IOWA

2026 Pharmacological and Pharmaceutical Sciences Research Retreat



August 14, 2026

College of Pharmacy

2026 Pharmacological and Pharmaceutical Sciences Research Retreat

Friday, August 14, 2026

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Fan Favorite Voting

Thank you for participating in the "Fan Favorite" of judging the oral presentations and posters presentation for the Pharmacological and Pharmaceutical Sciences T32 Research Retreat.

Upon check-in you should have recieved 9 tickets:
1 - Short Talk Presentation (white)
2 - Undergraduate Poster Presetation (gray)
2 - Graduate Student Poster Presentation (blue)
2 - Post-Doc Poster Presentation (yellow)
2 - Research Staff Poster Presentation (pink)

Voting Criteria & Scientific Conduct
To foster a rigorous and equitable experience for all presenters, we ask attendees to observe the following principles when casting votes:

Broad Engagement: Please visit and evaluate multiple posters across each category rather than limiting your interactions to familiar labs or colleagues.

Complete Attendance: For short talks, please listen to all presenters in the session before casting your vote.

Merit-Based Evaluation: Consider clarity of presentation, scientific novelty, experimental design, and visual organization when selecting your winner.

How to Vote

Short Talks: Hand your ticket to admin staff near the 210 CPB exit doors as you leave for the break and group photo.

Poster Sessions: Place your ticket into the collection bowl near the entrance of 110A/B CPB for the presenter who earned your vote at the conclusion of each poster session.

Friday, August 14, 2026

College of Pharmacy Building – 110 A&B, and 210

8:00 am	Registration and Poster setup – CPB Atrium Continental breakfast
9:00 am	Welcome and Opening Remarks – CPB 210 Stefan Strack, PhD, Co-Director of T32
9:10 am	Short Talk Presentations by T32 Trainees *(12-minute talks + 3 minutes Q&A)*
10:50 am	Break & Group Photo
11:10 am	Poster session 1 – 110 A&B Odd number posters
12:00 pm	Lunch – CPB Atrium
12:50 pm	Poster session 2 – 110 A&B Even number posters
1:45 pm	Career Development – Explore Future Careers with T32 Trainee Alumni –210
2:45 pm	Keynote Speaker – 210 *(35-minute talk + 10-minute Q&A)* Yao Chen, PhD *"Molecular Ballet: Decoding Biochemical Signaling Dynamics in the Brain"*
3:35 pm	Closing Remarks & Awards – 210 Stefan Strack, PhD and Dave Roman, PhD

Keynote Speaker



Yao Chen, PhD

Assistant Professor,
Department of Neuroscience -
Washington University

Keynote Lecture: *Molecular Ballet: Decoding Biochemical Signaling Dynamics in the Brain*

Program Leadership



David Roman, PhD
Co-Director

Professor, Associate Dean of Research and Graduate Education, College of Pharmacy



Stefan Strack, PhD
Co-Director

Professor, Interim DEO of Neuroscience and Pharmacology, Carver College of Medicine



Michael G. Anderson, PhD

Professor, Molecular Physiology and Biophysics



Rebecca Dodd, PhD

Associate Professor
Internal Medicine - Hematology, Oncology, and Blood and Marrow Transplantation



Ernesto Fuentes, PhD

Professor
Biochemistry and Molecular Biology



Nels Gerstner, PhD

Assistant Professor
Chemistry



Dawn E. Quelle, PhD

Professor
Neuroscience and Pharmacology



Brenna Powers

Junior Student Representative
Trainee, Pharmacology Graduate Program



Hannah Hazzard

Senior Student Representative
Trainee, Neuroscience Graduate Program

Short Talk Presentations by Second Year T32 Trainees

College of Pharmacy Building - 210
9:10 - 10:45 am

Order	Presenter
1	Sean Brobston, Human Toxicology Grad Program *"Establishing a Baseline Human Relevant PCB Metabolite Profile for Future Studies of Microbiome-Mediated Neurotoxicity"* Mentor: Hans-Joachim Lehmler, PhD
2	Brenna Powers, Pharmacology Grad Program *"D2 Autoreceptors Regulate the Dopamine Transporter in a Sex- and Region-Dependent Manner"* Mentor: Adele Stewart, PhD
3	Alexander Dumas, Biochemistry Grad Program *"The Count of Cardiac Metabolism: Omega-3 Fatty Acids, Cardiac Fatty acid Uptake, and Hart Health"* Mentor: Brandon Davies, PhD
4	Sarah Preston, DDET Program *"NRF2 Signaling as a Mechanism of Protection Against Organophosphate Toxicity"* Mentor: Jon Doorn, PhD
5	Victor Kilonzo, Neurosci/MSTP Grad Program *"Dopaminergic Modulation of Prefrontal Oscillations and D1 Ensembles in Working Memory"* Mentor: Nandakumar Narayanan, MD, PhD

1. *"Establishing a Baseline Human Relevant PCB Metabolite Profile for Future Studies of Microbiome-Mediated Neurotoxicity"*

Sean Brobston[1], Xueshu Li[2], Youjun P. Suh[3], Julia Cui[3], Sujitha Ravulapalli[2], Nicole Breese[1,2], Hans-Joachim Lehmler[1,2]

[1]Interdisciplinary Graduate Program in Human Toxicology, The University of Iowa, [2]Department of Occupational and Environmental Health, College of Public Health, The University of Iowa, [3]Department of Environmental and Molecular Toxicology, College of Agricultural Science, Oregon State University

Polychlorinated biphenyls (PCBs) are persistent environmental contaminants with known neurotoxic effects. Environmental exposure to PCBs is rarely limited to a single congener, and the resulting toxic effects can vary depending on the mixture of PCB congeners an individual is exposed to. While several environmentally relevant mixtures have been assessed for toxicity, these simulated PCB mixtures differ from the PCBs present in the human brain. To more accurately assess PCB neurotoxicity, we generated a Human-Relevant PCB (HR-PCB) mixture based on PCBs found in postmortem human brain tissue. Furthermore, the gut microbiome can act on PCBs, influencing metabolic outcomes. Individual variation in gut microbial composition and function could result in different neurological outcomes. Our larger scope is to evaluate how differences in the human gut microbiome alter the PCB metabolite profile and resulting memory deficits from exposure to the HR-PCB mixture. This study was an early look into the PCB metabolite profile in regular mice exposed to the HR-PCB mixture.

2. *"D2 Autoreceptors Regulate the Dopamine Transporter in a Sex- and Region-Dependent Manner"*

Brenna A. Powers[1], Nathan Fassett[1], Jacob Bernholtz[1], Danlin Liu[2], Julia I. Neuharth[2], and Adele Stewart[1,3]

[1]Department of Neuroscience and Pharmacology, [2]Interdisciplinary Graduate Program in Neuroscience
[3]Iowa Neuroscience Institute, University of Iowa

Dysregulation of dopaminergic neurotransmission has been linked to numerous neuropsychiatric disorders, virtually all of which display a sex bias in prevalence, age of onset, and/or clinical symptomology. The presynaptic dopamine (DA) transporter (DAT), the primary facilitator of rapid synaptic DA clearance, is essential for maintenance of DA homeostasis. DAT is controlled by several regulatory mechanisms including an important negative feedback loop mediated by D2-type DA autoreceptors (D2ARs), which, when activated, increase surface trafficking of DAT to aid in the inactivation of synaptic DA. Previous work has identified sex-biased and region-specific coupling between DAT and D2AR wherein D2AR-dependent DAT threonine 53 (T53) phosphorylation and membrane trafficking occur exclusively in the nigrostriatal DA circuit in male mice but are only observed in the mesolimbic DA circuit in females. However, it remains unclear how or if changes in DAT phosphoregulation or surface expression translate into alterations in DA clearance. To functionally assess the impact of D2ARs on DAT mediated DA reuptake, we isolated brain slices containing dorsal striatum (DStr), nucleus accumbens (NAc), pre-frontal cortex (PFC), projection regions defining the three major DA circuits, from both male and female WT C57BL/6 mice and performed ex vivo [3H] DA uptake assays following bath application of the D2/D3 agonist quinpirole or D2/D3 antagonist sulpiride. Our results show that quinpirole exerts a concentration-dependent effect on DAT function specific to the male DStr. More specifically, low dose quinpirole (0.1 mM) resulted in a significant decrease in DA uptake and a higher concentration (1 mM) led to a significant increase in DA uptake. Interestingly, quinpirole failed to impact DA uptake in the NAc or PFC of males or in any of the assessed female projection regions. Sulpiride treatment also inhibited DA uptake exclusively in the male DStr. The lack of autoreceptor dependent feedback driving enhanced DA reuptake in females is predicted to dramatically shape DA dynamics in vivo and highlights a disconnect between DAT phosphorylation state, surface expression, and transporter function. Ongoing work focuses on generation of a novel tamoxifen inducible D2AR knockout mouse model (Drd2flx/flx:DATiCreER) to evaluate the in vivo consequences of differential autoreceptor action between the sexes on DA release and reuptake as well as animal behavior. Importantly, we hypothesize that sex biased D2AR-DAT coupling might represent a key mechanism determining sex specific susceptibility to neuropsychiatric disorders many of which have been linked to DA dysregulation.

3. *"The Count of Cardiac Metabolism: Omega-3 Fatty Acids, Cardiac Fatty acid Uptake, and Hart Health"*

Alexander Dumas, B.S., Brandon Davies, Ph.D.

University of Iowa Department of Biochemistry and Molecular Biology

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in the world with ~19.4 millions of deaths in 2021, an 18% increase from 2011. The incidence of cardiovascular risk and disease is predicted to continue climbing in the coming decades. Dietary omega-3 (n-3) poly-unsaturated fatty acids have gained popularity as a means of lowering circulating triglycerides and potentially reducing the risk for CVD. Increasing evidence from rodent and human studies have demonstrated that n-3s reduce circulating triglycerides and increase insulin sensitivity but only a subset of human studies have found that n-3s reduce the risk of CVD. The heterogeneity found in these clinical studies suggests that our understanding of the physiological effects of n-3s is incomplete. Data from our lab shows that mice fed a diet enriched in n-3 fatty acids have a striking increase in cardiac triglyceride-derived fatty acid uptake while also showing lower plasma insulin levels and increased insulin sensitivity. This is intriguing as increased cardiac fatty acid uptake is commonly associated with increased risk of lipotoxicity and cardiomyopathy. Furthermore, these data suggest three interesting and important questions: 1) Is the increased cardiac fatty acid uptake induced by n-3 feeding beneficial or detrimental to heart health? 2) Does increased fatty acid uptake in the heart contribute to the increased systemic insulin sensitivity in mice on an n-3 based diet? 3) How does an n-3 enriched diet increase fatty acid uptake in the heart? To answer these questions, I fed wildtype mice and mice with reduced cardiac fatty acid uptake a diet enriched in n-3s or control diets. Following 10 and 26 weeks on diet, I measured systemic insulin and glucose tolerance and systolic heart function. Reducing cardiac fatty acid uptake did not influence plasma insulin or glucose tolerance in n-3 fed mice but did decrease systemic insulin sensitivity in n-3 fed male mice after 10 weeks on diet. Despite increased cardiac fatty acid uptake, n-3 fed mice showed no disruption of systolic heart function after 10 or 26 weeks on diet. We also analyzed RNA-sequencing from hearts of n-3 fed mice and found that PPARa target genes are more strongly enriched following an n-3 diet compared to control diets suggesting a possible regulatory pathway. My findings show that the n-3 induced increase in cardiac fatty acid uptake is not necessary for the beneficial systemic metabolic phenotypes also related to n-3s. n-3 fed mice also appear to be more tolerable to heart dysfunction caused by increased cardiac fatty acid uptake.

4. *"NRF2 Signaling as a Mechanism of Protection Against Organophosphate Toxicity"*

Sarah E. Preston[1], Michael A. Garcia-Mares[1], Anna E. Bartman[1], David B.C. Martin[2], Jonathan A. Doorn[1]

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

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in the world with ~19.4 millions of deaths in 2021, an 18% increase from 2011. The incidence of cardiovascular risk and disease is predicted to continue climbing in the coming decades. Dietary omega-3 (n-3) poly-unsaturated fatty acids have gained popularity as a means of lowering circulating triglycerides and potentially reducing the risk for CVD. Increasing evidence from rodent and human studies have demonstrated that n-3s reduce circulating triglycerides and increase insulin sensitivity but only a subset of human studies have found that n-3s reduce the risk of CVD. The heterogeneity found in these clinical studies suggests that our understanding of the physiological effects of n-3s is incomplete. Data from our lab shows that mice fed a diet enriched in n-3 fatty acids have a striking increase in cardiac triglyceride-derived fatty acid uptake while also showing lower plasma insulin levels and increased insulin sensitivity. This is intriguing as increased cardiac fatty acid uptake is commonly associated with increased risk of lipotoxicity and cardiomyopathy. Furthermore, these data suggest three interesting and important questions: 1) Is the increased cardiac fatty acid uptake induced by n-3 feeding beneficial or detrimental to heart health? 2) Does increased fatty acid uptake in the heart contribute to the increased systemic insulin sensitivity in mice on an n-3 based diet? 3) How does an n-3 enriched diet increase fatty acid uptake in the heart? To answer these questions, I fed wildtype mice and mice with reduced cardiac fatty acid uptake a diet enriched in n-3s or control diets. Following 10 and 26 weeks on diet, I measured systemic insulin and glucose tolerance and systolic heart function. Reducing cardiac fatty acid uptake did not influence plasma insulin or glucose tolerance in n-3 fed mice but did decrease systemic insulin sensitivity in n-3 fed male mice after 10 weeks on diet. Despite increased cardiac fatty acid uptake, n-3 fed mice showed no disruption of systolic heart function after 10 or 26 weeks on diet. We also analyzed RNA-sequencing from hearts of n-3 fed mice and found that PPARa target genes are more strongly enriched following an n-3 diet compared to control diets suggesting a possible regulatory pathway. My findings show that the n-3 induced increase in cardiac fatty acid uptake is not necessary for the beneficial systemic metabolic phenotypes also related to n-3s. n-3 fed mice also appear to be more tolerable to heart dysfunction caused by increased cardiac fatty acid uptake.

5. *"Dopaminergic Modulation of Prefrontal Oscillations and D1 Ensembles in Working Memory"*

Victor Kilonzo, Irina Zaslavskaya, Matthew Weber, Alexandra Bova, Young-Cho Kim, Nandakumar Narayanan

University of Iowa CCOM Department of Neurology, Department of Neuroscience, Medical Scientist Training Program

Working memory is orchestrated by the prefrontal cortex (PFC), which supports persistent activity patterns as a mechanism for temporary storage, maintaining task-relevant information in the absence of external cues. Prefrontal dopamine facilitates the stabilization of prefrontal activity states by binding to D1 dopamine receptors (D1DRs). Prefrontal activity and dopamine signaling work in concert to regulate working memory, yet the mechanisms by which they cooperate are unclear. Advances in GPCR activation-based dopamine (GRAB DA) sensors that fluoresce at low dopamine concentrations enable temporally precise sampling of dopamine dynamics in the mouse PFC. To assess working memory, D1-cre mice were trained on a delayed non-match to position (DNMTP) working memory task and, once trained, underwent stereotaxic surgery and injection of GRAB DA sensor variant gDA3m and Cre-dependent ChrimsonR into the PFC. Mice were also implanted with a combined optogenetic and multielectrode recording array to simultaneously record prefrontal dopamine dynamics via fiber photometry and prefrontal ensemble activity during the DNMTP task. After recording, prefrontal D1DR-expressing neurons were optogenetically tagged via ChrimsonR to identify putative D1DR neurons. We examined whether increasing delay intervals during the DNMTP task amplified prefrontal dopamine, as well as low-frequency oscillations that accompany optimal working memory performance. Furthermore, we investigated how prefrontal dopamine input contributes to oscillatory signatures of cognitive control, and neuron spiking activity associated with working memory. Our work highlights how neuromodulatory dopamine signals in the PFC engage specific D1DR populations to orchestrate neuronal activity and network rhythms underlying working memory.



