoded as labile short-term memories and then converted into stable long-term memory by transcription- and translation-dependent processes. Gene expression after learning involves a transient wave of transcription that is critical for memory consolidation. One group of transcription factors induced during the first wave of transcriptional events are the Nr4A subfamily of nuclear receptors. In this study, we show that transgenic mice expressing a dominant-negative form of Nr4A (Nr4ADN) in forebrain excitatory neurons have impaired long-term spatial memory. Total RNA and single neuronal nuclear RNA sequencing (sn-nuc RNA seq) following a spatial object recognition task revealed that genes related to endoplasmic reticulum (ER) protein chaperones are downregulated in Nr4ADN mice especially in dorsal CA1 excitatory neurons. The RNA sequencing results were validated at the single-cell level using RNA scope flourescent *in situ* hybridization techniques. We also found that several ER protein processing genes regulated by Nr4ADN are also induced by learning in wild-type mice. AAV-mediated overexpression of Nr4ADN exclusively in CA1 excitatory neurons resulted in impaired long-term spatial memory suggesting Nr4A regulates CA1 gene expression to form long-term memory. To study if the downregulated genes in Nr4ADN mice are directly regulated by Nr4A, we generated a Nr4A1-Tavi knock-in mice using CRISPR technology. Nr4A1-Tavi mice were infused with biotin ligase enzyme (BirA) in the dorsal hippocampus to biotinylate Tavi sequence fused with Nr4A1. Chromatin pulldown using streptavidin beads showed enriched occupancy of Nr4A1 on ER chaperone gene promoters following learning. As learning leads to protein translation, the newly synthesized proteins in naïve form require folding by ER chaperones into their correct conformation to be transported to destinations. Therefore, our study reveals a critical mechanism by which Nr4A regulates learning induced expression of ER protein processing genes which ultimately fold the newly synthesized proteins required for memory.

Keywords: Memory, CA1, transcription, hippocampus, chromatin, single cell.

35 **Skeletal muscle estrogen-related receptor alpha overexpression leads to lower lean mass and muscle weakening**

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Mitochondrial dysfunction in skeletal muscle has been implicated in development of various metabolic disorders like insulin resistance and type 2 diabetes. In skeletal muscle of type 2 diabetes patients, the expression levels of genes in mitochondrial oxidative phosphorylation are reduced and some of them are regulated by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α). Estrogen-related receptor alpha (Esrra) is an orphan nuclear receptor with strong homology to estrogen receptor alpha (ERα), and involves in the transcriptional regulation of genes in glucose metabolism, mitochondrial biogenesis, oxidative phosphorylation, and fatty acid oxidation. The inhibition of Esrra in skeletal muscle cells induced a type 2 diabetes-like phenotype, indicating that modulation of Esrra in skeletal muscle may be an attractive drug target of this disease. Beyond pathological conditions, Esrra is required for the skeletal muscle metabolic responses in physiological conditions. However, the impact of skeletal muscle-specific roles of Esrra on energy homeostasis has not been thoroughly studied in vivo. Here we generated a conditional transgenic mouse model in which Esrra is overexpressed in tamoxifen-inducible and skeletal muscle-specific manner and show that skeletal muscle-specific Esrra overexpression led to lower lean, fat, and the body masses under high-fat diet challenge. Energy expenditure normalized by lean mass is higher in the overexpression mice than control mice, but the overall energy expenditure is lower in the overexpression mice than the control mice. At seven weeks after tamoxifen treatment, the body weight of the overexpression mice under high fat diet remained the level before tamoxifen treatment. Lean mass of the overexpression mice was even lower than the level before tamoxifen treatment, indicating muscle wasting. In consistent with their lower lean mass, the skeletal Esrra overexpression mice showed weaker grip strength. Our ongoing study so far indicates that overactivation or chronic activation of skeletal muscle Esrra may be harmful or outweigh the benefit that Esrra activates oxidative phosphorylation.

