

# Abstract Program

*Postdoc Poster Session &  
Graduate Student Poster Session*

15<sup>th</sup> Annual Trainee Research Day

Monday April 8, 2024

University of Kentucky Gatton Student Center  
Grand Ballrooms



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## The regulatory role of asprosin in hypertension

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Asprosin is a novel adipokine, identified through the study of genetic disease called neonatal progeroid syndrome (NPS). So far, two spatio-temporally distinct functions of asprosin have been discovered. Asprosin cell-autonomously induces hepatic glucose release and stimulates appetite via the activation of agouti-related protein (AgRP) neurons of the hypothalamus. Asprosin performs these two spatio-temporally distinct functions via two different receptors. *Ptprd* (Protein Tyrosine Phosphatase type  $\delta$ ), a membrane bound phosphatase receptor mediates asprosin's orexigenic function, while a G-protein coupled receptor, *Olf734* (mouse ortholog of OR4M1), acts as the hepatic receptor for its glucogenic function. In this ongoing study we are assessing the role of asprosin in regulation of blood pressure (BP). Our preliminary results show that asprosin deficient female mice (NPS) present with significantly lower BP, which can be completely rescued with intra-nasal treatment of recombinant asprosin. Further, at the mechanism level, our preliminary data shows that asprosin's hypertensive effects are mediated by *Ptprd* signaling in the oxytocin neurons. Female mice with genetic loss of *Ptprd* from oxytocin<sup>+</sup> neurons (*Oxy-cre<sup>+</sup>;Ptprd<sup>flox/flox</sup>*) had significantly lower MAP (mean arterial pressure) when compared to wild type littermate controls (*Oxy-cre<sup>+</sup>;Ptprd<sup>+/+</sup>*). This study identifies a novel function of asprosin and represents a new avenue for therapeutic development for treatment of hypertension.

## **Profiling peripheral glial cells from intact and injured human nerves for grafting in the central nervous system**

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The unique pro-regenerative capability of peripheral nervous system (PNS) cells, including repair Schwann cells (SCs) from injured nerves, has been exploited clinically in cell transplantation therapies to treat central nervous system (CNS) trauma and neurodegenerative diseases. However, the characteristics of peripheral nerve cells has not yet been addressed thoroughly in humans. Therefore, the goal of this study was to identify specific markers able to reveal the identity and stage of differentiation of cells from intact and injured human nerves before and after implantation within CNS tissues. To study injury-associated changes in the expression of SC markers, we developed an in vitro model of human nerve degeneration to be compared with injured nerve grafts and brain tissues from participants enrolled in a nerve transplantation clinical trial for Parkinson's disease (NCT02369003, NCT05377281). Overall, our results confirmed the value of using antibodies against S100 $\beta$ , myelin protein zero (MPZ), periaxin (PRX), NGFR, GFAP, and Sox10, alone and in combination with axonal markers and myelin-selective fluorophores, to identify mature (intact) and repair (injured) human SCs in relationship to axons, myelin, and nonglial cells. In particular, our histological analysis revealed that: (1) NGFR was a reliable marker to discriminate PNS-derived cells, including repair SCs, from CNS neurons and glial cells; (2) S100B, GFAP, and Sox10 were useful to specifically identify SCs within intact and injured nerve tissues, with the caveat that they also revealed glial populations in the CNS (astrocytes and oligodendrocytes); and (3) MPZ and PRX were equally useful to identify human myelin sheaths derived from SCs rather than oligodendrocytes. To conclude, the above-mentioned markers can be used in different combinations to reveal grafted PNS cells, mainly SCs, in the human CNS to study their survival, differentiation, and relationship to host tissue.

## The Protective Effect of VAMP8 Deficiency in Aortic Aneurysms: Potential Impact of Impaired Platelet Cargo Release

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**Introduction and Objective:** Platelet activation and subsequent cargo secretion play a critical role in thrombus formation and aortic remodeling, factors potentially influencing the growth and rupture of abdominal aortic aneurysms (AAA). Despite the compelling data that platelet activation is associated with AAA and rupture, the underlying mechanisms remain poorly understood. This study hypothesizes that VAMP8, a key element of platelets' secretory machinery, significantly contributes to AAA formation.