Eli Frakes, PhD

National Field Director
for Women's Health
AbbVie

Mentor: Donna Hammond, PhD - 2006

Eli Frakes has 19+ years of experience in the pharmaceutical industry, with a career spanning field medical, US and Global medical affairs, as well as team management. After earning his PhD in Pharmacology from the University of Iowa, he began his career at Eli Lilly and Company, where he spent more than a decade in roles of increasing responsibility. He has experience as a Neuroscience Medical Science Liaison, working in U.S. Medical Affairs supporting multiple chronic pain launches, leading a scientific communications team for the Alzheimer's and Psychosis portfolio, and global medical affairs in the development of the Alzheimer's global launch strategy.

In October 2017, Eli joined AbbVie as National Field Director for Women's Health, where he returned to field medical leadership and helped launch a new asset. Since then, he has continued to grow his scope and responsibility across medical affairs. He is currently Head of Specialty in Medical Affairs and Health Impact, where he leads an organization of approximately 60 people responsible for more than 50 global on-market and pipeline assets across more than 10 therapeutic areas.

Eli also holds a B.S. in Biology and Chemistry from Buena Vista University and completed an executive leadership program at Indiana University's Kelley School of Business. He lives in Long Grove, Illinois, with his wife, Mandy, and their two children. Outside of work, he enjoys spending evenings and weekends at basketball and softball games.



Rondine Allen, PhD

Pharmacology/Toxicology Team Lead, Office of Therapeutic Products, Center for Biologics Evaluation and Research, Food and Drug Administration

Mentor: Kevin Rice, PhD - 2018

Dr. Rondine Allen earned her Ph.D. in 2018 from the University of Iowa College of Pharmacy, where she trained in the laboratory of Dr. Kevin Rice. Following graduation, Dr. Allen conducted research as a postdoctoral fellow at the Food and Drug Administration (FDA). In 2020, she transitioned away from the bench into a regulatory role within the FDA as a Pharmacology/Toxicology (P/T) reviewer. In this capacity, she evaluated nonclinical data to support clinical trial decisions for cell and gene therapies. In 2024, she was promoted to Senior P/T Reviewer and subsequently to Team Lead, where she guides a team of reviewers responsible for assessing the pharmacology and toxicology of a wide range of innovative cell and gene therapy products. In addition to her review responsibilities, Dr. Allen contributes to multiple FDA and interagency working groups and has co-authored FDA draft guidance documents and publications on the nonclinical regulatory perspective. Dr. Allen is deeply committed to mentorship and is passionate about helping the next generation of scientists discover diverse, impactful career paths in public service and regulatory science.



Valeria Cota, PhD

Associate Clinical Trial Manager
Medpace

Mentor: Nicole Brogden, PhD - 2025

Valeria Cota completed her doctoral training at the University of Iowa in May 2025 with a degree in Human Toxicology.

She has been with Medpace for a year as an Associate Clinical Trial Manager and has overseen five Phase II-III clinical trials. Within her first year in Cincinnati, she has completed the Flying Pig Marathon and volunteered with TOPSoccer.



Dipali Deshpande, PhD

Senior Director
Protein Expression Sciences
Regeneron Pharmaceuticals, Inc.

Mentor: Fred Quelle, PhD - 2004

Word of advice: Follow the 3A's in life, Aspire, achieve, and acknowledge….

Coming from a professional working family originally from Mumbai, India, education was always given the highest priority. My Bachelors degree in Pharmacy was a stepping stone that motivated me to pursue a scientific career in the United states. With Bachelors in Pharmacy, I thought I was getting a good scientific foundation. After that I was still on the fence about a PhD so I first applied for a Masters program in California. I took classes in cancer biology and became interested in the scientific aspects of tumor growth. That is when I started my eastward journey, first to University of Iowa for PhD in Immunology and Molecular Pharmacology followed by a postdoc training at Northwestern University, Chicago and finally to Regeneron Pharmaceuticals in Tarrytown, NY.

I started my industry career as a cell line development Scientist at Regeneron Pharmaceuticals, Inc in 2009. 17 years later, I lead a team of 25 scientists responsible for generating clinical cell lines for Regeneron's pipeline.



Aaron Mickle, PhD

Associate Professor
Depts. of Physiology,
Neurosurgery & Urology
Medical College of Wisconsin

Mentor: DP Mohapatra, PhD - 2014

Aaron Mickle is currently an Associate Professor in the Departments of Physiology, Neurosurgery, and Urology, where he leads a research program investigating the role of the nervous system in the regulation of bladder sensation, pain, and control. He received a PhD in pharmacology from the University of Iowa under the mentorship of Dr. DP Mohapatra, studying nociceptor sensitization in the context of metastasized cancer pain. He then completed his postdoctoral fellowship at Washington University in Saint Louis with Dr. Robert Gereau, where he collaborated with materials, electrical, and biomedical engineers to develop closed-loop optogenetic-based neuromodulatory technologies targeting the lower urinary tract. Then in 2019, he joined the Department of Physiological Sciences at the University of Florida to establish his own lab. In 2024, he moved his lab to the Medical College of Wisconsin and was promoted to associate professor. He has been NIH-funded for the majority of his career, starting with an F31, F32, and R21, and is currently the PI on two R01 grants studying bladder neurophysiology.

Yao Chen, PhD

Assistant Professor,
Department of Neuroscience
Washington University

Molecular Ballet: Decoding Biochemical Signaling Dynamics in the Brain



Yao Chen is an Assistant Professor in Neuroscience, and affiliated member in Biomedical Engineering, Cell Biology and Physiology, and Electrical and Systems Engineering at Washington University in St. Louis. During her Ph.D. in John Flanagan's laboratory at Harvard University, she contributed to developmental neurobiology by elucidating mechanisms that establish the correct pattern of neural mapping cues during brain wiring. As a postdoc in Bernardo Sabatini's laboratory at Harvard Medical School, she developed optical reporters and built fluorescence lifetime-based systems to visualize molecular signals, in both live brain slices and freely moving mice. Using the reporters, she uncovered new principles in neuromodulator actions. In her laboratory at Washington University, her team aims to develop new technology and use these novel tools to uncover the spatiotemporal dynamics of biochemical signals and investigate how these previously unknown features contribute to sleep, learning, and functions of neuromodulators.

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1. *"Sex-dependent regulation of the Dopamine Transporter (DAT) by insulin"*

Nathan Fassett[1], Anya Melson[1], Julia Neuharth[2], Brenna Powers[1], Adele Stewart[1,3]

[1]Department of Neuroscience and Pharmacology, [2]Interdisciplinary Graduate Program in Neuroscience, [3]Iowa Neuroscience Institute, University of Iowa

Obesity affects over 70% of U.S adults over the age of 20. Weight gain in obesity results from maladaptive food consumption irrespective of homeostatic needs. Though the prevalence of obesity is similar between sexes, women are more likely to develop severe obesity (BMI > 35) suggesting that biological sex may influence compulsive eating. Obesity has been linked to altered activity in dopamine (DA)-regulated brain circuits, which play key roles in motivation, reinforcement learning, and habit formation. The dopamine transporter (DAT) is the chief determinant of synaptic DA availability and exposure to obesogenic diets high in fat and sugar has been shown to impair DA clearance in the nucleus accumbens (NAc). However, prior work excluded female subjects and failed to systemically control for diet composition. We have now shown that high fat diet (HFD) feeding does not alter baseline DAT activity, pointing to sugar consumption as an influencing factor of DA clearance. Insulin is released as blood glucose rises following a meal and is known to be actively transported across the blood-brain barrier. Single cell nuclear RNA seq confirmed that insulin receptors (IRs) are expressed in DA neurons in rodents and humans, and insulin has been shown to increase DAT-driven DA uptake in vitro. We now demonstrate that insulin (1nM) increases uptake of [3H] DA in male ex vivo brain slices containing dorsal striatum (DStr), but not NAc or prefrontal cortex (PFC). However, this effect of insulin was absent in the DStr of females. Remarkably, chronic inhibition of nuclear progesterone receptors (PGRs) with mifepristone restored insulin-dependent DAT regulation in the female DStr. Our data demonstrate that the ability of insulin to modulate DAT is a sex- and region-specific and under hormonal control in females. Ongoing work seeks to establish the molecular basis for insulin- and progesterone-dependent DAT regulation and the impact of these mechanisms on food-driven habits. Ultimately, this work will yield valuable insights into how maladaptive changes in DAT function drive compulsive eating in individuals with obesity in a sex-biased manner.

2. *"Sex-dependent regulation of DAT by progesterone receptors"*

Jaxson Hicks[1], Julia I. Neuharth[1,2], Brenna A. Powers[1], Nathan Fassett[1], Jacob Bernholtz[1], Adele Stewart[1,3]

[1]Department of Neuroscience & Pharmacology, [2]Interdisciplinary Neuroscience Graduate Program, 3 Iowa Neuroscience Institute, University of Iowa

Virtually all neuropsychiatric disorders exhibit sex bias in prevalence, age of onset, or symptomology including several linked to dysregulation of dopaminergic neurotransmission. However, the molecular mechanism(s) by which biological sex shapes dopamine (DA) action in the brain remain unclear. Here, we provide new single cell nuclear RNA sequencing (scRNAseq) data demonstrating that dopaminergic neurons in both the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) express receptors for sex steroid hormones including the progesterone receptor (PGR) and androgen receptor (AR), but not estrogen receptors (ERα/β). Notably, Pgr was the sole steroid hormone receptor identified as DA neuron enriched in a translating ribosome affinity purification (TRAP) dataset. PGR is also highly co-expressed with the DA transporter (DAT), which controls extracellular DA levels by facilitating rapid DA reuptake. In fact, data from our group has demonstrated that inhibition of PGR with mifepristone leads to an increase in DAT activity and surface expression selectively in the dorsal striatum (dStr), a brain region that receives dense innervation from SNc DA neurons. Here, we provide new histochemical evidence that PGRs are expressed on dopaminergic neurons in both the SNc and VTA in mice. Ongoing efforts seek to quantify PGR+/DAT+ neurons in the SNc and VTA of male and female WT mice, analysis that will be critical for elucidating how progesterone may shape DA dynamics in mice of both sexes. Our work also identified progesterone as a likely mediator of sex differences in DA action with relevance to psychiatric disorders such as schizophrenia, attention-deficit/hyperactivity disorder (ADHD), and substance use disorders.

3. *"Single-bouton presynaptic gating revealed in ex vivo dual-color two-photon calcium imaging"*

Yaroslav Andrianov[1], Anastasiia Tokhtamysh[1], Alex Keyes[1], Pavel Belan[2], Nana Voitenko[3,4], Yuriy Usachev[1]

[1]Department of Neuroscience & Pharmacology and The Iowa Neuroscience Institute, University of Iowa Carver College of Medicine, [2]Department of Molecular Biophysics, Bogomoletz Institute of Physiology, Kyiv, Ukraine, [3]Department of Biomedicine and Neuroscience, Kyiv Academic University, Kyiv, Ukraine, [4]Dobrobut Academy Medical School, Kyiv, Ukraine

Presynaptic inhibition at dorsal root ganglion terminals defines sensory processing both in health (e.g., tactile discrimination) and disease (e.g., hyperalgesia and allodynia in chronic pain) by regulating neurotransmitter release. Since Ca2+ directly regulates exocytosis of synaptic vesicles, its imaging provides the most direct readout of presynaptic inhibition, unlike indirect electrophysiological methods (whole cell postsynaptic recordings and DRPs) requiring interpretation of complex phenomena such as Cl- shunting inhibition. Calcium imaging of individual boutons allows us to directly observe presynaptic inhibition at this complex central relay where all peripheral sensory information converges, detangling it from the ambiguity of pooled measurements and ongoing synaptic plasticity. We developed a specific mouse line expressing two different Ca2+ indicators in both presynaptic primary afferents and postsynaptic spinal dorsal horn neurons. We performed dual-color two-photon Ca2+-imaging using ex vivo mouse spinal cord preparation with preserved dorsal roots for primary afferent fiber electrical stimulation and measured the effect in healthy and SNI models, as well as applied GABA and Glu receptor blockers (Bicuculline, CNQX, and AP5). This approach gives us enough spatial and temporal resolution to measure Ca2+ elevations in large numbers (up to ~300) of synaptic boutons per image. Our experiments demonstrate that the pre-stimulation of dorsal root A-fibers - within the characteristic duration of the dorsal root potential (DRP) of 50-300 ms - reduces the subsequent calcium elevation in electrically stimulated C-fibers and corresponding synaptic boutons in spinal dorsal horn laminae. Furthermore, the strength of the inhibition is proportional to the rate of electrically recruited Aβ-fibers and continues to increase as stimulation intensity rises into the recruitment range of low-threshold (LT) Aδ afferents. The aforementioned inhibition relies simultaneously on both GABA and glutamate transmission, suggesting the presence of inhibitory interneurons within the circuit. We directly image presynaptic inhibition at its most fundamental level — the primary afferent terminal. The studied effect resonates with the Gate Control Theory of Pain; however, it is revealed underneath a complicated interconnection.

4. *"Mechanism-Guided Therapeutic Targeting in Knock-in Mouse Models of PPP2R5D-Associated Neurodevelopmental Disorder "*

Tehreem Haider, Chian Ju Jong (PhD), Chunling Chen (PhD), Stefan Strack (PhD)

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

Houge-Janssens-Syndrome 1 (aka Jordan's Syndrome, JS) is a rare neurodevelopmental disorder caused by de novo missense mutation in the PPP2R5D gene, which encodes the B56δ regulatory subunit of the PP2A complex. This disorder is marked by developmental delay, cognitive impairment, and autism. PP2A regulatory subunits are responsible for determining enzyme localization and substrate specificity. Currently, there are no approved disease-modifying therapies for JS. Therefore, to enable a mechanism-guided therapeutic evaluation, we generated a knock-in mouse models with JS mutations (E198K, E420K). In this study, we employed a mechanism-guided approach to screen two pharmacologically distinct therapeutic candidates targeting complementary neural pathways. Zatolmilast, a selective PDE4D inhibitor, was chosen to enhance cAMP signalling, which is involved in synaptic plasticity and gene regulation. R-baclofen, a GABA-B receptor agonist, was selected to modulate inhibitory neurotransmission and network excitability. The behavioural assessment of E198K and E420K mice was conducted using a battery of behavioural assays, including the Open field test to evaluate locomotor activity and sedation, the 2-Trial Y-maze to assess short term memory, the Nestlet shredding/nest building assay, and the Phenotyper home cage system to quantify innate home cage behaviour, locomotor activity, and circadian activity patterns. The JS mouse models demonstrated robust deficits in cognition, home cage behaviours, and activity patterns, consistent with clinical manifestations observed in patients. Zatolmilast treatment improved cognitive deficits and reduced hyperactivity, reflecting that cAMP pathway modulation can be a viable therapeutic strategy for Jordan Syndrome. In contrast, R-baclofen failed to rescue the cognitive impairment and hyperactivity in E198K mice. Acute R-baclofen produced a dose-dependent sedation, which could be overcome with repeated dose administration. However, R-baclofen did not result in sustained improvements in cognitive and hyperactivity behaviours. Together, these findings demonstrate that PPP2R5D mutations contribute to neurodevelopmental disorders by disruption of the PP2A-regulated signalling pathways. The E198K and E420K knock-in models recapitulate the significant clinical representation of this disorder and serves as a valuable model for therapeutic evaluation. Importantly, pharmacological modulation of pathways can differentially influence disease-relevant phenotypes. These results highlight the importance of pathway-specific and mechanism-guided drug selection and support continued exploration of cAMP-targeted therapies for Jordan Syndrome.