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36 **Regulator of G protein signaling 6 (RGS6) suppresses pathological α-synuclein accumulation and Parkinson's disease**

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Parkinson's disease (PD) is a devastating, primarily non-familial, age-related neurodegenerative disorder characterized by the progressive loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNc). Unfortunately, why these SNc DA neurons die and why their loss is largely associated with aging remains unknown, therefore highlighting a *critical need* to further elucidate the mechanisms underlying SNc DA neuronal death. Accumulation of α-synuclein (αSyn) aggregates in Lewy bodies is believed to play a crucial role in PD pathogenesis. In support of this hypothesis, β-agonists dramatically and concomitantly reduce αSyn expression and the incidence of human PD, as well as repress MPTP-induced SNc DA neuron loss in mice (Mittal *et al.*, *Science, 2017*). RGS proteins modulate G protein-coupled receptor (GPCR) signaling by facilitating heterotrimeric G protein inactivation through their GTPase-activating activity (GAP) toward Gα subunits. RGS6 ($G\alpha_{i/o}$-GAP), a member of the R7 RGS protein subfamily, is exclusively expressed in SNc DA neurons, and loss of RGS6 in mice leads to several PD hallmarks, including: late-age-onset SNc DA neuron degeneration, reduced nigrostriatal DA, motor deficits, and pathological accumulation αSyn in the SNc. $RGS6^{-/-}$ mice also exhibit hyperactive DA D_2 autoreceptor (D_2R) signaling and late-age-onset reduction in cAMP/PKA activity in SNc DA neurons. Given that β-agonists repress αSyn expression through their activation of β2-adrenorecetor-$G\alpha_s$—a GPCR that increases cAMP—and RGS6's role in inhibiting D_2R-$G\alpha_{i/o}$ signaling—a GPCR that reduces cAMP—we *hypothesized* that RGS6 may also function to suppress the pathological accumulation of αSyn through modulation of cAMP signaling. Here, we show that RGS6 plays a critical role in protecting against late-age-onset pathological αSyn accumulation in the SNc of 12 and 18mo mice. RGS6 suppression of late-age-onset αSyn expression and PD is likely due to its ability to increase cAMP signaling through inhibition of D_2R-$G\alpha_{i/o}$ signaling in SNc DA neurons. These findings demonstrate that RGS6 is a critical neuroprotective protein in PD pathogenesis and illuminate an entirely novel signaling pathway underlying pathological αSyn accumulation in PD.

37 **Generation and characterization of mice harboring Jordan's syndrome alleles causing intellectual disability with autism**

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BACKGROUND: Protein phosphatase 2A (PP2A) activity is essential for eukaryotic cells and is controlled through its many regulatory subunits. Mutations in regulatory subunits have been associated with a wide variety of human diseases from cancer to intellectual disability. To date, more than sixty individuals have been identified that have *de novo* mutations in the *PPP2R5D* gene which encodes the PP2A regulatory protein B'delta. The affected individuals' symptoms vary but can include intellectual disability, language delay, autism spectrum disorder, and seizures. **METHODS:** The most common and severe disease-causing mutation in humans is the E198K charge-reversal mutation, and therefore, we generated a mouse harboring this mutation in *Ppp2r5d* using CRISPR-Cas9 gene editing at the University of Iowa Genome Editing Core Facility. **RESULTS:** Mice harboring one E198K allele are viable but are born below expected frequency with either parent being carrying the mutation. Additionally, these mice often die as juveniles and show craniofacial abnormalities including cranial bossing. We have examined the brain structures by MRI and CT scans and have begun to investigate changes in glucose regulation/uptake and metabolomics. **CONCLUSION:** Mice heterozygous for the *Ppp2r5d* E198K mutation (reflecting the condition of Jordan's Syndrome patients) have highly penetrant phenotypes and will likely represent a faithful model for studying the human disease.

38 **Allosteric inhibitors of apicoplast DNA polymerase: New antimalarials that bind a novel allosteric pocket**

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Plasmodium spp. are the causative agents of malaria, killing nearly 600,000 people each year. Resistance of *Plasmodium* to current therapies accentuates the need for new drugs that target novel aspects of the parasite's biology. Parasites in the phylum *Apicocomplexa* have an unusual organelle; apicoplast, which participates in the biosynthesis of fatty acids, heme, iron-sulfur clusters, and isoprenoids. Any defect in apicoplast metabolism or its failure to replicate leads to the death of the parasite. Additionally, lack of a human counterpart makes apicoplast a promising drug target. The apicoplast genome is replicated by select DNA replication enzymes, of which apicoplast DNA polymerase (apPOL) is unique to the parasite. The apPOLs from *P. falciparum* and *P. vivax* have 84% homology, while the most similar human DNA polymerases are the lesion bypass polymerases theta and nu (23 and 22% identity, respectively). Towards identifying inhibitors of apPOL, a high throughput screen of 400 compounds from the Open Malaria Box provided by (Medicines for Malaria Venture (MMV) identified a sub-micromolar inhibitor of apPOL. Our studies indicate that MMV666123 is specific for apPOL, with no inhibition of human DNA Pol or *E. coli* DNA Pol I. Additionally, being a malaria-box compound substantiates the anti-malarial activity of MMV666123. Presented here are our current design, synthesis, crystallographic, and *in vitro* evaluation efforts toward understanding the structural requirements of MMV666123 for inhibition of apPOL, identifying the binding site and designing more potent and drug-like apPOL inhibitor derivatives.