**Approach and Results:** A retrospective, single-center multiple comparisons study of adult aneurysmal patients admitted to UKHealthCare between 2004 and 2023 revealed a significantly lower platelet count in AAA patients ( $n=3131$ ,  $213.76 \pm 3.46$ ) vs. healthy individuals ( $n=6309$ ,  $243.18 \pm 4.09$ ) suggesting an increase in platelets consumption, potentially in the aneurysmal sack. Thoracic aortic aneurysm and aortic dissection patients also indicated lower platelet count. *In vivo*, platelets were shown to accumulate at elastin break sites and false lumens of abdominal aortas from 28-day AngII-infused hypercholesteremic mice. To assess the role of platelets, specifically platelet secretion, we utilized VAMP8 null mice. We first validated the platelet secretion defect in these mice and then used RNASeq and proteomics array to define the role of VAMP8 in platelet cargo packaging. Next, VAMP8<sup>-/-</sup> or WT hypercholesterolemic male mice fed a Western diet were infused with AngII (1,000 ng/kg/min) for 4 weeks. Our results revealed that VAMP8<sup>-/-</sup> mice are protected against AngII-driven aortic rupture. Additionally, aortic *ex vivo* analysis indicated that the VAMP8 deficiency ( $n=15$ , aortic diameter:  $0.9 \text{ mm} \pm 0.03$ ) profoundly attenuated AngII-induced AAA compared to control ( $n=9$ , aortic diameter:  $2.0 \text{ mm} \pm 0.2$ ). Further analysis also indicated a marked reduction in thoracic aortic aneurysm and atherosclerosis development in VAMP8<sup>-/-</sup> vs. Control group.

**Conclusion:** Our results elucidates that a deficiency in VAMP8 results in the profound attenuation of aortic aneurysms and atherosclerosis development, introducing a novel paradigm for understanding the impact of platelet cargo secretion in the development of aortopathies.

## Targeting the Neddylolation Pathway: Bridged Piperidine Derivatives as Potent Inhibitors of DCN1-UBE2M Interaction for Cancer Therapy

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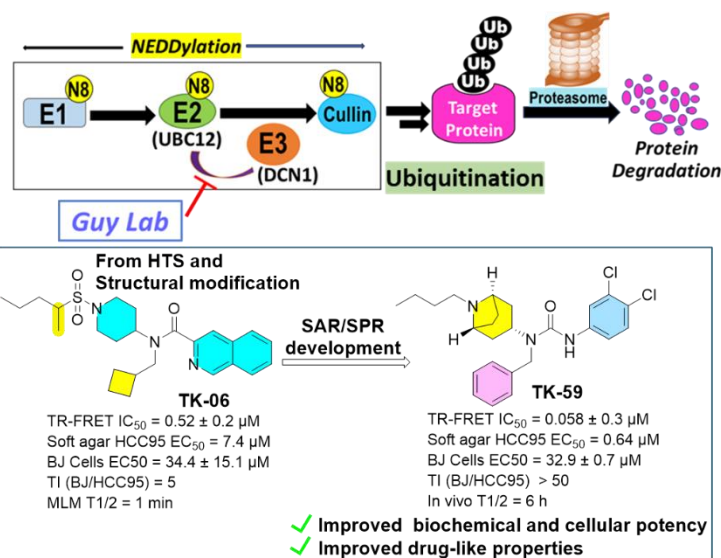
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### Abstract

Neddylation, a post-translational modification akin to the ubiquitin-proteasome system (UPS), plays a crucial role in intracellular regulation by controlling the activity of cullin-RING ubiquitin E3 ligases (CRLs). During neddylation, the ubiquitin-like protein NEDD8 is conjugated to target proteins such as cullins. Neddylated cullins then combine with a multiprotein complex to create activated CRLs, which control the UPS pathway for the degradation of numerous proteins. Disruption of CRLs function can lead to the development of various diseases, including cancer. The NEDD8-activating enzyme (E1) inhibitor Pevonedistat prevents the neddylation of the CRLs and has been clinically validated as a therapeutic approach targeting the neddylation pathway in oncology.