5. *"NRF2 Signaling as a Mechanism of Protection against Organophosphate Toxicity"*

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Human exposure to organophosphate (OP) pesticides such as chlorpyrifos (CPF) occurs in agricultural, occupational and military settings worldwide. OP pesticides, such as CPF, can lead to cholinergic crisis via acetylcholinesterase (AChE) inhibition and accumulation of the neurotransmitter acetylcholine. Downstream effects of OP intoxication include seizures and production of reactive oxygen species (ROS), likely due to glutamate (Glu) excitotoxicity. In addition to cholinergic crisis as a toxic endpoint, there is evidence that CPF exposure correlates with additional adverse outcomes, including depression and neurodegenerative diseases. Molecular mechanisms for such conditions are elusive and may involve targets other than AChE. Metabolism of CPF is critical to cholinergic toxicity as it can yield either bioactivation to the toxic chlorpyrifos-oxon (CPO), detoxication via glutathione (GSH)/glutathione-S-transferase (GST; dechlorination), or hydrolysis of CPO via the esterases, paraoxonase 1 (PON1) and carboxylesterase 1 and 2 (CES), with CES serving as a “sink" to protect AChE. Interestingly, several of the enzymes critical to the metabolism of CPF are under the control of the transcription factor NRF2, including: GSH/GST, PON1 and CES. Activation of NRF2 is known to induce expression of numerous antioxidant enzymes to mitigate aberrant ROS production. The goal of our work is to explore the role of NRF2 modulation in mitigating risk of toxicity and adverse effects to CPF/OP agents and the therapeutic potential to target this pathway to mitigate CPF/OP toxicity. Our central hypothesis is that NRF2 activation alters CPF disposition and potential for toxicity via induction of enzymes that modulate OP metabolism and cellular damage, thus providing protection against OP insult. We have found that pretreatment of PC12 cells with potent activators of NRF2 provides protection against acute CPF and CPO treatment at sub-micromolar concentrations. In addition, we have shown that NRF2 activators induce the expression of both CES and PON1 in HepG2 cells (liver) as well as increasing general intracellular esterase activity. We found that NRF2 activation appears to protect HepG2 hepatic cells as well as SH-SY5Y neuronal cells and reduce overall ROS production upon insult with CPF. Finally, we found that NRF2 activation protects HepG2 cells from CPF induced AChE inhibition. These data indicate the potential for NRF2 as a target to mitigate short- and long-term OP/CPF toxicity.

6. *"Extracellular Vesicle Interactions with Fetal Brain Microglia in a Mouse Model of Maternal Stress"*

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Maternal stress during pregnancy is associated with increased risk for neurodevelopmental diagnoses in children such as Autism and ADHD. This prenatal stress (PS) can be modeled in mice with maternal restraint, which leads to anxious and avoidant offspring phenotypes and neuroimmune changes. Microglia, the brain's immune cells, are altered in PS and sculpt fetal brain circuits relevant to neurodevelopmental disorders. Maternal and placental biology are also changed by stress and modify fetal neurodevelopment, though molecular communication mechanisms remain unclear. One potential communication mechanism between maternal and fetal systems are extracellular vesicles (EVs). EVs are lipid-bilayer bound nanoparticles produced by the placenta which carry regulatory micro-RNAs (miRNAs) through maternal and fetal circulation. Our unpublished clinical cohort studies have revealed that EV miRNA cargos are altered in pregnancies resulting in children with neurodevelopmental disorders, and miRNA changes implicate neurodevelopment targets. Here we investigate the effects of PS-EVs on microglia in culture and assess how endogenous placental EVs interact with maternal and fetal tissues in mice.

For in vitro work (n=3), BV2 (murine microglia) cells were exposed to PKH26 labeled EVs from PS and control conditions for 12 hours. In CD1 dams, stress was administered for the last week of gestation. EVs were isolated by submandibular bleed and purified using size exclusion chromatography (IZON). EV uptake was assessed using spectral flow cytometry and ImageStream. For in vivo work (n=4 litters/group), we crossed a female B6.FVB-Tg(Ada*-Cre)5Xiay/J ("AdaP-Cre"; transgenic expressing cre under Ada promoter and chimeric Tpbpa/Ada placental trophoblast enhancer) with a male B6;129S1-Gt(ROSA)26Sortm1(CAG-CD9/GFP)Dmfel/J ("TIGER"; Cre-inducible His-tagged CD9/TurboGFP reporter targeted to EVs) to produce a pregnancy with GFP+ trophoblast-derived EVs. At gestational day (GD)14, 16, and 18, maternal and fetal brain, spleen, and blood were collected for flow cytometry. Macrophages, microglia, and monocytes were identified, and EV+ events were determined by comparing GFP+ fluorescent profiles to female controls.

Our results show EVs from prenatal stress (PS-EVs) have downregulated miRNAs with targets relevant to fetal neurodevelopment (miR3107, miR451b, miR374c, miR30f, $p<0.1$). RNA sequencing of microglia treated with PS-EV reveals 52 DEGs compared to nonstress EV controls ($p<0.1$). Functional enrichment analysis shows DEGs downregulated in PS (50) are overrepresented in pathways involving apical junctions and WNT signaling, while upregulated DEGs (2) involve oxidative phosphorylation pathways. Further, PS-EV exposed microglia scored significantly lower on the microglia development index, suggesting a more immature phenotype. Microglia exposed to PS-EVs also showed decreased EV phagocytosis ($p=.002$) compared to controls, a phenotype observed in other prenatal stress models. In non-stressed mice, we observe a significant increase in uptake of endogenous placental EVs by fetal brain microglia, but not other tissue-specific macrophages, maternal microglia, or monocytes at GD14 ($p<.001$), GD16 ($p<.01$), and GD18, $p<.001$). This associated was not dependent on Cre expression in each fetus, suggesting placental EVs are thoroughly distributed between fetuses. These results indicate PS conditioned EVs are sufficient to alter microglia phenotypes in culture, and placental EVs interact with fetal brain microglia in mid-late gestation. These findings implicate EVs as potential mediators of fetal neuroimmune programming.

7. *"IL-1 alpha expression induces drug resistance to all generations of EGFR TKIs in HNSCC"*

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Background: Epidermal Growth Factor Receptor (EGFR) expression is upregulated in head and neck squamous cell carcinomas (HNSCCs) and is associated with poor clinical outcomes. Unfortunately, the incorporation of EGFR inhibitors (EGFRIs) into the management of HNSCC has not improved long term survival rates in HNSCC patients. Identifying mechanisms that limit EGFRI efficacy is therefore critical for improving therapeutic outcomes. Previous work from our laboratory demonstrated the first-generation EGFR tyrosine kinase inhibitor (TKI), erlotinib induces expression and release of the pro-inflammatory cytokine interleukin-1α (IL-1α), promoting inflammation and drug resistance. This study examined whether IL-1α-mediated resistance extends across later generations of EGFR TKIs.

Methods: EGFR wildtype-positive HNSCC cells (FaDu, Cal-27 and SQ20B) were treated with erlotinib (first-generation afatinib (second-generation), osimertinib (third-generation), and silevertinib (fourth generation) for 48 h. Cell viability responses to EGFR TKIs were assessed using MTT assays. IL-1α and IL-6 cytokine expression was detected by ELISA and Western blot. IL-1 signaling was modulated using neutralizing antibodies to human IL-1α antibody (nIL-1α) and IL-1β (nIL-1β), and the human IL-1 receptor antagonist - anakinra.

Results and Conclusion: All four EGFR TKIs induced release of IL-1α, but not IL-1β, and increased IL-6 secretion (IL-1α signaling endpoint) in all cell lines. These effects were suppressed by IL-1α neutralization and anakinra (but not nIL-1β). IL-1α overexpression in Cal-27 cells conferred resistance to all EGFR TKIs tested. Lastly, erlotinib-resistant Cal-27 and SQ20B cells displayed cross-resistance to later-generation TKIs. Collectively, these findings identify IL-1α as a central, EGFR mutation-independent mediator of resistance to EGFR TKIs in HNSCC and highlight IL-1α signaling as a promising therapeutic target.

8. *"The Count of Cardiac Metabolism: Omega-3 Fatty Acids, Cardiac Fatty Acid Uptake, and Heart Health"*

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Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in the world with ~19.4 millions of deaths in 2021, an 18% increase from 2011. The incidence of cardiovascular risk and disease is predicted to continue climbing in the coming decades. Dietary omega-3 (n-3) poly-unsaturated fatty acids have gained popularity as a means of lowering circulating triglycerides and potentially reducing the risk for CVD. Increasing evidence from rodent and human studies have demonstrated that n-3s reduce circulating triglycerides and increase insulin sensitivity but only a subset of human studies have found that n-3s reduce the risk of CVD. The heterogeneity found in these clinical studies suggests that our understanding of the physiological effects of n-3s is incomplete. Data from our lab shows that mice fed a diet enriched in n-3 fatty acids have a striking increase in cardiac triglyceride-derived fatty acid uptake while also showing lower plasma insulin levels and increased insulin sensitivity. This is intriguing as increased cardiac fatty acid uptake is commonly associated with increased risk of lipotoxicity and cardiomyopathy. Furthermore, these data suggest three interesting and important questions: 1) Is the increased cardiac fatty acid uptake induced by n-3 feeding beneficial or detrimental to heart health? 2) Does increased fatty acid uptake in the heart contribute to the increased systemic insulin sensitivity in mice on an n-3 based diet? 3) How does an n-3 enriched diet increase fatty acid uptake in the heart? To answer these questions, I fed wildtype mice and mice with reduced cardiac fatty acid uptake a diet enriched in n-3s or control diets. Following 10 and 26 weeks on diet, I measured systemic insulin and glucose tolerance and systolic heart function. Reducing cardiac fatty acid uptake did not influence plasma insulin or glucose tolerance in n-3 fed mice but did decrease systemic insulin sensitivity in n-3 fed male mice after 10 weeks on diet. Despite increased cardiac fatty acid uptake, n-3 fed mice showed no disruption of systolic heart function after 10 or 26 weeks on diet. We also analyzed RNA-sequencing from hearts of n-3 fed mice and found that PPARa target genes are more strongly enriched following an n-3 diet compared to control diets suggesting a possible regulatory pathway. My findings show that the n-3 induced increase in cardiac fatty acid uptake is not necessary for the beneficial systemic metabolic phenotypes also related to n-3s. n-3 fed mice also appear to be more tolerable to heart dysfunction caused by increased cardiac fatty acid uptake.

9. *"Establishing a Baseline Human Relevant PCB Metabolite Profile for Future Studies of Microbiome-Mediated Neurotoxicity"*

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Polychlorinated biphenyls (PCBs) are persistent environmental contaminants with known neurotoxic effects. Environmental exposure to PCBs is rarely limited to a single congener, and the resulting toxic effects can vary depending on the mixture of PCB congeners an individual is exposed to. While several environmentally relevant mixtures have been assessed for toxicity, these synthetic PCB mixtures differ from the PCBs present in the human brain. To address this gap, we developed a human-relevant PCB (HR-PCB) mixture based on PCB congeners identified in postmortem human brain samples. Emerging evidence suggests that the gut microbiome can influence PCB biotransformation, potentially contributing to interindividual variability in metabolite profiles and neurotoxic outcomes. Our long-term goal is to determine how differences in the gut microbiome influence PCB metabolism and subsequent memory deficits following exposure to the HR-PCB mixture. The objective of this study was to characterize the PCB metabolite profile in mice exposed to the HR-PCB mixture. Male and female 7-week-old mice were orally exposed to the HR-PCB mixture (6 mg/kg/day, administered in peanut butter) for 7 weeks. We identified and quantified relative PCB metabolite levels in the serum using liquid-chromatography-high resolution mass spectrometry-based suspect screening. We identified several PCB metabolites in serum, including hydroxylated PCB (OH-PCB) metabolites ranging from tri- to octa-chlorinated congeners and PCB sulfate metabolites ranging from tri- to hexa-chlorinated congeners. This data will serve as the baseline for studying how the human microbiome influences metabolite profiles and ultimately affects toxicity in human microbiome-associated mice. This work addresses a critical knowledge gap and supports future investigations into the role of the gut microbiome in modulating PCB-induced adverse neurological outcomes.

10. *"Per- and Polyfluoroalkyl Substances Alter GPCR Activity"*

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Human exposure to synthetic chemicals is well documented, and exposure to these chemical pollutants is associated with a myriad of adverse health outcomes. Per- and polyfluoroalkyl substances (PFAS) are a large class of chemicals used in industrial applications and have been garnering considerable attention for their apparent human exposure and potential toxicity. These “forever chemicals” are tied to a range of toxic outcomes, including but not limited to certain cancers, diabetes, impeded immune function, liver and kidney diseases, and neurological disorders. However, the mechanisms of activity and the molecular initiating events that contribute to pollutant toxicity are largely unknown. G protein-coupled receptors (GPCRs) are signaling proteins that regulate a plethora of physiological activities and interact with highly chemically diverse structures, making them suitable targets for mediating the biological activity of pollutants. Structurally, many of the compounds resemble bioactive lipids that signal through specific GPCRs. Therefore, we hypothesize that PFAS may modulate lipid-recognizing GPCRs. In this work, we conducted a focused screen of PFAS pollutants at a sub-set of GPCRs. We selected relevant PFAS representing diverse chemical space from a list prioritized by the US Environmental Protection Agency. The selected GPCRs recognize ligands that structurally resemble PFAS and are implicated in physiological processes adjacent to the adverse outcomes of PFAS exposure. By leveraging contemporary screening resources, we have identified novel PFAS activity at specific GPCRs, acting in an agonist or antagonist manner.

11. *"Dissolving Microneedles for Transdermal Epinephrine Delivery"*

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Introduction: Anaphylaxis is an acute, life-threatening systemic allergic reaction affecting millions of people globally. Epinephrine, commonly delivered with autoinjectors, is the gold standard treatment of anaphylaxis. Autoinjectors deliver a large dose of epinephrine quickly but are bulky, painful, and associated with needle phobia. Additionally, with a half-life of less than 5 minutes, patients are advised to carry multiple devices and readminister the epinephrine while awaiting further medical care. To overcome these challenges, we are developing polymeric dissolving microneedle (dMN) arrays that can provide a painless, biphasic delivery of epinephrine with an initial burst within 5 minutes and continued release over 1 hour.

Methods: Gel formulations were prepared with polymers including pullulan (PL), hyaluronic acid (HA), and polyvinyl pyrrolidone (PVP) and loaded with epinephrine. In vitro release studies were conducted with Franz diffusion cells to assess the epinephrine release profile from each formulation. The gels were then cast into dMN arrays via a mold centrifugation method, and the mechanical strength was evaluated with axial compression testing. Dissolution tests were performed by inserting the dMN array into excised porcine skin and removing after 30, 60, and 120 seconds to characterize the extent of dissolution in a short time frame. Permeation studies were conducted with gel applied to intact skin, gel applied to solid MN treated skin, and dMN treated skin loaded into flow-through permeation cells.