39 **Novel Endocrine Circuit Regulating Sugar Satiety**

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Metabolic syndrome is a major health risk in the United States. Imbalances in macronutrient intake have been shown to be a contributing factor in the development of this disease. While there is evidence in humans for independent appetites for macronutrients (fats, proteins, and carbohydrates), the mechanisms that determine appetites for specific macronutrients is lacking. Preferences for carbohydrates in humans can be strong as sugars contribute a major source of energy and are therefore rewarding. Sugars activate different components of the mesolimbic dopamine system in the brain, including the nucleus accumbens (NAc) and ventral tegmental area (VTA). These regions receive various central inputs that allow for the stimulation and downregulation of rewarding stimuli. Little is known, however, about the integration of peripheral nutrient signals to this reward center. The Potthoff lab studies fibroblast growth factor 21 (FGF21), a hepatic endocrine hormone that crosses the blood brain barrier to lower carbohydrate intake and preference in mice. FGF21 signals through a receptor complex comprised of the canonical FGF receptor, FGF receptor complex 1 (FGFR1c), and its co-receptor, β-klotho (KLB). While FGFR1c is ubiquitously expressed throughout the body, KLB is more selectively expressed, conferring FGF21's specificity. Previous work from our lab has shown that FGF21 signals to the paraventricular nucleus of the hypothalamus to regulate carbohydrate intake in mice. Here, we demonstrate that FGF21 regulates simple sugar intake and sweet taste preference through its actions on neurons that potentially modulate the mesolimbic dopamine system. Using behavioral experiments, we show that FGF21 mediates sugar satiety by regulating motivation in mice, likely through modulating the mesolimbic dopamine reward pathway. Through these studies, we have identified new mechanistic information into this liver-brain hormonal axis regulating central pathways controlling energy homeostasis and reward.

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40 **Hepatic Bbs1 gene is a critical determinant of glucose metabolism and insulin sensitivity**

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Bardet-Biedl syndrome (BBS) is a pleiotropic autosomal recessive human disorder associated with several features including obesity and diabetes mellitus. Mice lacking *Bbs* genes are also obese and display diabetes as indicated by the increased blood glucose, impaired glucose clearance and insulin resistance. These changes in glucose and insulin sensitivity are independent of obesity. We hypothesized that BBS proteins in insulin-sensitive tissues contribute to the development of diabetes in BBS. To address this, we deleted the hepatic *Bbs1* gene by crossing the $Bbs1^{flox}$ mice with liver-specific (albumin) Alb^{Cre} mice. Interestingly, body weight of $Alb^{Cre}/Bbs1^{flox}$ mice was slightly increased compared to control littermates (males: 40.8±1.6 vs 36.2±2 g, females: 29.6±1.9 vs 25.8±1.2 g, at 25 weeks of age). Fat mass, but not lean mass, was higher in $Alb^{Cre}/Bbs1^{flox}$ mice relative to controls. $Alb^{Cre}/Bbs1^{flox}$ mice exhibited elevated fasting glucose (169± 14 vs 136±8 mg/dL, $P<0.05$), but glucose tolerance test showed no significant difference in glucose clearance between $Adipo^{Cre}/Bbs1^{flox}$ mice and controls. On the other hand, insulin tolerance test revealed impaired insulin sensitivity in $Alb^{Cre}/Bbs1^{flox}$ mice compared to controls. Insulin-induced activation of AKT was significantly decreased in the liver (2.6±0.07 vs 6.6± 0.34 AU), but not in white adipose tissue and skeletal muscle, of $Alb^{Cre}/Bbs1^{flox}$ mice. Using immunohistochemistry, we found that insulin receptor level in plasma membrane of primary cultured hepatocytes derived from $Alb^{Cre}/Bbs1^{flox}$ mice was decreased by 24.2±6.8%. Expression of glucogenesis genes such as FBP1, G6Pase, MCAD, FASN, ACOX1 and SREBP1 was increased by 2-4 folds in the liver of $Alb^{Cre}/Bbs1^{flox}$ mice relative to control animals. Taken together, these findings demonstrate that hepatic *Bbs1* gene is critical for glucose homeostasis and insulin sensitivity.