Through an HTS involving over 600,000 molecules and employing structural optimization strategies, we identified a potent small molecule inhibitor **TK-06** (IC<sub>50</sub> = 0.52 μM). This inhibitor targets CRL activation by disrupting the DCN1-UBE2M protein-protein interaction. However, the isoquinoline sulfonamide compound (**TK-06**) rapidly metabolized in mouse liver microsomal models (T<sub>1/2</sub> = 1 min; CL<sub>int</sub> = 5416 mL/min/kg). X-ray co-structures of bound inhibitor guided specific structural modifications to improve physicochemical properties and DCN1 binding. Careful attention to structural constraints, sites of metabolism, and structure-property relationships allowed the discovery of an orally bioavailable bridged piperidine analogue (**TK-59**). This inhibitor not only significantly improved potency (both biochemical and soft agar cellular potency) but also demonstrated enhanced stability in both in vitro and in vivo models.



## **Contusion Spinal Cord Injury Model Characterization for Autonomic dysreflexia: A pilot study**

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### **Abstract**

Complete high thoracic spinal cord injury (SCI) often leads to autonomic dysreflexia (AD), a condition that manifests as acute, episodic hypertension with and without bradycardia. AD is characterized by excessive discharge of sympathetic preganglionic neurons (SPN) in the intermediolateral cell column (IML) that are reflexively activated by noxious stimuli below the injury level. Most animal models of AD utilize a complete spinal cord transection at the T3 spinal level or above, however, anatomically complete spinal transection is relatively uncommon clinically, and individuals with incomplete injury still experience AD. We hypothesized that rats will develop AD over time following severe contusion SCI at the T3 spinal level. Thus, we designed an 8-week study with two different injury severities and evaluated the development of AD.

Adult female Wistar rats were subjected to T3 contusion SCI with two different forces (300 kdyn (5s dwell time) and 400 kdyn (5s dwell time)). Injured rats were subjected to weekly behavioral testing using Basso Beattie Bresnahan (BBB) locomotor rating scale for hindlimb function as well as the tail spasticity test for eight weeks. To evaluate changes in blood pressure, a telemetric probe was implanted in the descending aorta 2 weeks after the injury. Spontaneous AD events were recorded 3, 5, and 7 weeks post-injury. We also monitored induced AD by colorectal distension (CRD) at 4, 6, and 8 weeks post-SCI. We observed significant locomotor dysfunction and the development of spasticity regardless of injury severity. Both injury severities also resulted in significant increases in mean arterial pressure (MAP) but no change in heart rate (HR) after AD by CRD. This evoked AD was not significantly different between injury severities. There was no significant difference in spontaneous AD events between injury severities. Tissue histology will be correlated with the magnitude of induced AD for both SCI severities. Collectively, our data support severe SCI contusion as a correlate for SCI-induced AD in humans.

## **Bioequivalence of Oil-in-Water Adjuvant Prepared with Synthetic Biology-derived Squalene to that Prepared with Shark-derived Squalene Based on Immunogenicity with Quadrivalent Influenza and SARS-CoV-2 S1 Vaccines in Mice**