Results: After 30 minutes, the 10% and 15% PL and 10% and 20% PVP gels released the most epinephrine, reaching up to 63.3% ± 5.8% of their drug loading. These gels also released over 15% of their loading within 5 minutes. Of the formulations investigated, both PL gels and the 4% HA-30% PVP gel produced well-formed dMN arrays for mechanical tests. These three formulations showed strong mechanical properties with average needle height reductions less than 15.2% ± 7.2%, but the HA-PVP arrays fractured under as little as 3.7 N of force whereas the PL arrays had no cracking up to 20 N. Upon insertion into excised porcine skin, the 10% PL dMN began dissolving within 30 seconds but did not dissolve further after 120 seconds. Pre-treatment of skin with a solid MN followed by applying the gel increased the amount of epinephrine permeated compared to the gel applied to intact skin or skin treated with the dMN formulation. However, permeation began only after 120 minutes. Further formulation development is necessary to permeate clinically relevant amounts of epinephrine within 5 minutes.

12. *"Inducing the pathogenic variant (E420K) of a PP2A regulatory subunit (PPP2R5D) in GABAergic neurons drives behavioral abnormalities in a mouse model of Houge-Janssens syndrome"*

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Houge-Janssens syndrome(Jordan syndrome) is caused by de novo mutations in protein phosphatase(PP2A) regulatory subunit PPP2R5D, leading to intellectual disability with developmental delay. However, cell specific mechanisms underlying disease phenotypes remain poorly understood.

To determine contributions of inhibitory neurons to disease pathogenesis, our laboratory generated Gad2-Cre mediated recombination driven E420K conditional knock-in mice by using CRISPR/Cas9. Our previous evidence suggests dysregulated cAMP-PKA signaling downstream of PP2A dysfunction, evaluating therapeutic potential of phosphodiesterase 4 (PDE4) inhibition. Pharmacological elevation of cAMP partially ameliorated hyperactivity, supporting a role for cAMP-dependent signaling in disease pathogenesis.

While newborn developmental milestones were grossly normal for Gad2 TVJ mice, adult mice exhibited robust hyperactivity, suggesting intact neurodevelopment in early stage but circuit dysfunction emerges later in life.

We constructed Gad2 TVJ mice cross with Nutrap mice to figure out cell specific mechanisms. EGFP expression was confirmed in Gad2+ neurons across major brain regions, providing opportunities for isolation of ribosome mRNA from inhibitory neurons for translating profiling. Ongoing Ribo-seq experiments aim to identify variants of PP2R5D induced translation dysfunction.

Together, these findings suggest that selective disruption of PP2A signaling in GABAergic neurons is sufficient to drive behavioral abnormalities and establish a framework for cell specific translational analysis and therapeutic targeting.

13. *"Characterizing the impact of Western diet on long-term memory and tau pathology in a humanized Alzheimer's disease mouse model"*

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory loss. AD is driven by the interplay of genetic and environmental risk factors, with diet identified as one factor linked to AD incidence. Longitudinal studies have identified an association between frequent consumption of saturated fats and ultra-processed foods during midlife and increased risk of AD later in life. The Western diet (WD) is a dietary pattern common in the United States characterized by high consumption of processed foods, saturated fats, and added sugars. In AD patients and mouse models, chronic WD consumption is associated with increased cognitive decline and long-term memory impairment. Consuming a diet high in fat also exacerbates the levels of phosphorylated tau, a key pathological hallmark of AD, in the hippocampus of an AD mouse model. While WD consumption has been shown to impair long-term memory and exacerbate hippocampal tau pathology, the molecular mechanism by which WD impairs memory is not clearly known. To address this knowledge gap, we first investigated the impact of WD consumption on long-term memory and tau pathology in a humanized mutant tau gene replacement mouse model (MAPT-GR.P301L) that eliminates the confounding effects of tau overexpression and gene deletion. To accomplish this, MAPT-GR.P301L mice were randomly assigned to consume a normal diet (ND) with 18% fat and no added sugars, or a 40% fat and 29% sugar WD from 4 to 12 months of age. Next, long-term spatial memory was assessed using the Barnes maze task. After behavioral assessment, spatial proteomic characterization of phosphorylated tau in different hippocampal neuronal cell types was performed using the Akoya PhenoCycler-Fusion. Our preliminary behavior data from female MAPT-GR.P301L mice suggest decreased long-term spatial memory performance in WD-fed mice compared to ND-fed mice, as evidenced by an increase in distance traveled to locate the target hole during testing. Spatial proteomic analysis revealed hippocampal subregion-specific increases in phosphorylated tau levels in MAPT-GR.P301L mice consuming a WD compared to ND, specifically phosphorylated tau (S202/T205) in the CA3 and DG subregions. Overall, these findings suggest that WD consumption impacts spatial memory and exacerbates tau pathology in a newly developed gene replacement mouse model of AD. To identify the molecular mechanism of memory impairment in MAPT-GR.P301L mice fed a WD, we will characterize the transcriptomic and epigenetic signatures of ND and WD mice after learning. Candidate memory-related genes and proteins will be pharmacologically targeted to establish a mechanism by which WD impacts memory.

14. *"HPLC Method Development for Analysis of Ubiquinol-Cyclodextrin Complexes"*

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Ubiquinol, the reduced and active form of Coenzyme Q10, plays a critical role in the electron transport chain and neutralization of reactive oxygen species (ROS) in the cell. It also directly inhibits mitochondrial permeability transition pore opening and apoptosis. Despite its high therapeutic potential, ubiquinol product development is hindered by its extremely low aqueous solubility and rapid oxidation to the inactive form ubiquinone. Previously, our group described the development of inclusion complexes of ubiquinol and g-cyclodextrin to address these issues, with treatment application focused on preserving corneal endothelial cell vitality. The cyclodextrin complex greatly improved stability and water solubility of ubiquinol, but the analytical characterization has relied on a tedious extraction process. Despite providing a clean sample, extraction complicates the analytical workflow due to potential analyte degradation and intensive validation requirements, presenting a substantial hurdle towards regulatory approval.

This ongoing work aims to develop a High-Performance Liquid Chromatography (HPLC) method that can bypass the need for extraction and minimize analytical uncertainty. Initial analytical method iterations involved testing different C18 analytical columns (Waters Symmetry, Agilent ZORBAX, Phenomenex Luna) using a mobile phase mixture of 60:35:5 Acetonitrile (ACN): Tetrahydrofuran (THF): Water, which faced several challenges such as high column pressure and subpar sensitivity. Subsequent work has transitioned to use of a Phenomenex Kinetex EVO C18 column, focusing on optimization of sample preparation.

A screening of various solvent combinations identified 60:40 Water:THF as the optimal diluent, exhibiting highest solubility visually. Paradoxically, complex dissolved in this mixture exhibited lower UV absorbance at 290 nm than previously tested samples diluted with mobile phase. Preliminary data suggests that the complex inherently exhibits a UV signal ~22x lower than the neat drug at equivalent concentrations. Accordingly, our research now utilizes UV-based optimization to address sample detection discrepancies and facilitate the development of a robust, regulatory-compliant protocol.

15. *"HPLC Method Development for Analysis of Ubiquinol-Cyclodextrin Complexes"*

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Alpha 1-adrenergic receptors (α1-AR) are G protein-coupled receptors highly expressed in the autonomic and central nervous systems. While the physiological role of α1-AR is well-characterized in smooth muscle innervated by the sympathetic nervous system, these receptors are also prevalent in the brain where their activation induces synaptic plasticity and mediates excitatory neurotransmission. In cell lines and heterologous expression systems, protein kinase C (PKC) phosphorylates α1-AR and decreases receptor activity, primarily via receptor internalization or desensitization. Whether PKC decreases α1-AR activity in the brain is not known. Here, we used whole-cell patch-clamp electrophysiology from serotonin neurons in acute mouse brain slices containing the dorsal raphe nucleus. Electrical stimulation of the brain slice causes the release of noradrenaline from axon terminals which activates α1-AR and produces an α1-AR-dependent excitatory postsynaptic current (α1-AR-EPSC). In order to determine if PKC decreases α1-AR activity in the brain, we used phorbol esters to activate PKC. Phorbol esters bind to the C1 domain of PKC promoting its translocation to the membrane and activation. Application of the phorbol ester, PDBu, eliminated the α1-AR-EPSC. When slices were pretreated with the PKC inhibitor, Gö 6983, the effect by PDBu on the α1-AR-EPSC was strongly reduced. When α1-AR-dependent inward currents were evoked by photolysis of caged-noradrenaline (I-NA), application of PDBu also eliminated I-NA, indicating that activation of PKC with PDBu eliminated the α1-AR-EPSC through a postsynaptic mechanism and not through a decrease in noradrenaline release. In contrast to the effect of PDBu, activation of PKC with another phorbol ester, PMA, had no effect on the synaptically evoked α1-AR-EPSC and only partially inhibited I-NA. The decreased efficacy of PMA compared to PDBu may be related to stronger lipophilicity of PMA which causes greater activation of PKC within the plasma membrane compared to the cytosol. The primary endogenous activator of conventional and novel PKCs is the lipid second messenger diacylglycerol (DAG), which also binds to the C1 domain of PKC and promotes its membrane association. We used a DAG mimetic,1-oleoyl-2-acetyl-sn-glycerol (OAG), to mimic the acute and physiological activation of PKC. Unlike PDBu and PMA, OAG induced a moderate augmentation of the α1-AR-EPSC. Synthetic DAG analogues and phorbol esters can bind and activate other C1 domain-containing proteins, therefore PKC-independent effects of C1 domain ligands will also be discussed. Together, the results of PDBu demonstrate that PKC is a negative regulator of α1-AR activity in the central nervous system. However, the differing effects of OAG and PMA indicate that non-PKC activity may also have a role in the modulation of α1-AR-dependent synaptic transmission.

16. *"Mechanisms of PCB52 Metabolite-Induced Glial Bioenergetic Dysfunction"*

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Background and Purpose: Polychlorinated biphenyls (PCBs) are persistent environmental toxicants linked to developmental neurotoxicity. Lower-chlorinated congeners such as PCB52 are increasingly relevant due to inhalation exposure and biotransformation to human-relevant metabolites (4-OH-PCB52, 4-OH-PCB52-S). Critical gaps remain regarding how these species perturb glial bioenergetics and redox signaling. This study tests whether PCB52 and metabolites produce mitochondria-centered toxicity in astrocytes and microglia, and whether astrocyte differentiation heightens susceptibility.

Methods: C6 astroglioma cells were differentiated with dibutyryl-cAMP/theophylline. Viability, (CellTiter-Blue®) was measured in glucose and galactose media (Glc→Gal) to force oxidative phosphorylation (OXPHOS) and derive IC_{50} values. BV-2 microglia were assayed in parallel. Cellular ATP was quantified under glucose vs galactose (CellTiterGlo 2.0®) over the course of 0, 1, 2, and 4 hours. Oxidative/nitrosative stress was imaged in differentiated C6 and primary rat astrocytes by MitoSOX (mitochondrial superoxide), and DAF-FM (NO/RNS; SNAP positive control).

Results: Differentiation increased astrocyte susceptibility and set the potency order to 4-OH-PCB52 > 4-OH-PCB52-S > PCB52. In C6 cells, IC_{50} values (μM) shifted from 11.7 (undiff) to 12.1 (PCB52), 3.1 to 2.1 (4-OH-PCB52), and 8.9 to 4.7 (4-OH-PCB52-S), indicating a differentiation-linked vulnerability most pronounced for the hydroxy metabolite. Mitochondrial dependence was evident in the Glc→Gal paradigm and mirrored by ATP. For differentiated C6 in galactose, IC_{50} left-shifted to 10.1 μM (PCB52), 0.9 μM (4-OH-PCB52), and 2.1 μM (4-OH-PCB52-S).

Correspondingly, cellular ATP declined sharply under galactose, with collapse near ~2 μM 4-OH-PCB52 in both differentiated C6 and BV-2, aligning temporally with the viability loss and pointing to OXPHOS liability rather than nonspecific cytotoxicity. BV-2 microglia followed the same potency order but were modestly more tolerant (IC_{50}, μM: PCB52 10.3, 4-OH-PCB52 3.0, 4-OH-PCB52-S 8.7). In differentiated C6, MitoSOX showed the strongest mitochondrial superoxide signal with 4-OH-PCB52 and moderate increases with the parent and sulfate. BV-2 microglia and Primary rat astrocytes recapitulated these patterns, showing mito-ROS elevations with 4-OH-PCB52 (~4.7 μM) and 4-OH-PCB52-S (~7 μM). DAF-FM at 2 h detected punctate-to-perinuclear NO/RNS under 4-OH-PCB52 at IC_{50}, exceeding both vehicle and SNAP positive control signal.

Conclusions: Human-relevant PCB52 metabolites act as glial-directed mitochondrial stressors. 4-OH-PCB52 is the dominant driver, producing OXPHOS-dependent ATP loss, mitochondrial superoxide, and rapid NO/RNS elevations. Ongoing work will quantify electron-transport-chain liabilities in Glc→Gal, implement HPLC-ECD dopamine uptake/turnover to functionally connect glial bioenergetic failure to neuron-relevant outcomes and evaluate origin of RNS as potential unique mechanism of bioenergetic collapse in astrocytes.

17. *"Abstract Title: Optimization of NanoBRET Platform for a Screen of Selective Adenylyl Cyclase 8 Inhibitors to Treat Alcohol Use Disorders"*

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Adenylyl cyclase 8 (AC8) is a membrane bound protein that catalyzes the formation of cAMP from ATP. AC8 has been implicated in various neurological disorders, including alcohol use disorders (AUDs). Additionally, in an alcohol preferring mouse model, AC8-/- knockout decreases alcohol seeking behaviors. Attempts to inhibit AC8, however, have proven challenging. As a member of the adenylyl cyclase family, achieving selective inhibition of AC8 is at the root of this challenge. To achieve selective inhibition of AC8 over other AC isoforms, we have focused on disrupting calmodulin (CaM) mediated activation of AC8. This mechanism of activation is shared only by one other isoform: AC1. The binding sites of CaM on AC8 and AC1 are not conserved, and their differences provide hope that selective inhibition is possible. Identifying a selective and efficacious inhibitor of the AC8-CaM interaction could provide a novel first-in-class molecule that shows selectivity for AC8 inhibition to treat AUDs. To accomplish this we are developing a high throughput screen using NanoBRET to identify inhibitors of the AC8-CaM PPI.

18. *"Characterization of Human Choroidal Macrophages via Immunohistochemistry"*

Zoë Hettinger, Robert F, Mullins, Kelly Mulfaul

Department of Neuroscience and Pharmacology

Purpose: Dry age-related macular degeneration (AMD) is a multifactorial neurodegenerative disease of the outer retina, RPE, and choriocapillaris. Loss of the endothelial cells which line the choriocapillaris is increasingly recognized as an initiating event in disease pathogenesis. An increased number of activated macrophages are observed in the choroid of early AMD donor eyes, however the composition of such macrophages is yet to be detailed. Historically, macrophages are described as tissue-resident M2-like ($CD206^+/Arg1^+$, pro-repair) or monocyte-derived M1-like ($iNOS^+/CD80^+$, pro-inflammatory). Nevertheless, in vitro macrophage phenotypes are more complex and are governed by microenvironmental ques. This study examined marker-specific macrophage number, morphology, and localization relative to Bruch's membrane (BrM) in AMD versus control donor tissue.

Methods: Postmortem donor eyes (12 AMD, 14 age-matched controls) from the Iowa Lions Eye Bank were sectioned (7 µm) and immunostained with phenotypic markers, such as iNOS, CD206, CD80, and IBA1. Sections were imaged by epifluorescence/confocal microscopy and quantified in ImageJ (FIJI) for macrophage count, cell morphology, and distance from BrM.

Groups were assessed for normality and compared by t-tests.