41 NADPH and glutathione redox link TCA activity to ER stress

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Endoplasmic reticulum (ER) stress is associated with diseases of dysregulated metabolism, and is also tied to feeding and fasting cycles in tissues such as the liver. Yet little is known about how the activity of catabolic pathways—particularly those housed in mitochondria, such as the TCA cycle—is sensed by the ER. Here, we show that decreasing the availability of acetyl-CoA from either fatty acid oxidation or glycolysis diminished NADPH production, favored oxidized glutathione (GSSG), and attenuated ER stress, while driving TCA activity had the opposite effects and caused ER stress. We show that ER stress was alleviated by knockdown of NADPH-producing TCA cycle enzymes, and by inhibition of glutathione reductase. Conversely, enforced glutathione reduction blocked the ability of diminished TCA cycle activity to alleviate ER stress. Finally, we validated these findings in cells lacking the mitochondrial pyruvate carrier, deletion of which is known to protect the liver from diet-induced injury, providing compelling genetic evidence that mitochondrial oxidative metabolism is linked to ER homeostasis. Our results elucidate a novel pathway of communication between mitochondria and the ER, through relay of redox metabolites.

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42 Mapping a novel endocrine circuit regulating alcohol consumption

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In the United States alcohol use disorder (AUD) affects ~15% of adults with the prevalence of binge drinking on the rise in adolescents and young adults. AUD represents a major issue to healthcare given that chronic excessive alcohol consumption in humans is associated with the development of cardiovascular disease, obesity and diabetes, liver disease, and various types of cancer. Furthermore, ~2,000 people die in the United States each year due to acute alcohol poisoning. Economically, AUD represents a massive burden resulting from loss of productivity and associated healthcare and legal costs to the tune of $249 billion each year. However, despite the massive impact AUD has on almost every aspect of society we still lack effective therapies for mediating AUD. Recently, the endocrine hormone fibroblast growth factor 21 (FGF21), well known for its potent metabolic effects, was illustrated to significantly reduce alcohol consumption in mice via an undescribed mechanism requiring expression of the obligate FGF21 co-receptor β-klotho (KLB) in the brain. Suggestive of a role for FGF21 signaling in regulating alcohol consumption in humans, mutations in the genes encoding FGF21 and KLB are highly associated with increased alcohol consumption in humans. Therefore, investigation of how FGF21 signaling to the brain regulates alcohol consumption represents a unique opportunity to understand the biology underlying alcohol consumption in addition to potentially providing novel therapeutic targets. Importantly, we find that FGF21 reverses alcohol consumption even in mice allowed to freely consume alcohol for weeks prior to FGF21 administration. Furthermore, excessive alcohol consumption promotes FGF21 secretion from the liver into circulation, ultimately signaling to the brain, representing a negative feedback loop for physiologic regulation of alcohol consumption. However, the direct target of FGF21 in the brain mediating reduction in alcohol consumption remains undefined. Excitingly, we find that removal of KLB from Vglut2^{+} neurons blocks the effects of FGF21 on alcohol consumption in mice representing a potential direct target of FGF21 in regulation of alcohol preference. Further, we have identified a previously undescribed population of neurons residing in the basolateral amygdala (BLA) which express β-klotho and send projections to the nucleus accumbens (NAc), a region known to contribute to regulation of alcohol consumption and reward processing. Excitingly, neurons which express β-klotho in the BLA also express Vglut2^{+} which previous work has identified as a marker for BLA neurons which project to the NAc to promote reward signaling. In support of this, FGF21 administration enhances ethanol conditioned place preference in mice suggesting FGF21 enhances ethanol associated reward. This finding suggests FGF21 may actually decrease alcohol consumption by lowering the threshold of alcohol consumption required for rewarding effects as opposed to being aversive. Additionally, we find that FGF21 alters dopaminergic signaling in the NAc, a process largely thought to regulate reward-based decision making. Importantly, this effect does not appear to be a result of FGF21 signaling directly to either the NAc or dopaminergic neurons. Excitingly however, we find infusion of FGF21 into the BLA inhibits alcohol consumption in mice, in line with previous work suggesting projections from the BLA to the NAc can regulate voluntary alcohol consumption. Thus, we hypothesize the direct target of FGF21 in the brain regulating alcohol consumption may be excitatory neurons which reside in the BLA and project to the NAc to modulate reward. Future work will focus on how FGF21 influences activity of KLB expressing neurons in the BLA and what the molecular identity of the neurons required for inhibition of alcohol consumption are.