Kelly Oriakhi

Influenza virus remains a global health threat, necessitating effective vaccination strategies to mitigate its impact. Squalene, a triterpene, has proven efficacy as an adjuvant in influenza and SARS-CoV-2 vaccines. Unfortunately, the conventional practice of sourcing squalene from sharks has triggered environmental and ethical concerns. Wildlife advocates express alarm over the endangerment of shark species, exacerbated by a 71% decline in oceanic sharks primarily attributed to fishing practices. Moreover, human activities in aquatic environments pose safety issues, potentially contaminating shark-derived squalene and limiting its application in diagnostic use. However, squalene produced from yeast not only eliminates the need for animal products but also expands the accessibility of squalene, benefiting its widespread use and large-scale vaccine production. In this study, we demonstrate that immunization of Balb/c mice with MF59 or its mimetic AddaVax and EVAXS plus soluble antigen (quadrivalent influenza virus hemagglutinin protein) results in robust antigen-specific antibody and T cell response in the splenic tissues. Results show that the synthetic adjuvants significantly increased total IgG concentrations at day 21 (21 days after prime immunization), day 42 (21 days after boost immunization) and day 168 when compared to mice given antigen only for the respective days. Similarly, squalene-based adjuvant vaccines elicited IgG1, and IgG2a titres when compared to mice administered antigen only. It was also observed that the squalene-based adjuvant vaccines (EVAXS, Addavax, and MF59) increased IgG1/IgG2 ratios and elicited peptide epitope-specific CD4 T cells when quantified by interferon gamma (IFN- $\gamma$ ) ELISpot assays which correlate with the stimulation of Th1. In conclusion, findings from our results suggest that the squalene-based adjuvant vaccines stimulated durable, long-lasting memorable immune responses with evident memory B and T cells.

## **Environmental Effects on the Heart Rate and Core Body Temperature in Diabetic Mice**

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**Objective:** Environmental factors affect the 24-hour regulation of the heart rate (HR) and core body temperature (T<sub>b</sub>). Db/db mice have been shown to have irregular thermoregulation. We explored the effect of housing temperature and light conditions on the HR and T<sub>b</sub> in db/db mice.

**Methods:** Four to six-month-old db/db and control female mice (n=5-6/genotype) were implanted with telemetry devices to continuously record the electrocardiogram (ECG), core body temperature, and activity. Mice were housed at room temperature (25 °C) followed by thermoneutrality (30 °C) in 12 h light: 12 h dark cycles (LD, 200 lux: 0 lux) with ad libitum access to food and water. Mice were then subjected to 12 h light: 12 h dim light at night cycles (dLAN; 200 lux: 5 lux) for one week.

**Results:** Db/db mice had blunted day/night rhythm in heart rate and core body temperature compared to control mice, as 50% of db/db mice did not show a robust 24-hour variation in the heart rate. Thermoneutrality increased the day/night variation and 24-hour rhythmicity in the heart rate. Specifically, thermoneutrality decreased mean heart rate in both the genotypes and increased average core body temperature in db/db mice up to the control levels. dLAN blunted the day/night differences i.e., amplitude of HR, T<sub>b</sub>, and activity in db/db and the control mice.

**Conclusion:** dLAN disrupted the 24-hour rhythm in both HR and T<sub>b</sub> in db/db and control mice, underscoring the critical role of environmental conditions in 24-hour heart rate and core body temperature regulation.

## Monitoring the conformational ensembles of a Bifurcating Electron Transfer Flavoprotein in solution

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Bifurcating electron transfer flavoproteins (Bf-ETFs) perform electron transfer 'bifurcation' in a variety of microorganisms, including both bacteria and archaea. Bf-ETFs contain two flavin adenine dinucleotide (FAD) molecules. The bifurcating FAD accepts a pair of electrons from NADH and dispenses them to two separate pathways.<sup>(1)</sup> An exothermic electron transfer (ET) reaction to a high-potential acceptor, mediated by the second FAD termed ET-FAD, is used to drive endothermic transfer of the second electron to a lower potential acceptor: ferredoxin or flavodoxin semiquinone. Conformational dynamics are essential in maximizing the energy efficiency of bifurcation. The open and closed conformations of *Acidaminococcus fermentans* ETF (*AfeETF*) and *Acetobacterium woodii* (*AwoETF*) have been resolved by X-ray crystallography.<sup>(2)</sup>

<sup>19</sup>F NMR provides high sensitivity spectra in which the observed chemical shifts are highly responsive to changes in local chemical environment.<sup>(3)</sup> Therefore we are advancing this method to permit observation of Bf-ETF's conformational dynamics. In the current study, incorporation of 5-Fluo-Trp in wild type and mutant *AfeETF* was also accomplished, and <sup>19</sup>F NMR measured with the objective of monitoring the conformational ensembles. The effect of changes in solvent polarity on <sup>19</sup>F NMR spectrum of mutant W317Y was investigated to understand the differences between buried and solvent exposed Trp residues. Future directions include investigating the spectral responses upon partner protein binding and/or population of different redox states. Additionally, we are assigning the observed signals to specific residues based on mutagenesis, to obtain insight into local structural features.