Results: We observed M1 (iNOS+ and CD80+) and M2 (CD206+) macrophages in both control and AMD donors. M1 macrophages were increased in AMD donors compared to controls ($p=0.041$), while M2 numbers did not differ. M1 macrophages in AMD shifted toward a more ameboid morphology, whereas M2 morphology and spatial distribution were similar between groups. Interestingly, we identified a population of macrophages that labelled iNOS+/CD206+, as well as CD80+/CD206+, present in the human choroid. These mixed phenotype macrophages were more abundant and localized closer to BrM in AMD tissue ($p<0.0005$ and $p<0.005$, respectively).

Conclusions: Increased M1 and mixed phenotype, but not M2, macrophages increase in

AMD suggesting that monocyte-derived macrophage recruitment drives immune changes in AMD disease, rather than resident macrophages. The proximity of mixed phenotype macrophages to BrM in AMD points to a potential role in structural remodeling, warranting further investigation into the signaling pathways governing macrophage polarization and as therapeutic targets.

19. *"Regulator of G-protein Signaling 6 (RGS6) Controls Dopamine Reuptake via the Dopamine Transporter (DAT) in Nigrostriatal Dopamine Neurons"*

D Liu[1,2], Z Luo[1], J Yang[1], D Li[1], M Guo[1], J Bernholtz[1], N Fassett[1], R Fisher[1] & A Stewart[1,2,3]

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Parkinson's disease (PD) is one of the most common and debilitating neurodegenerative diseases, and its prevalence continues to climb globally. Although dopamine (DA)-restoring therapies can alleviate motor symptoms, no disease-modifying treatment for PD is currently available. Therefore, delineating pathogenic drivers of PD is crucial for the identification of novel treatment targets. Our previous study found that expression of regulator of G protein signaling 6 (RGS6) is diminished in dopamine neurons of the substantia nigra pars compacta (SNc) from postmortem PD brains. In establishing if RGS6 loss is causative in PD pathogenesis, we determined that RGS6-/- mice exhibit age-dependent loss of tyrosine hydroxylase + (TH+) DA neurons, disruptions in DA metabolism, α-synuclein aggregation, and motor coordination deficits. DA neurotransmission is dynamically controlled by the actions of D2 autoreceptors (D2ARs), which suppress neuronal firing, inhibit vesicular release, and facilitate DA reuptake via the dopamine transporter (DAT). Rgs6, Drd2, and Slc6a3 expression are enriched in DA neurons of the SNc relative to the ventral tegmental area (VTA), and RGS6 canonically functions to limit the signaling response to Gαi/o-coupled receptors (GPCRs), including D2ARs. Here, we demonstrate that uptake of [3H]DA is elevated selectively in the dorsal striatum (DStr) of male RGS6-/- mice, a phenotype absent in females. DStr DA uptake is also insensitive to quinpirole, a D2R agonist, in the absence of RGS6. To test if DAT activity is altered in RGS6 knockouts in vivo, we injected the oxidation-prone DA analog 6-hydroxydopamine (6-OHDA), a DAT substrate, into the SNc of RGS6-/- mice. As expected, loss of RGS6 sensitized mice to the deleterious impacts of 6-OHDA, including disrupted DA metabolism, DA depletion, and motor coordination deficits. RGS6 and DAT also form a co-precipitable complex in a heterologous expression system, and ongoing work seeks to determine if the ability of RGS6 to block DAT function requires RGS6's GTPase-activating protein (GAP) function and D2AR regulatory capacity. We further seek to establish the role of RGS6-dependent DAT regulation in DA neuron degeneration as well as in vivo DA dynamics and behavior as a function of both age and biological sex.

20. *"Sex-dependent alterations in psychostimulant-elicited behavior following progesterone receptor inhibition"*

Julia I. Neuharth[1,2], Jaxson Hicks[1], Jacob Bernholtz[1], Nathan Fassett[1], Brenna A. Powers[1], Danlin Liu[1,2], Adele Stewart[1,3]

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Dysregulation of the dopamine (DA) transporter (DAT), which regulates synaptic DA uptake, is linked to several psychiatric disorders that show sex-biased prevalence. Sex differences in the architecture of the DA system may have a hormonal basis, and recent single cell sequencing studies have revealed that DA neurons express progesterone receptors (PGRs), but not estrogen receptors, with PGR expression mirroring DAT distribution across DA subpopulations. Prior work in our lab demonstrated that administration of the PGR blocker mifepristone to WT C57BL/6 mice increased surface DAT and [3H]DA uptake in female dorsal striatum (dStr), but not male dStr or other brain regions innervated by dopaminergic projections from the midbrain. Acute mifepristone treatment did not impact DAT activity suggesting PGR likely influences DAT through transcriptional regulation, which requires extended drug exposure. Here, we evaluate how inhibition of PGR shapes the behavioral response to psychostimulant drugs, which alter DA neurotransmission via DAT inhibition. Although mifepristone failed to impact DAT activity in male mice, the locomotor stimulating actions of amphetamine (AMPH) were elevated in WT mice of both sexes following chronic mifepristone treatment. In contrast, mifepristone administration blunted the ability of cocaine (COC), a DAT antagonist, to increase locomotion only in WT females. We next evaluated the impact of mifepristone on COC conditioned place preference (CPP) and locomotor sensitization, which require repeated psychostimulant treatment. In both male and female mice, mifepristone prevented escalation of locomotor activity following repeated COC dosing. We noted no impact of PGR blockade on acquisition or extinction of COC CPP. However, mifepristone completely prevented COC CPP reinstatement in females only. The sex- and drug-dependent impacts of PGR on psychostimulant-elicited behaviors highlights the critical modulatory role of sex steroids in shaping dopaminergic neurotransmission.

21. *"Conditional knockdown of prohibitin-1 in cardiomyocytes reveals a sex dimorphic role for mTORC1 in pathogenesis of dilated cardiomyopathy"*

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Prohibitins 1 and 2 (PHB1/2) are ubiquitously expressed lipid raft proteins that form a multimeric ring supercomplex in cellular membranes where they regulate numerous processes including cellular metabolism, growth, signaling, gene expression and death. The role of the PHB supercomplex, and particularly PHB1, in maintaining homeostasis, metabolism and electromechanical function in the heart is not well understood, though emerging evidence suggests that mammalian target of rapamycin complex 1 (mTORC1) may be involved. To address this question, we created a cardiomyocyte-specific PHB1 knockdown (cPHB1ko) mouse model using i.p. tamoxifen injection in homozygous floxxed PHB1 (PHB1fl/fl) MER-Cre-MER positive adult mice (aged ~8 weeks). Six weeks after inducing Cre recombinase expression with tamoxifen, cardiomyocyte PHB1 and PHB2 levels were decreased by >90% in the cPHB1ko mice compared with WT. Cardiac ejection fraction (EF) in the cPHB1ko mice progressively declined and by 12 weeks post-tamoxifen, mice had developed severe dilated cardiomyopathy and were dead by 14 weeks. Females were more severely impacted by the PHB knockdown, with more rapid onset of cardiomyopathy and mortality compared with males. Integrated transcript-/metabolomic analysis of cPHB1ko hearts at 6 weeks post-tamoxifen showed up-regulation of amino acid metabolism and signaling pathways, coupled with a decline in fatty acid oxidation and glycolysis-related genes. Importantly, these metabolic changes in the cPHB1ko hearts occurred in parallel with substantial up-regulation of mTORC1 activity that was minimally responsive to fasting. This hyper-activated mTORC1 led to impaired autophagy in cPHB1ko hearts, characterized by increased p62, LC3I/II, and Lamp1 levels. Treatment with mTORC1 inhibitor rapamycin at 2 mg/kg every other day via intraperitoneal injection mitigated hypertrophy and delayed the decline in EF and onset of cardiomyopathy in female, but not male cPHB1ko mice. These findings demonstrate that the PHB complex is a critical regulator of mTORC1 in cardiomyocytes. They further suggest that persistent mTORC1 activity contributes to pathogenesis of cardiomyopathy in female but not male mice, consistent with prior reports of the sexually dimorphic effects of PHB's in other cardiometabolic disorders.

22. *"Sex- and Region-Dependent Inhibition of the Dopamine Transporter (DAT) by Tamoxifen"*

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Inducible Cre recombinase/estrogen receptor (CreER) driver lines have become a staple in biomedical research, providing investigators with temporal control over initiation of Cre-dependent genetic recombination. The Jackson Laboratory, the world's largest repository of transgenic mouse lines, currently recommends a 5 day, high dose treatment course of the ER modulator tamoxifen (TAM) to activate CreER with a subsequent 1-week washout period prior to experiment initiation to allow for drug clearance. Notably, pharmacokinetic analysis of TAM indicates that active TAM metabolites persist in the circulation well beyond 1 week. Furthermore, previous work in heterologous systems has shown that TAM, as well as its active metabolites, can inhibit the dopamine (DA) transporter (DAT), the primary facilitator of rapid synaptic DA clearance, through an estrogen receptor independent mechanism. It remained unclear, however, if these impacts would be detectable in vivo following systemic drug administration. To functionally assess the effects of TAM on DAT in both sexes, male and female C57BL/6 mice were treated with TAM (100 mg/kg, i.p.) or corn oil vehicle for 5 days followed by a drug washout period (1 week or 3 weeks). We then isolated brain slices containing dorsal striatum (DStr), nucleus accumbens (NAc), pre-frontal cortex (PFC), substantia nigra pars compacta (SNc) and ventral tegmental area (VTA), encompassing the projection regions and dopaminergic cell bodies of the three major DA circuits, and performed ex vivo [3H] DA uptake assays. To determine the behavioral impact of TAM's effect on DAT function, and additional cohort of mice underwent open-field locomotor testing 1 week after conclusion of treatment. A significant decrease in [3H] DA uptake was observed selectively in the female DStr following 1 week of drug washout that resolved after 3 weeks. No significant changes in DAT function were detected in males or in the other female projection regions examined at either timepoint. Despite reductions in DAT function, TAM treatment had no significant effect on amphetamine-induced locomotor activity in either sex. These findings suggest that TAM modulates DAT through a sex-dependent mechanism and highlights the potential role of TAM metabolites in driving the observed sex differences. Importantly, these findings indicate that 1 week, the timepoint recommend for TAM washout for inducible Cre-ER lines, is not sufficient to eliminate the effects of TAM on DAT, a potential confound for using these models to study brain function.

23. *"From Acute Response to Stable Resistance: Temporal ABC Transporter Dynamics in Paclitaxel-Resistant Ovarian Cancer"*

Atonu Chakrabortty, Kaleb Feia, Melissa Fath, David Roman, Jill Kolesar, Jared McLendon

Dept. Pharmaceutical Sciences and Experimental Therapeutics, Univ. of Iowa

Chemoresistance to paclitaxel and cisplatin remains a major obstacle to durable treatment response in ovarian cancer. ATP-binding cassette (ABC) transporters are established mediators of drug efflux, yet how their expression evolves as tumor cells transition from a drug-sensitive to a resistant state is not well understood. In this study, we characterized chemosensitivity profiles across a panel of ovarian cancer cell lines (OVCAR3, OVCAR8, TOV-21G, OV90) and quantified acute transcriptional changes in ABCB1, ABCA8, ABCG2, and ABCB5 following paclitaxel and cisplatin exposure. Using these baseline profiles, we generated paclitaxel-resistant sublines through continuous drug selection and confirmed resistance by IC50 shift relative to parental lines. To resolve how resistance emerges over time rather than as a fixed endpoint, we performed temporal RNA sequencing across sensitive and resistant cell states at early acute (6h) and late acute (48h) timepoints following drug exposure. Together, this approach captures the transition from acute transporter dysregulation to stable resistance, providing a time-resolved framework for identifying transporter-driven mechanisms and candidate biomarkers of chemoresistance in ovarian cancer.

24. *"Monocyte recruitment in early age-related macular degeneration by secretion of inflammatory molecules from complement injured choroidal endothelial cells."*

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Purpose

Age-related macular degeneration (AMD) is a leading cause of vision loss worldwide. AMD is characterized by complement activation and increased macrophage infiltration in the choroid, the vascular tissue located at the posterior pole of the eye. The immune signaling pathways involved in recruitment of monocyte derived macrophages in early AMD remain to be determined. We sought to identify cytokine or chemokine molecules released from complement injured choroidal endothelial cells (CECs) and determine the extent to which these molecules influence monocyte migration towards complement injured CECs.

Methods

Induced pluripotent stem cells (iPSCs), from a donor with AMD, were used to generate CECs which were incubated with either (a) normal human serum (NHS) for 2 hours to induce complement membrane attack complex (MAC) formation or (b) heat inactivated NHS as a “no MAC” control. After 24 hours, the supernatant of MAC injured CECs was collected and screened for inflammatory molecules using a human inflammatory array. Changes in mRNA expression of target canidates were confirmed by qPCR between 3-24 hours post MAC formation. iPSC-derived monocytes were added to the apical chamber of a Matrigel coated 5 µm transwell insert. Invasion towards MAC injured CECs was assayed after 24 hours and visualized by crystal violet. Invaded monocytes were counted using Image J. Monocyte derived macrophages were cultured with control or MAC injured CECs for 24 hours. After incubation, total RNA was extracted from the macrophages and sent to the University of Iowa Genomics Division for library prep and bulk RNA-sequencing. We utilized Rstudio for genome alignment and downstream analysis.

Results

We found that MAC injury of iPSC derived CECs induced secretion of cytokines MCP-1, IL-8, and chemokine CCL5. We observed increased expression of MCP-1, IL-8, and CCL5 mRNA in CECs post MAC injury when compared to the “no MAC” control CECs. We observed increased invasion of iPSC derived monocytes towards MAC injured CECs after 24 hours in co-culture. Exposure of “naïve” MF macrophages to MAC injured CECs upregulated the expression of genes involved in inflammatory pathways.

Conclusion

AMD iPSC derived CECs secrete MCP-1, IL-8, and CCL5 inflammatory molecules in response to MAC injury. This secretion by MAC injured CECs contributes to increased monocyte invasion. Naïve macrophages develop a more pro-inflammatory phenotype in response to MAC injured CECs. Complement induced CEC injury plays a role in driving an inflammatory microenvironment and monocyte recruitment by secretion of MCP-1, IL-8, and CCL5 in AMD.