43 Interrogating Activity of Environmental Contaminants Against the Druggable GPCR-ome

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Exposure to environmental contaminants can result in a myriad of conditions including developmental disorders, pulmonary conditions, and neurological pathologies. Literature precedent and new, emerging data, demonstrates specific GPCRs can be activated by some classes of environmental contaminants, including PCBs, pesticides, chloropropanols, and others. When interrogating GPCR's with small-molecule based screens, the traditional paradigm is to test hundreds of thousands of compounds against a single target. In recent literature, an open-source method **"PRESTO-TANGO"** (Parallel Receptor-ome Expression and Screening via Transcriptional Output – TANGO) has been described as a feasible approach to simultaneously screen the entire druggable human GPCR-ome against a smaller collection of compounds via a G protein-independent β-arrestin recruitment assay. In this work, we have adopted a similar approach to the PRESTO-TANGO methodology, enabling us to screen activity of a number of environmental contaminates against the known druggable GPCR-ome of 314 GPCRs. From our initial screen, we identified several potential ligand-receptor interactions. Secondary dose response experiments uncovered what we believe to be a novel ligand-receptor relationship between PCBs and the Sphingosine-1-phoshpate receptor (S1PR) family. Experiments to further validate and probe the relationship between PCBs and S1PR's, and the subsequent biological effects are currently underway. Ultimately, this work utilizes a modified form of the PRESTO-TANGO assay as a novel screening platform to identify the bioactivity of environmental contaminants.

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44 Polysynaptic neurotracing of autonomic nuclei involved in liver and kidney function

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Obesity and related conditions such as hypertension represent a major health concern. Studies have demonstrated that energy homeostasis which is altered in obesity is in part related to liver function while renal dysfunction contribute to obesity-related hypertension. Although some of the physiology associated with adverse liver and renal function in obesity and related hypertension is understood, the complexity and extent of central nervous system (CNS) involvement is not well understood. Previous studies employing pseudorabies viruses (PRV) for polysynaptic tracing of the CNS autonomic network have provided insight into the complexity of these networks related to the autonomic control of liver, kidney, or adipose tissue. However, comparison of these studies had not shown complete agreement in the nuclei identified and no studies have directly compared the autonomic network nuclei for the liver and kidney. Therefore, we used two groups of animals that received PRV injections into the left lobe of the liver or the two kidneys to compare labeled nuclei between the two groups. The animals were sacrificed 5, 6, or 7 days after PRV injections, perfused, and the brains extracted. The brains were sectioned at 50-micron thickness, stained, and imaged with confocal microscopy. Sections were matched to the mouse atlas (Franklin & Paxinos, 3rd ed, 2008) and nuclei identified. Consistent with published work, we identified several nuclei that project to both the liver and kidney although they may represent different orders (expression appearing on different days). Moreover, we identified several nuclei such as the subfornical organ, nucleus accumbens, and insular cortex associated with the liver or kidney which have not been previously reported and a few nuclei that were specific to the liver or kidney. Our study demonstrates the existence of an overlap in the projections that sub-serve the liver and kidneys. The relevance of the newly identified autonomic projections for the control of hepatic and renal functions is under investigation.

45 **Novel *de novo* mutations in the PP2A regulatory subunit PPP2R2B causing neurodevelopmental disorders**

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Serine/threonine protein phosphatase 2A (PP2A), is a heterotrimeric protein comprised of a conserved scaffold A subunit, catalytic C subunit, and a variable regulatory B subunit. The regulatory subunit determines PP2A substrate specificity and subcellular localization. Mutations in PPP2R2B, one of twelve regulatory subunit genes are mammals, causes spinocerebellar ataxia 12 (SCA12), a rare neurodegenerative disorder. PPP2R2B encodes neuron specific and alternatively spliced cytosolic Bβ1 and mitochondria-targeted Bβ2 splice variants. The N-terminal mitochondrial targeting sequence of Bβ2 recruits it to the outer mitochondrial membrane (OMM) and induces mitochondria fragmentation and increases susceptibility to neuronal insults. Furthermore, a mouse knockout (KO) of Bβ2 shows elongated mitochondria and neuroprotection. Recently, Hamdan et al. performed whole exome sequencing on severe to moderate intellectually disabled patients and identified a possibly damaging de novo missense mutation, Arg149Pro, in the PPP2R2B gene in one patient. The patient suffered from intellectual disability (ID), intractable seizures and autistic features. Two different, yet unpublished *de novo* missense mutations (N310K And T246K) were identified in patients from New Zealand, present autistic features, tremors, hyperactivity disorder, microcephaly, cerebellar atrophy and spastic diplegia. We generated and expressed these mutations by itself and in complex with A subunit in mammalian HEK cells and initial co-immunoprecipitation experiments indicate that one of the mutations of the three, R149P does not bind to A and C subunit to form a holoenzyme assembly. We have previously shown that under cellular stress, Bβ2 rapidly translocates to mitochondria to promote apoptosis. To understand the effect of these mutations, we studied the co-localization of these mutants in Hela cells and do not observe any significant difference in case of N310K and T246K mutations, however the R138P mutant tends to be more cytoplasmic than mitochondrial localized and when expressed in hippocampal neurons, the mitochondrial length determined by form factor seems significantly longer compared to WT Bβ2 and other mutants. These initial findings suggest the neuroprotective nature of R149P mutation in Bβ2. We are also generating stable mammalian cell lines to identify cellular substrates by phospho-proteomics

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46 **Testosterone and resistance training exercise protects against the development of chronic widespread muscle pain**

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Chronic widespread pain conditions are more common in women, with a greater female presentation of movement-related pain suggesting sexual differences in the developmental mechanisms of widespread pain. Female mice develop bilateral, more severe and longer duration hyperalgesia compared to male mice in a model of activity-induced muscle pain. We examined if testosterone protects against the development of widespread fatigue-induced muscle pain. Muscle hyperalgesia was induced by two sub-threshold muscle insults (20µl normal saline, pH 5.0, 5 days apart) and fatiguing muscle contractions (6 min electrically-induced contractions) in C57BL6/J mice (n=97). Hyperalgesia was assessed as decreases in muscle withdrawal thresholds to pressure applied over the gastrocnemius muscle by force-sensitive tweezers. We examined if 1) orchiectomy, 2 weeks before induction of the model, prevented the development of bilateral and long-lasting hyperalgesia, when compared to intact male and female mice, and 2) 2 weeks of testosterone administration reversed effects of orchiectomy in males and reduced widespread hyperalgesia in females. We examined 3 conditions 1) muscle insult and fatigue given at the same time in the same muscle, 2) muscle insult and fatigue given at the same time in opposite muscles, and 3) muscle fatigue given 24h before muscle insult. Female mice developed bilateral hyperalgesia in all 3 conditions, which was longer lasting than males. Male mice only developed unilateral hyperalgesia when the muscle insult and fatigue were given in the same muscle at the same time. In contrast, orchiectomized male mice developed bilateral hyperalgesia in all 3 conditions, and the hyperalgesia was longer-lasting, similar to that observed in female mice. Testosterone in female and orchiectomized male mice, shortened the hyperalgesia similar to that observed in intact male mice. A resistance training protocol also prevented the development of activity-induced hyperalgesia in male and female mice, which was reversed through the administration of the androgen receptor blocker flutamide. These results suggest that testosterone and resistance training protect male and female mice from developing long-lasting widespread mechanical hyperalgesia in a model of chronic muscle pain.