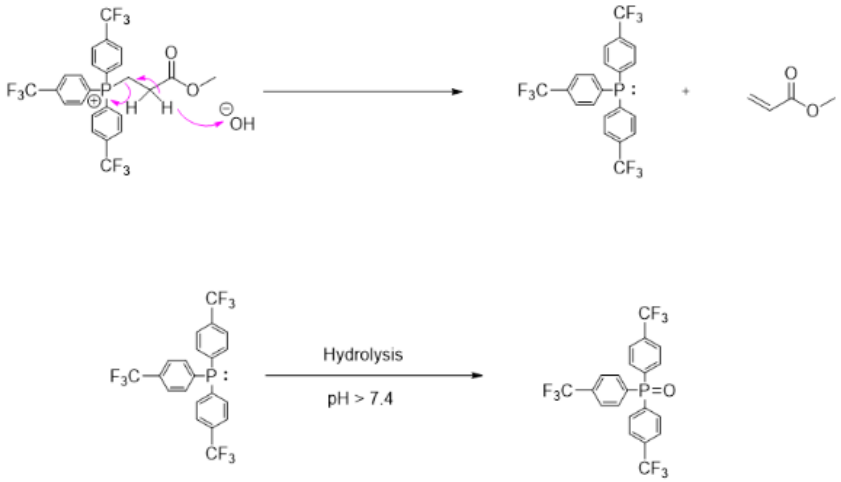
25. *"The Synthesis of Triphenylphosphonium (TPP+) Conjugates with Modified Linkers for Improved Mitochondrial Targeting "*

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Mitochondrial dysfunction plays a role in the pathology of many cardiovascular, metabolic, and neurodegenerative disorders. To effectively target dysfunctional mitochondria, intramitochondrial delivery of therapeutics and molecular probes has been explored. Delocalized lipophilic cations (DLCs) and mitochondrial penetrating peptides (MPPs) have been shown to effectively target a variety of pharmacological agents to the mitochondria. Specifically, lipophilic triphenyl phosphonium (TPP+) cations accumulate in mitochondria due to their large ionic radius, net positive charge, and a lipophilic chain. In response to high mitochondrial membrane potential (ΔΨ), TPP+ conjugates rapidly accumulate in the mitochondria. Compared to a 5-to-10-fold increase in TPP+ cations in the cytosol, there is a 100-to-1000-fold increase in the concentration of TPP+ cations in the mitochondria. Longer-chain alkyl TPP+ derivatives, such as those with a decyl chain, that are optimal for mitochondrial uptake, have been shown to increase proton leak, uncouple oxidative phosphorylation (OXPHOS), and decrease mitochondrial respiration. Therefore, lipophilic cations, such as TPP+, exhibit increased cytotoxicity. Previously, para-substitution of the phenyl rings of TPP+ conjugates was varied to identify derivatives that would be less potent uncouplers of OXPHOS, while maintaining mitochondrial uptake-– therefore ablating inherent cytotoxicity. Specifically, introducing a para-trifluoromethyl group on each phenyl ring of TPP+ (4-CF3-TPP+) significantly reduced OXPHOS uncoupling while maintaining delivery of cargo into the mitochondria. However, in the presence of aqueous DMSO, under mildly basic conditions (pH > 7), 4-CF3-TPP+ derivatives were found to undergo DMSO-mediated conversion to phosphine oxide products. Guided by this discovery, the design and synthesis of TPP+ conjugates that will “self-inactivate” under mitochondria-specific conditions is being pursued. Presented here are the synthesis and evaluation of novel TPP+ derivatives for potential β-elimination under mildly basic conditions, that exist inside the mitochondria.

β-Elimination Reaction:



26. *"Epigenetic Remodeling During Pregnancy and Response to Stress."*

Emma Simpson-Wade[1], Tiffani Roberts[2], and Marie Gaine[3,4]

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The perinatal period, encompassing gestation through one-year post-partum, represents one of the most profound hormonal and physiological transitions experienced by pregnant individuals, including dynamic remodeling of the maternal brain. Furthermore, this transition has been associated with increased susceptibility to, or exacerbation of, psychiatric disorders, profoundly impacting maternal-fetal health and mortality. Given previous evidence showing a role for DNA methylation (DNAm) in modulating psychiatric behaviors, we sought to understand gestation-specific regulation of DNAm, and its response to stress, to better distinguish the molecular mechanisms driving perinatal behavioral deficits. Female C57Bl/6J mice (8 weeks) were randomly assigned into four categories: 1) Non-pregnant/Non-stressed (NPNS; n = 9); 2) Non-pregnant/Stressed (NPS; n = 11); 3) Pregnant/Non-stressed (PNS; n = 14); 4) Pregnant/Stressed (PS; n = 14) with stressed dams subjected to daily micro stressors via a modified version of the unpredictable chronic mild stress paradigm from GD10-GD18. Prior to parturition (GD16-17), pregnant dams underwent behavioral testing to identify alterations in anxiety- and/or depressive-like phenotypes. Hippocampal and blood samples were used to examine DNAm patterns via Illumina Mouse Methylation Beadchip. We found evidence of gestation-specific DNAm changes in both blood (3 CpG sites; 12 differentially methylated regions (DMRs) and the hippocampus (35 DMRs), suggesting increased epigenetic remodeling in the hippocampus associated with matrescence. Examination of perinatal stress behavior showed stressed dams spent significantly more time in the open arms of the elevated zero maze compared to controls ($p = 0.0414$). Furthermore, while it did not survive multiple correction, the top differentially methylated hippocampal CpG site (cg31984807_TC21) showed hypermethylation of PS vs PNS dams and was significantly correlated to proportion of time in the open arms ($p = 0.0481$; $R2 = 0.1420$); thus highlighting the interaction between DNAm and perinatal behavior in this cohort. In addition, we identified 10 significantly hypomethylated DMRs in hippocampal samples between stress phenotypes that were associated with genes involved in neuroplasticity and indirect redox regulation. Notably, these DMRs showed diverging status from pregnancy-specific states, suggesting perinatal stress disrupts gestational epigenetic regulation. Overall, these results provide novel insights regarding hippocampal specific epigenetic regulation associated with gestation and perinatal stress and lay the groundwork for future studies examining mechanisms inducing stress-associated hypomethylation including one-carbon metabolism.

27. *"Mechanisms of Behavioral Rescue Following Excess Neonatal Testosterone in Wild-Type Mice"*

Pravda Quiñones-Labernik[1,2], Michael Hasso[1,2], Emily Hagan[1,3], Nikša Poleksić[2], Ted Abel[1,2], Sarah Ferri[2,3]

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Neurodevelopmental disorders (NDDs) are complex conditions affecting the developing brain and exhibit a pronounced male bias in prevalence and symptom profile. During fetal development, a surge in testosterone is critical for establishing sex-specific neural circuits that shape lifelong behavior. We previously demonstrated that a single neonatal testosterone injection induces male-specific social and contextual fear memory deficits in wild-type mice; however, the underlying mechanisms remain unclear.

C57BL/6J pups received testosterone propionate vehicle on the day of birth. To assess pharmacological rescue, mice received D-cycloserine (DCS) or saline prior to behavioral testing. Additional cohorts underwent spatial object recognition and cued fear conditioning to evaluate hippocampal- and amygdala-dependent memory, respectively. Whole-cell electrophysiology, Western blotting, and immunohistochemistry (IHC) were used to investigate hippocampal synaptic- and serine shuttling-related mechanisms.

DCS rescued social and contextual fear memory deficits in testosterone-treated males. Testosterone-treated males also exhibited impaired spatial object recognition but intact cued fear memory, suggesting selective hippocampal dysfunction. Whole-cell recordings from CA1 pyramidal neurons in behaviorally naïve juvenile mice revealed no differences in evoked excitatory postsynaptic currents or AMPA/NMDA ratios between testosterone-treated and control males. Consistent with these findings, Western blot analysis revealed no detectable differences in PSD95, or NMDA receptor (NMDAR) subunit protein expression between groups. Interestingly, IHC revealed increased GFAP immunoreactivity in testosterone-treated males compared to controls.

Because DCS is a partial NMDAR co-agonist that can enhance NMDAR signaling, these findings suggest that excess neonatal testosterone may disrupt astrocyte-mediated regulation of NMDAR function, potentially through altered D-serine signaling. Ongoing studies will determine whether excess neonatal testosterone alters activity-dependent hippocampal plasticity and astrocyte-mediated mechanisms underlying these behavioral deficits. Elucidating these mechanisms is critical for understanding how early hormonal dysregulation alters circuit function and for informing targeted therapeutic strategies.

28. *"Excess Neonatal Testosterone Alters Neurodevelopment and Social Behavior in a Sex-Dependent Manner"*

Emily Hagan, MS[1,2], Anthony Lange[2,3], PhD; Danielle Preuschl[2], Niksa Poleksic[4], Brooke Hoogland[2], Brenna Powers[1], Megan Weis[1], Alicia Li[2], Charlotte Tesar[2], Pravda Quinones-Labernik[1,4], Sarah Ferri, PhD[2]

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Neurodevelopmental disorders (NDDs) are complex conditions that arise during early development that disrupt brain development, resulting in impairments in motor, cognitive and social functioning. NDDs affect approximately 1 in 6 individuals and consistently exhibit a male-biased prevalence, although the mechanisms underlying this sex difference remain unclear. We utilize a novel experimental system of neonatal injections of the gonadal hormone, testosterone, to disrupt sex-specific developmental pathways in mice to determine their effects on development and behaviors relevant to NDDs. We have previously demonstrated that a single administration of testosterone on the day of birth causes social deficits in juvenile and adult wild-type male, but not female mice. Here, we examined the effects of excess neonatal testosterone on developmental milestones, neuromuscular reflex, ultrasonic vocalization, and adult behaviors relevant to NDDs. Neonatal testosterone delayed physical and neuromuscular development in both sexes, including eye opening, fur development, grasping reflex and horizontal suspension. However, reduced ultrasonic vocalizations and impaired adult motor learning were observed only in testosterone-treated males. Adult behavioral testing further revealed reduced grooming behavior in both sexes and impaired motor coordination in males. To identify the brain circuity involved in this male susceptibility, we have used brain wide activity mapping using the iDisco clearing method with cFos to identify differential expression throughout the brain following social interaction. Our cFos data has revealed that excess testosterone during neonatal development causes brain activation changes in regions associated with social behavior such as the basolateral amygdala (BLA). Our results suggest that brief periods of hormonal dysregulation during specific critical developmental periods can induce proximate and long-lasting neurodevelopmental disruptions. Currently we are investigating the mechanisms of these sex specific vulnerabilities, including changes in steroid hormone receptor expression and the involvement of aromatized estradiol. These findings will aid in advancing the current understanding of how the brain is susceptible to social impairments and help identify novel treatment targets.

29. *"Organophosphate Pesticide Study in Oregon Orchard Community"*

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Children residing in agricultural communities may be exposed to organophosphate pesticides through multiple pathways, including spray drift, contaminated household environments, dietary intake, and take-home exposure from parents working in agricultural workplaces. Although urinary metabolites are commonly used biomarkers of organophosphate exposure, few studies have used intensive repeated sampling linked to documented spray events to characterize exposure dynamics in children. Metabolite persistence and variability are likely underestimated in studies relying on single spot urine samples. Seventeen children aged 5-13 years residing in orchard communities were enrolled in this pilot repeated-measures study. Bedtime and morning urine samples were collected at least two times during each pre-spray, spray, and post-spray period. Urine samples were analyzed for six metabolites. Survey data were collected to characterize demographic factors and potential exposure pathways including parental agricultural employment history, residential proximity to treated areas, and household pesticide use. Urinary metabolite concentrations demonstrated substantial temporal variability across sampling periods. Mean metabolite concentrations increased during orchard pesticide application periods and generally declined after application season. Most peak concentrations occurred during documented spray intervals. Children experiencing repeated or closely spaced spray events exhibited prolonged metabolite persistence compared with children associated with single spray events. Survey-derived indicators suggested stronger associations between metabolite elevations and parental agricultural employment history than with residential proximity measures. Repeated biomonitoring identified short-term fluctuations in urinary organophosphate metabolites that would likely not have been captured using conventional single-sample approaches. These findings highlight the importance of sampling frequency, spray-event timing, and occupational factors in characterizing pesticide exposure among children living in orchard communities.

30. *"Sorbs2 regulates vascular smooth muscle cell contraction and mechanotransduction"*

Kailey Said-Lathrum[1], Henry Lin[1], Grace Rocco[1], Ben Stimson[1], Ryan Boudreau[2,3], Kris DeMali4, Isabella Grumbach[2,3], Kamal Rahmouni[2,3], Jared McLendon[1,2,3]

[1]Pharmaceutical Sciences and Experimental Therapeutics, University of Iowa College of Pharmacy, [2]Abboud Cardiovascular Research Center, University of Iowa Carver College of Medicine, [3]Department of Internal Medicine, University of Iowa Carver College of Medicine, [4]Biochemistry and Molecular Biology, University of Iowa Carver College of Medicine

Introduction:

Sorbs2 is a cytoskeletal adapter protein that is dysregulated in cardiovascular diseases (CVD) and is highly expressed in vascular smooth muscle cells (VSMCs). Our objective is to understand the role of Sorbs2 in vascular biology. We hypothesize that Sorbs2 regulates VSMC contraction and mechanotransduction responses.

Methods and Results:

To test if Sorbs2 knockout in VSMCs is associated with CVD phenotypes, we created a smooth muscle specific Sorbs2 knockout (smKO) mouse. Blood pressure radio telemeters were installed, and mice were treated with Angiotensin II (AngII) at 400 ng/kg/min for 10 days to induce hypertension. Sorbs2-smKOs showed a reduced change in mean arterial blood pressure (ΔMAP) after AngII administration relative to WT. However, after 5 days post-AngII administration, smKOs increased in ΔMAP relative to WT potentially through compensatory mechanisms.

To test if Sorbs2 knockout affects vascular tone in arteries, we performed wire myography experiments. Force of contraction was measured for arteries from WT and KO mice under increasing phenylephrine (PE) dose. KO aortas showed increased PE-induced contractility compared to WT (n=6). In passive length-tension relationships, KO aortas showed lower passive tension and higher elasticity than WT. The higher contractility and lower passive tension of KO aortas suggest Sorbs2 plays a role in maintaining the VSMC structural cytoskeleton.

To assess Sorbs2's role regarding the VSMC cytoskeleton, primary WT or KO VSMCs were plated on elastic membranes at static conditions, or with 10% bicyclic stretch at 1 hz for 24 hours. After, cells were lysed for protein and RNA expression analysis via western blots (WB) or RNA sequencing. Sorbs2 KO showed dysregulation of Calponin1 and Integrin beta-5 at a protein level and significant upregulation of phosphorylated ERK1/2 with no change in total ERK expression ($n=6$, $p<0.05$). RNA sequencing of static KO vs WT showed an increase in genes involved in contractile and focal adhesion pathways ($Z=5.8$, $p<0.0001$). Under stretch, KO VSMCs continued to show compensatory upregulation of genes associated in focal adhesion ($Z=4.9$, $p<0001$) compared to WT.

Conclusion:

Our results suggest Sorbs2 regulates vascular tone and VSMC mechanotransduction by regulating the VSMC structural cytoskeleton. We think this is achieved by mediating focal adhesion protein expression, actin cytoskeletal dynamics, and microtubule stability.

31. *"Delineating a Role for the Retrosplenial Cortex in Alzheimer's Disease and Related Dementias"*

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Alzheimer's Disease (AD) is a debilitating neurodegenerative disease that impairs cognition and memory. Spatial memory is one facet of memory impacted by AD and manifests as difficulties navigating familiar environments. Previous studies have focused their efforts on how AD pathology in the hippocampus, a brain region important for learning and memory, can contribute to memory impairment. However, given that AD is a very complex disease, it is imperative to identify other key regions impacted by AD pathology that contribute to spatial memory impairments. One candidate region is the retrosplenial cortex (RSC), as our lab and others have previously shown that the RSC is critical to spatial memory consolidation and retrieval. Our lab has also shown that the RSC displays a gene expression signature during memory consolidation that is comprised of many genes which are critical for long-term memory. Importantly, AD-related cognitive decline is correlated with an increase in pathological tau deposition in a brain region which includes the RSC, indicating that the RSC may play a critical role in AD-related spatial memory impairments. Yet, a direct role has yet to be uncovered. To address this, we aimed to investigate how tau pathology expressed exclusively in the RSC affects spatial memory. We used a viral construct (AAV-CAMKIIa-MAPT-P301L) to express a humanized mutant form of tau prone to hyperphosphorylation exclusively within the excitatory neurons of the RSC of adult male mice. Mice injected with either the P301L viral construct or a control viral construct (AAV-CAMKIIa-eGFP) were tested on the Barnes Maze, a behavioral strategy that is used to study spatial navigation. We found that animals injected with the P301L viral construct exhibited slower latency times to find the target hole and spent less time in the target quadrant when compared to control animals, suggesting hyperphosphorylated tau expressed exclusively in the RSC results in spatial memory impairments. Additionally, using a novel spatial proteomic platform we were able to assess changes in key inflammatory proteins that could be a result of the increased expression of phosphorylated tau. Utilizing a single-cell transcriptomic platform, we were able to show that key immediate early genes (IEG) necessary for memory consolidation were downregulated in the excitatory neurons of the RSC in an ADRD mouse model. Future directions will investigate the expression of these IEGs in excitatory neurons of the RSC in our virally injected animals. These experiments will determine if the impairments in spatial memory can be partially explained by a neuronal dysfunction that is present due to a reduced expression in genes that are critical for memory consolidation.