47 NTS Catecholamine Neurons Mediate Hypoglycemic Hunger Via Medial Hypothalamic Feeding Pathways

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Glucose deficit triggered by energy deprivation or therapeutic agents can have fatal consequences. Increased appetite is a key behavioral defense against hypoglycemia, however the central pathways involved are not well understood. Here we describe a glucoprivic feeding pathway by tyrosine hydroxylase (TH)-expressing neurons from nucleus of solitary tract (NTS) which project densely to the hypothalamus and elicit feeding through bidirectional adrenergic modulation of agouti related peptide (AgRP) and proopiomelanocortin (POMC)-expressing neurons. Acute chemogenetic inhibition of ARC projecting NTS^{TH} neurons or their target, AgRP neurons, impaired glucoprivic feeding induced by 2-Deoxy-D-glucose (2DG) injection. Neuroanatomical tracing results suggested that ARC-projecting orexigenic NTS^{TH} neurons are largely distinct from those parabrachial nucleus (PBN)-projecting anorexigenic NTS^{TH}-neurons. Collectively, we describe a circuit organization in which an ascending pathway from brainstem coordinates hypoglycemic appetite through key hunger neurons in the hypothalamus in response to hypoglycemia.

48 **Targeting GPCR signaling suppresses HER2+ breast cancer stemness and sensitize HER2-targeted therapies**

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Background: G-protein coupled receptors (GPCRs) are the largest family of cell surface receptors and highly desirable drug targets for diverse diseases. They are implicated in the progression of many cancers, including breast cancer. Despite their importance, our understanding of the role of GPCRs in tumorigenesis remains limited because there are over 350 non-sensory GPCRs. Moreover, many GPCRs are overexpressed in cancer cells, making it difficult to target individual GPCRs for cancer treatment. Thus, to target GPCRs as a point of therapeutic intervention for breast cancer, it will require understanding the function of the relevant subset of GPCRs and identifying key players and signaling molecules.

HER2$^+$ breast cancer is aggressive and usually treated with HER2-targeted therapy (e.g.,trastuzumab), which is effective in >50% of cases. Quickly, however, cancers develop resistance. The mechanisms driving resistance remain largely unknown but are attributed to a reservoir of stem-cell-like, cancer stem cells (CSCs). CSCs may escape therapy in part because they find ways to transactivate HER2 via other pathways—including pathways mediated by GPCRs. Ablating CSCs might enhance HER2-targeted therapy and prevent resistance. In this study, we tested whether targeting GPCRs that signal via a subset of G proteins, the Gi/o proteins, blocks HER2+ breast cancer progression and enhances tumor response to therapies.

Method: The expression of ~400 non-sensory GPCRs in MCF10A, MCF10A-overexpressing HER2, murine mammary epithelial cells isolated from FVB mice or premalignant MMTV-Neu transgenic mice were determined by qPCR arrays. Transgenic mouse lines were generated to block or upregulate Gi/o-GPCR signaling, in an inducible manner and specifically in mammary epithelial cells. The effects of manipulating GPCR signaling on tumor initiation and progression and tumor metastasis of Neu mice were monitored over time. The impact of Gi/o signaling on cancer stemness was investigated by flow cytometric analysis of cancer stem cell markers/reporter and tumorsphere formation assays. The cross talk between Gi/o-GPCRs and EGFR/HER2 signaling was characterized by biochemical analysis.

Results: As compared to control cells, many GPCRs, in particular, Gi/o-coupled GPCRs are upregulated in MCF10A cells overexpressing HER2 and premalignant mammary epithelial cells from Neu mice. By mammary gland-specific expression of a catalytic subunit of the pertussis toxin (PTx), a selective inhibitor that uncouples Gi/o proteins from their cognate GPCRs, in MMTV-Neu transgenic mice, we demonstrated that blocking Gi/o-GPCR signaling suppressed HER2-driven mammary tumor initiation and progression and decreased lung metastasis. Mechanically, we found that upregulated Gi/o-GPCRs in breast cancer cells transactivated the ErbB/HER family of protein-tyrosine kinases via Src and PI3K pathways to promote breast cancer stemness and tumor growth and metastasis. Targeting Gi/o-GPCR signaling by PTx, Src or PI3K inhibitors suppresses breast cancer stem cells (CSCs) and sensitizes HER2$^+$ breast cancer to HER2-targeted therapies. Together, our data demonstrate that targeting GPCR downstream signal pathways may represent a new approach to ablate CSCs and block tumor progression and augment HER2-targeted therapeutics.

Significance: This study provides new insight into how cancer stem cells acquire the ability to transactivate EGFR/HER2 via Gi/o-GPCR signaling, leading to tumor progressions and resistance to HER2-targeted therapies. Such knowledge might be exploited as we search for new ways to eliminate CSCs to augment HER2 therapeutics