32. *"Ir-SIMCat Compound Protection Against Rotenone-Induced Reactive Aldehyde Species in an H9C2 Cell Model"*

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Reactive aldehyde species (RASP), generated during oxidative stress, play a causal role in myocardial dysfunction and a range of other conditions, including neurodegenerative, fibrotic, and pulmonary diseases. Yet no clinically approved treatments exist. Traditional biological and stoichiometric small-molecule scavenging approaches have shown limited therapeutic promise. Organoiridium small-molecule intracellular metal catalyst (Ir-SIMCat) compounds, Ir-1 and Ir-2, offer an alternative route to RASP detoxification, acting as hydride donors and reducing agents that convert toxic aldehydes into non-toxic alcohols via non-biological catalysis. Unlike other RASP scavengers, they detoxify numerous aldehyde molecules without being consumed. Previous studies have shown Ir-SIMCat compounds can significantly reduce RASP accumulation and neuronal cell death in vitro. As RASP overload is implicated in both neurodegenerative disorders and myocardial dysfunction, the primary goal of this study is to investigate the protective potential of Ir-SIMCat compounds against RASP-induced cardiomyocytes death and myocardial dysfunction. An in vitro cell model utilizing H9C2 cells that were differentiated into a cardiomyocyte-like phenotype was used to assess the protective potential of Ir-SIMCat compounds against rotenone and norepinephrine at toxic concentrations, meant to mimic RASP overload and myocardial dysfunction. In cytotoxicity assays, specifically LDH (lactate dehydrogenase) and CTB (Cell Titer Blue) assays, cells underwent a 3 hour pre-treatment with Ir-SIMCat compounds and then treated with toxicants for 24 hours. Post-treatment, media from each cell treatment group was collected and analyzed for LDH release via fluorescence as a measure of cytotoxicity/cell death. Media was then replaced, and cells were incubated with CTB reagent for 1 hour to provide a fluorescent measure of viable cell count. The results revealed that the Ir-SIMCat compounds can significantly reduce cell death in cardiomyocytes in cytotoxicity assays. For example, the LDH assays found that the Ir-1 SIMCat compound reduced rotenone-induced cytotoxicity by up to 20% compared to toxicant-only controls. These findings establish the protective capacity of these non-biological catalysts in the cardiac setting and suggest their therapeutic potential. This demonstrates a promising new approach to combating myocardial dysfunction, warranting further investigation in in vivo studies.

33. *"Characterization of Post-Transcriptional Regulatory Elements Controlling MED13L Reveals a Mutation-Agnostic Therapeutic Strategy for MED13L Syndrome"*

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MED13L syndrome is a rare neurodevelopmental disorder characterized by developmental delay, intellectual disability, hypotonia, and distinct craniofacial abnormalities resulting from MED13L haploinsufficiency. The extensive allelic heterogeneity of this disorder, driven largely by de novo mutations, presents a major challenge for mutation-specific therapies and underscores the need for a mutation-agnostic approach that restores MED13L expression. Because MED13L is subject to post-transcriptional regulation, we investigated whether these regulatory mechanisms could be therapeutically targeted to increase MED13L expression. Specifically, we investigated upstream open reading frames (uORFs), which limit synthesis of the primary coding sequence by modulating translation initiation, and microRNAs (miRs), which repress gene expression by promoting translational inhibition and transcript destabilization. First, we identified a functional uORF that impedes MED13L translation. Disruption of the uORF start codon increased MED13L expression by 10-20-fold in cultured cells. To evaluate in vivo uORF-mediated regulation in mouse brains, we generated adeno-associated virus (AAV) reporters expressing either wild-type or uATG-to-uATA mutant MED13L minigene reporters. Following AAV delivery, mutant uORF constructs showed increased MED13L reporter expression by 3-4-fold in mice. Next, we assessed whether antisense oligonucleotides (ASOs) could block the uORF to derepress MED13L expression. We identified a lead uORF-targeting ASO that increases MED13L transgene reporters and endogenous MED13L expression in vitro. In parallel, we leveraged our high-resolution map of empirically defined Ago2:miR-mRNA interactions in human tissues to identify four high confidence miR binding sites within MED13L mRNA. Functional analyses confirmed that each site represses MED13L expression. We then designed ASOs to block miR binding at two of these sites and identified three lead ASOs that relieved miR-mediated repression and increased MED13L expression. Finally, a dual-ASO approach targeting miR binding sites and the uORF produced a significantly greater derepression and upregulation of MED13L than either strategy alone. Overall, our findings define novel post-transcriptional mechanisms governing MED13L expression and establish ASO-mediated inhibition of miR- and uORF- dependent repression as a promising mutation-agnostic therapeutic strategy for MED13L syndrome.

34. *"Neuronal mechanism of hunger-induced reward sensitization"*

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Addiction to rewarding stimuli, whether drugs of abuse or natural reinforcers like palatable food remains a growing public health concern. Both types of stimuli can alter the brain's dopamine (DA) reward circuitry, leading to compulsive reward-seeking behavior. This convergence suggests shared neural mechanisms may underlie drug and food related reinforcement. Agouti-related protein (AgRP)-expressing neurons in the hypothalamic arcuate nucleus (ARC) are essential for regulating feeding behavior as well as other behavioral physiological adaptations to energy deficit state. Their activity has also been shown to modulate mesolimbic DA circuits and alter rewarding value of food. Moreover, hunger is a well-known reward sensitizer, elevating drug reward and risk of relapse. However, it remains unclear how AgRP neurons potentiates non-food reward processing and mediates hunger's reward potentiation. To address this, we investigated how AgRP neuron activity influences the mesolimbic DA reward system during reward seeking. Our central hypothesis is that AgRP neuron activity enhances the sensitivity of the ventral tegmental area (VTA) DA → nucleus accumbens (NAc) pathway, thereby facilitating non-food reward-seeking behavior. To test this, we used fiber photometry and chemogenetics to monitor and manipulate AgRP and dopamine neurons in real time and assessed reward-seeking behavior in mice in the form of optogenetic intracranial self-stimulation (oICSS) of VTA:DA neurons. Our findings show that activation of AgRP neurons significantly increases non-food reward-seeking behavior in mice. Mice exhibited increased optogenetic intracranial self-stimulation (oICSS) during AgRP neuron activation and showed a preference for non-food rewards over food when given the choice. Additionally, we find that this effect may not be mediated by pre-synaptic potentiation of DA release in the VTA→NAc pathway, as AgRP activation did not alter DA release in the NAc following VTA stimulation. These results uncover a previously unrecognized interaction between homeostatic hunger signals and non-food reward processing.

35. *"Recurrent Hypoglycemia Alters Hypothalamic Neural Circuits and Metabolic Dynamics to Impair Counterregulatory Responses"*

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Recurrent hypoglycemia (RH) is a major limiting factor in intensive glycemic management for individuals with diabetes. Repeated exposure to low blood glucose induces Impaired Awareness of Hypoglycemia (IAH) and blunts the physiological counterregulatory response (CRR), creating a dangerous cycle of severe, unrecognized hypoglycemic events. However, the precise neural circuit adaptations driving this counterregulatory failure remain incompletely understood. In this study, we investigated how repeated hypoglycemic episodes remodel central counterregulatory pathways and hypothalamic metabolic dynamics to impair autonomic and behavioral responses to low blood glucose. To establish an IAH model, mice were administered daily insulin injections over consecutive days. Following RH, mice exhibited delayed peripheral glucose recovery, reduced food intake, and attenuated glucose dynamics within the mediobasal hypothalamus (MBH) compared to control groups. Circuit-level analysis revealed blunted neural activity in tyrosine hydroxylase-expressing neurons in the nucleus tractus solitarius projecting to the paraventricular nucleus of the hypothalamus (NTSTH→PVN). Dynamically monitoring MBH ATP levels revealed that, despite persistent low glucose levels, ATP replenishment occurred significantly faster after RH compared to the first day of insulin administration. These data indicate that RH induces functional impairment in NTSTH→PVN circuits, blunting central autonomic activation. Furthermore, the combination of low hypothalamic glucose alongside rapid ATP replenishment suggests that RH triggers a metabolic shift toward alternative, non-glucose energy substrates to compensate for energy deficiency. This blunted neural responsiveness and metabolic adaptation serve as primary drivers of IAH and defective CRR, highlighting central circuit and metabolic pathways as potential therapeutic targets to restore hypoglycemia awareness.

36. *"Investigating the cardiomyocyte ncMTOC and perinuclear proteostasis with PCM1"*

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This project addresses the paradox in heart failure where centrosomal genes are highly dysregulated despite the absence of canonical centrosomes in post-mitotic cardiomyocytes (CMs). Adult CMs undergo centrosomal reduction, where centrosomal proteins relocate to perinuclear space alongside a large pool of proteasomes. We hypothesize knockout of the centrosomal gene PCM1 in CMs will directly destabilize the cytoskeleton through ncMTOC disorganization, leading to disruption of perinuclear proteostasis. We created a pipeline to identify candidate genes, design CRISPR guides, clone and validate guides, and test them in vivo. To determine candidate genes with the highest potential, we screened genes using data from the IMPC, HHF, CDVKP, GTEx, SCP, HPA, and BioGRID databases. Of an estimated 716 centrosomal genes, bioinformatic screening narrowed our focus to pericentriolar material 1 (PCM1). We have found PCM1 to localize to the perinuclear ncMTOC, be associated with atrial fibrillation/flutter and blood pressure, show prolonged QRS duration and RR interval, and is upregulated in human ischemic and dilated cardiomyopathy, conditions where disrupted perinuclear proteosomes worsens outcomes. Together, this indicates that PCM1 links cardiac genetic risk, contractile and conduction phenotypes, and perinuclear biology, positioning it as a strong candidate for examining the connection between the ncMTOC and perinuclear proteostasis. To induce knockouts, we identified CRISPR guides on or flanking constitutive exons near the N-terminal region to induce frameshifts and Golden Gate Assembly cloning with type 3 restriction enzymes with Sanger sequencing verification. To determine cutting efficiency, we transfected guides into HT22 cells. AAV viruses with the chosen PCM1 guides were injected into Cas9-J mice to create a CM-specific PCM1-KO. To test cardiac function, we did EKGs of PCM1-KO and control mice and measured gravimetric data. Heart and liver tissues were flash frozen or preserved in OCT for further examination. Western blot, qPCR, and immunofluorescence confirmed successful PCM1 knockout, establishing a validated model and serving as a starting place to uncover the link between the ncMTOC and perinuclear proteostasis in heart failure. Future experiments aim to clarify the role of PCM1-KO in perinuclear proteostasis, cardiac electrophysiology, and CM contractile function. These experiments will examine PCM1 as a possible mechanistic link between centrosomal biology and cytoskeletal/proteostatic dysfunction under cardiac stress, clarifying the role of the ncMTOC in proteostasis and heart failure.

37. *"Studying the Mechanism of Action of Small Neurotrophic Molecules trans-Banglene and 4,10-ARDD"*

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Despite therapeutic breakthroughs, neurodegenerative dementias have remained untreatable, leaving patients to suffer mental and physical decline. As such, the synthesis and study of small molecules that can pass through the blood-brain barrier and facilitate neuritogenesis continues to be an important and influential area of research. Previous work in the Williams Lab studied small neurotrophic molecule trans-banglene (t-BG) and its enantiomers, finding they exhibited neuritogenesis and NGF-potentiation in the PC 12 cell line (a model neuronal cell line). To further elucidate the mechanism of NGF-potentiation, the neuritogenesis and potentiation properties of (-) trans-banglene were tested in the presence of Erk 1/2, PkC and Akt inhibitors. It was found that (-) trans-banglene rescued neuritogenesis in the presence of Erk1/2 and PkC inhibitors. This poster will outline further investigations, including the synthesis of a photolabile crosslinker to identify the cellular target of t-BG. This photolabile crosslinker contains a diazirine which will bind to the cellular target in the PC 12 cells upon irradiation, as well as a terminal alkyne which can couple to biotin and allow for extraction from the cell lysate. Fluorescent labeling will also be used to determine where the target of t-BG localizes in the cell. In addition, synthetic work to access another neurotrophic compound, (-)9,10-aromadendranediol [(-)-ARDD] will be outlined. Previous and current work on t-BG is important to the (-)-ARDD project, as much of the same neuritogenesis and potentiation properties will be tested. With these current aims, a strong hypothesis for the mechanisms of actions for these small neurotrophic molecules can be constructed, which will aid new therapeutic strategies.

38. *"Visceral Adipose-Derived EVs Drive Neurogenic Hypertension via Action in the Brain"*

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Obesity is a major risk factor for metabolic and cardiovascular disease. In particular, a close relationship exists between high morbidity and intra-abdominal (visceral) fat obesity, rather than extra-abdominal (subcutaneous) fat obesity, but the underlying mechanisms are not known. Extracellular vesicles (EVs) are small carriers released by virtually all cell types. They are responsible for transferring bioactive cargo to all cell types. However, the role of EVs in obesity and how adipose-derived EVs (AdEVs) affect the brain is still not fully understood. To investigate this, we isolated AdEVs from the visceral (gonadal fat pad, gAdEVs) and subcutaneous (inguinal fat pad, iAdEVs) white adipose tissues (WAT) of C57BL/6 male mice fed normal chow diet (NCD, lean controls) or high-fat high-sucrose diet (HFHSD)-induced obese mice. AdEVs were obtained through gradient ultracentrifugation from the conditioned media of cultured adipocytes derived from both WAT depots. Transmission electron microscopy (TEM) and scanning electron microscopy confirmed the vesicular morphology consistent with EVs, and Western blot analysis validated the expression of the EV markers CD81 and HSP70. Nanoparticle tracking analysis of gAdEVs showed a significantly higher yield (particles/g tissue, $p<0.01$) in obese mice, with no significant differences in median or peak diameter, and no difference was observed for iAdEVs. To assess the cardiovascular effects of gAdEVs and iAdEVs derived from obese mice, C57BL/6 mice underwent baseline recording of telemetry blood pressure or direct multifiber recording of renal sympathetic nerve activity (SNA) followed by intracerebroventricular (ICV, 7 µg) injection, and the effects were evaluated over the subsequent 4 hours. Notably, ICV administration of obese gAdEVs induced a significant and sustained increase in blood pressure compared to obese iAdEVs ($p<0.001$). A similar pattern was observed for renal SNA, which showed a progressive increase of ~50–95% 4 hours following obese gAdEVs administration ($p<0.0001$), while obese iAdEVs showed no significant effect or a modest decrease. Lean gAdEVs did not affect blood pressure or renal SNA indicating that the pressor and sympathoexcitatoy effects of gAdEVs are acquired during obesity. Ganglionic blockade with hexamethonium significantly attenuated the pressor response evoked by obese gAdEVs, confirming autonomic nervous system involvement. These findings demonstrate that visceral gonadal, but not subcutaneous inguinal, AdEVs of obese mice are sufficient to drive neurogenic hypertension and renal sympathoexcitation, identifying visceral adipose-derived EVs as depot-specific mediators of autonomic cardiovascular dysregulation in visceral obesity.

39. *"Function and Expression of Complement C5aR1 Receptor in the Spinal Cord in Neuropathic Pain"*

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Chronic neuropathic pain affects approximately 10% of the population and only a minority of patients experience satisfactory relief of their pain with currently available pharmaceutics. Recent meta-analyses have identified pain-regulated genes, most notably complement system activation. Among the complement products, C5a seems to be especially important in the development and maintenance of neuropathic pain. Indeed, C5a receptor (C5aR1), a G-protein coupled receptor that mediates a majority of the effects of C5a, is expressed primarily on microglia in the CNS and is upregulated along with C5a in the dorsal horn of the spinal cord after peripheral nerve injury. Here, we address the role of C5a/C5aR1 signaling in mechanical pain following spared nerve injury (SNI) via behavioral pharmacology. We evaluated molecular changes occurring after SNI by RNASeq. We additionally examine changes in microglial signaling in the dorsal horn by performing 2-photon Ca2+ imaging in microglia in an ex vivo intact spinal cord preparation. Pharmacological or genetic inhibition of C5a/C5aR1 signaling decreased mechanical allodynia. C5a induced Ca2+ signaling in microglia in a dose-dependent manner and was blocked by co-application of a C5aR1 specific antagonist, PMX205. Overall, our findings suggest that C5a/C5aR1 signaling in the spinal dorsal horn plays a key role in modulation of pain hypersensitivity following SNI. Molecular and cellular mechanisms that link C5a/C5aR1 signaling with neuropathic pain are under investigation. Funded by National Institutes of Health (R01 NS127428, T32 DK112751).

40. *"Mutation of PRKAR1B produces robust deficits in development, learning, and nociception"*

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Marbach-Schaaf Neurodevelopmental Syndrome (MASNS) is a rare developmental disorder produced by de novo mutations of PRKAR1B, the RIβ regulatory subunit of protein kinase A (PKA). While PKA is a renowned kinase, the regulation of its activity by the RIβ subunit during neurodevelopment remains elusive. To better understand the molecular mechanisms of MASNS, we created a germline, heterozygous mouse model of the most pervasive PRKAR1B variant (p.R335W) using CRISPR-Cas9 Homology-Directed Repair. Here, we demonstrate this model replicates several phenotypes displayed in human patients: delayed growth and development of reflexes, obesity, reduced pain sensitivity, and impaired learning and memory. In electrophysiological experiments, R335W+/- mice had attenuated responses to long-term potentiation induced by theta-burst stimulation, but not high-frequency tetani. Western blotting revealed decreased proportions of GluR1 phosphorylation at PKA-dependent sites (S845). Finally, we show administration of BPN14770, a clinically relevant PDE4D-specific inhibitor, can rescue spatial memory deficits in R335W mice in a 2-Trial Y-Maze.

41. *"Two is better than one: Sex- and genotype-dependent alterations in psychostimulant driven locomotion in DAT Val559 mice on a C57BL/6 background"*

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Disruptions in dopaminergic neurotransmission have been implicated in multiple neuropsychiatric disorders including attention-deficit/hyperactivity disorder (ADHD), which affects males at higher rate than females. Notably, psychostimulant drugs, first line therapies for ADHD treatment, prevent synaptic dopamine (DA) reuptake by blocking the dopamine transporter (DAT), the primary mediator of DA clearance. Rare variants in the DAT gene (SLC6A3) have also been identified in ADHD probands including the DAT Val559 substitution, which drives anomalous, non-vesicular DA efflux. Though all carriers of the DAT Val559 variant identified to date harbor a single mutated allele, prior studies of DAT Val559 mice have largely focused on differences between wildtype (WT) and homozygous (HMZ) animals, employed animals on a mixed genetic background, and utilized primarily male subjects. Here, we characterize the genetic penetrance of DATVal559 variant on a congenic C57BL/6 genetic background in both sexes by examining differences in psychostimulant-induced locomotor behavior. Consistent with previous studies, male HMZs exhibit blunted amphetamine (AMPH)-induced locomotion and stereotypy compared to WT and heterozygote (HET) males. In contrast, AMPH-dependent hyperactivity was increased in female HETs compared to their WT and HMZ littermates. Cocaine (COC)-dependent locomotor activation was also modulated by genotype and sex with male HETs and HMZs exhibiting decreased COC-induced locomotion whereas HMZ females exhibited increased COC-driven hyperactivity relative to WT controls. Strikingly, both male and female HETs displayed significantly altered time in the center of the open field with an increase observed in HET females and a decrease in HET males compared to WTs. AMPH restored center occupancy to WT levels in HET females. To provide mechanistic insight into altered psychostimulant-elicited behavior, we are working to establish how the DAT Val559 variant impacts baseline DA dynamics including DA reuptake as a function of sex, circuit, and genotype. Our results both confirm the penetrance of the DAT Val559 allele on the C57BL/6 background, paralleling prior work with mixed 129S6-C57BL/6 mice, and extend these findings by highlighting the unique phenotypes of HET mice of both sexes. As DAT Val559 HETs more faithfully recapitulate the clinical population, determining the mechanistic bases for our findings will provide critical insight into how DA dysregulation contributes to neuropsychiatric disorder risk.

42. *"Neuromodulation of serotonin drives presynaptic plasticity underlying reward learning"*

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Synaptic plasticity underlying learning is modulated by multiple monoaminergic circuits and neuromodulators. How these signals are integrated at the cellular and subcellular levels to enable behavioral flexibility is not well understood. Circuit dynamics and receptor subtypes are involved in the cross talk of these pathways. Across taxa, serotonin and dopamine play roles in associative learning with valence and prediction establishments. In mammalian models these systems work these signals can be both synergistic or antagonistic to one another. Medium spiny neurons of the nucleus accumbens integrate serotonin and dopamine signals which are antagonistic, but conversely dopamine release of the ventral tegmental area potentiate serotonin release of the dorsal striatum. Alternatively, serotonin and dopamine are redundant for signaling mimic glucose reward signals in the invertebrate model C. elegans. A key question to address is how convergence of these two neuromodulatory pathways is integrated to modulate plasticity. To address this, we have examined how signals from serotonergic and dopaminergic circuits mediate synaptic plasticity that underlies reward learning in Drosophila. During olfactory associative learning, the Drosophila mushroom body integrates information about olfactory stimuli with unconditioned stimuli (e.g, rewards). Mushroom body neurons are anatomically compartmentalized, with discrete cholinergic output zones down the length of the axons. Dopamine and serotonin are released in the mushroom body and mediate aspects of synaptic plasticity associated with learning. Using a combination of in vivo 2P acetylcholine, dopamine and serotonin imaging, combined with 3D holographic optogenetics, molecular manipulations, and behavioral approaches, we examined how serotonergic and dopaminergic signals are integrated at the subcellular level to modulate synaptic plasticity. Conditional knockdown of 5-HT1A/B receptors in adult mushroom body neurons impaired reward learning and increased odor-evoked acetylcholine release from mushroom body neurons, disrupting compartment-specific patterns of reward learning-induced synaptic plasticity. Axon compartment-specific holographic stimulation of the serotonergic ramifies into compartmentally tiled serotonin release forming a localized suppression of odor-evoked synaptic acetylcholine release. Activation of protocerebral anterior medial dopaminergic neurons, and MB neurons induced localized release of serotonin, demonstrating functional feedback and feedforward connectivity between the dopaminergic and serotonergic circuits within the mushroom body. These data suggest that dopamine and serotonin functionally interact at the subcellular level to regulate synaptic plasticity underlying reward learning.

43. *"Sex- and Region-Specific Effects of the DAT Val559 Variant on Dopaminergic Function"*

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Perturbations in dopaminergic neurotransmission have been implicated in several neuropsychiatric disorders including attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD). The dopamine (DA) transporter (DAT) facilitates rapid clearance of extracellular DA. Notably, DAT is the pharmacological target of drugs used in ADHD treatment and genetic variants in the SLC6A3 gene, which encodes DAT, have been linked to psychiatric disorder risk. Several rare SLC6A3 coding variants have been identified including the Ala559Val substitution, found in individuals with ADHD, ASD, and bipolar disorder (BPD). The DAT Val559 mutation triggers anomalous DA leak resulting in perturbations in DA dynamics in mice harboring the mutation. Previous studies have focused on DAT Val559 homozygous (HMZ) and wild-type (WT) male mice on a mixed genetic background (~80% 129S6, ~20% C57BL/6J). For this study, we examined WT, HMZ, and heterozygous (HET) male and female mice on a congenic C57BL/6J background. As DA is critical for motor behavior, we first assessed locomotor activity at baseline in the open field. HMZ males showed reduced total distance traveled compared to WT and HET mice whereas HMZ females exhibited decreased stereotypic behavior relative littermate controls. Intriguingly, upon repeated exposure to the chamber, HET males spent less time in the center than WT and HMZ mice. These sex- and genotype-dependent changes in locomotor activity led us to evaluate baseline markers of DA neuron function including total and phosphorylated (Ser40) tyrosine hydroxylase (TH) as well as total and phosphorylated (Thr53) DAT across DA projection regions and in midbrain dopaminergic nuclei. In the dorsal striatum (DStr), we noted a decrease in both DAT and TH expression in HET and HMZ males relative to WT controls that was largely absent in females apart from a significant decrease in DAT in HET females. In the mesolimbic circuit, we noted a decrease in TH phosphorylation in HMZ males relative to control males in the nucleus accumbens (NAc). In contrast, female HETs exhibit decreased DAT expression in DA cell bodies in the ventral tegmental area (VTA). The unique molecular alterations in HETs, particularly females, is notable given DAT Val559 carriers almost universally harbor a single mutant allele. Future studies will continue to evaluate differences across sex, brain region, and genotype to better understand how DAT Val559 influences dopaminergic signaling and mouse behavior.

44. "Investigating Gut-Brain Mechanisms Underlying Behavioral Deficits Following Excess Neonatal Testosterone Exposure"

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Neurodevelopmental disorders (NDDs) are a heterogenous group of conditions that affect the developing brain and exhibit a pronounced male sex bias. Brain masculinization is driven by a surge of testosterone during a critical period of brain development. Disruption of this tightly regulated process, by factors including exposure to excess testosterone, has been associated with increased NDD diagnoses. Consistent with this, prior work from the Ferri lab demonstrated that excess neonatal testosterone in wild-type mice induces male-specific deficits in social behavior in juveniles and hippocampus-dependent fear memory in adulthood. However, the molecular and cellular mechanisms underlying these behavioral outcomes remain unclear. Testosterone exposure during early postnatal development can alter the gut microbiome, which may in turn influence neurodevelopment through the gut-brain axis. Here, we identified an enrichment of Lachnospiraceae UCG-006 in the gut microbiome of testosterone-treated mice. Lachnospiraceae are a major family of short chain fatty acid (SFCA)-producing gut bacteria involved in host immune regulation. Because microbiota-derived SFCAs are known to regulate microglial maturation and activation, we investigated whether excess neonatal testosterone alters microglial morphology. Testosterone-treated mice demonstrated a shift toward microglial morphologies associated with a more reactive phenotype. These findings suggest that excess neonatal testosterone exposure may alter gut-brain signaling and promote shifts in microglial activation states. Future studies will continue to characterize microglial morphology to confirm these findings and examine neonatal brain tissue to determine if microglial morphologies are altered during the critical developmental window following testosterone exposure.

45. *"Dynamin-Related Protein 1 in Leptin Receptor Neurons Regulates Metabolic Homeostasis and Cardiovascular Function"*

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Leptin receptor (LepRb) signaling in the brain plays a pivotal role in the regulation of metabolism and blood pressure. Mitochondria dysfunction is implicated in many diseases including obesity and cardiovascular diseases. Dynamin-related protein 1 (DRP1) which plays essential role for mitochondria fission/fusion has been implicated in mitochondria dysfunction. However, the role of DRP1 in LepRb neurons in the regulation of metabolic and cardiovascular functions is unknown. Here, we investigated whether loss of DRP1 in LepRb-expressing neurons affects metabolic and cardiovascular homeostasis. For this, we crossed female mice harboring floxed alleles of the Drp1 gene (Drp1fl/fl) with male mice expressing Cre recombinase in LepRb (LepRbCre) cells. Using immunofluorescence staining, we confirmed that DRP1 is expressed in the LepRb+ cells of the hypothalamus and that conditional knockout (CKO) mice have selective loss of DRP1 protein expression in the LepRb+ cells. LepRb+ cells of the CKO mice exhibited significantly elongated mitochondria by electron microscopy. Next, we found that both male and female CKO mice fed normal chow diet have similar body weight compared to control littermates. However, fat mass was significantly higher in both male (5.9±0.9 vs 3.6±0.6g) and female (3.1±0.4 vs 2.0±0.3g) CKO mice compared to controls. Of note, both male and female CKO mice displayed impaired insulin sensitivity compared to control animals, whereas glucose tolerance was similar between the two groups. Striking, CKO mice were sensitized to diet-induced obesity. Indeed, body weight of male and female CKO mice fed high-fat high-sucrose diet (HFHSD) was significantly increased compared to controls (47.4±1.6 vs 40.4± 2.1g for males, and 40.8±2.6 vs 34±1.6g for females). This excess weight gain was primary due to increased fat mass whereas lean mass was not altered. Decreased brown adipose tissue sympathetic activity indicates that reduced energy expenditure may contribute to the development of obesity in CKO mice fed HFHSD. Female, but not male, CKO mice fed HFHSD displayed insulin intolerance, but no change in glucose tolerance. Surprisingly, telemetry systolic blood pressure of male CKO mice fed normal chow diet was significantly lower relative to controls (107.9±5.9 vs 116.6±2.6 mmHg). Renal sympathetic nerve activity was also significantly lower in male CKO mice compared to controls under normal chow diet (75±2.5 vs 93.4±5.1 spike/sec) or HFHSD (67.2±8.3 vs 102.5±6.8 spike/sec), suggesting that reduced renal sympathetic nerve activity may contribute to lower blood pressure in CKO mice. We are currently testing blood pressure in mice fed HFHSD. These results demonstrate that DRP1 in leptin receptor-expressing cells regulate body weight, glucose homeostasis and blood pressure.

46. *"Brainstem PDYN neurons mediate feeding suppression and are required for taste aversion"*

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Glucagon-like peptide-1 receptor agonists (GLP-1RAs) are used world-wide as an effective mechanism to suppress appetite and manage obesity; however, they commonly cause aversive responses including nausea and vomiting. Hence, separating satiety from aversion is an important next frontier to treat obesity and related disease without these side effects. Dorsal vagal complex (DVC) including the nucleus solitary tract (NTS) has been shown to be the target of GLP-1R agonism induced satiety and nausea; however, downstream pathways mediating these effects are not fully understood. We found that a subset of NTS neurons defined by Prodynorphin (PDYN) expression are activated by the GLP-1R agonist exendin-4 (EX4) even though these neurons themselves are negative for GLP-1Rs. We hypothesized that NTSPDYN neurons could mediate GLP-1R agonism dependent suppression of food intake and/or aversion. Consistently, when activated, NTSPDYN neurons strongly suppressed food intake, decreased ARCNPY neuron activity, and promoted aversion in active place preference paradigm. We also show that NTSPDYN neurons are necessary for LiCl and EX4 mediated taste aversion, and their projections to the parabrachial nucleus (PBN) are activated by food consumption as well as by LiCl or EX4 injection. These results show that NTSPDYN neurons are downstream effectors of GLP1-R neurons that mediate feeding suppression via aversion.

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Thank you for your hard work and particpation during this year's Pharmacological and Pharmaceutical Sciences Research Retreat.