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## **Half-sarcomeres relax with a biphasic time-course in spatially-explicit simulations with series compliance**

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Experiments with isolated myofibrils or muscle cells show that force relaxes with a biphasic profile when the intracellular  $\text{Ca}^{2+}$  concentration is reduced. Force initially declines slowly and linearly before the preparation transitions to a different mode where force falls with an exponential time-course. The transition is associated with a sudden increase in half-sarcomere heterogeneity. Prior computer modeling has shown that the biphasic relaxation profile can be reproduced if the half-sarcomeres shorten against a series compliance. These calculations were performed using a distribution-based approach (MyoSim, PMID 26840730) to simulate cross-bridge cycling. In this new work, the spatially-explicit FiberSim model (PMID 34932957) has been extended to simulate myofibrils composed of half-sarcomeres connected in series with an elastic element. These new calculations confirm that series compliance can accelerate relaxation by allowing the thick filaments to move relative to actin. An important feature of spatially-explicit models is that they can mimic different potential functions of myosin binding protein-C, like stabilize the SRX state of myosin dimer and/or bind the thin filament. In our simulations different modes of myosin binding protein-C produce different effects on relaxation: if it binds to the actin filament, the linear phase of relaxation is prolonged, while if it stabilize SRX the linear phase it get shorter.

## EcoHIV-Infected Mice Show No Signs of Platelet Activation

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### Abstract

Platelets express several surface receptors that could interact with different viruses. To understand the mechanisms of HIV-1's interaction with platelets, we chose the EcoHIV model. While EcoHIV is an established model for neuroAIDS, its effects on platelets are ill-defined. Our results indicate that EcoHIV behaves differently from HIV-1 and is cleared from circulation after 48 h post-infection. The EcoHIV course of infection resembles an HIV-1 infection under the effects of combined antiretroviral therapy (cART) since infected mice stayed immunocompetent and the virus was readily detected in the spleen. EcoHIV-infected mice failed to become thrombocytopenic and showed no signs of platelet activation. One explanation is that mouse platelets lack the EcoHIV receptor, murine Cationic Amino acid Transporter-1 (mCAT-1). No mCAT-1 was detected on their surface, nor was any mCAT-1 mRNA detected. Thus, mouse platelets would not bind or become activated by EcoHIV. However, impure virus preparations, generated by Polyethylene Glycol (PEG) precipitation, do activate platelets, suggesting that nonspecific PEG-precipitates may contain other platelet activators (e.g., histones and cell debris). Our data do not support the concept that platelets, through general surface proteins such as DC-SIGN or CLEC-2, have a wide recognition for different viruses and suggest that direct platelet/pathogen interactions are receptor/ligand specific.

## **Serotonin, Psychedelics, and Claustrum Signaling to the Anterior Cingulate Cortex**

Anderson, Tanner (University of Kentucky); Ortinski, Pavel (University of Kentucky)

The claustrum (CLA), a subcortical nucleus, has the highest density of the serotonin 2A receptor (5HT2AR) in the brain with extensive connections to other brain areas, most prominently the anterior cingulate cortex (ACC) that is involved in both cognitive flexibility and drug-seeking behaviors. Though the CLA is gaining increasing attention for its' potential importance in regulating several aspects of cognition, almost nothing is known about the role of its' robust serotonergic innervation. Here, we target several 5-HTRs in the CLA with RT-qPCR, RNAscope, and whole cell patch clamp electrophysiology to characterize the function of 5-HTRs in CLA-ACC signaling.

5-HT caused dramatic inhibition in CLA-ACC neurons. Decreases in sEPSC frequency and amplitude were observed, as well as decreases in action potential firing rate and hyperpolarization of the resting membrane potential. Next, we used qPCR to observe the relative abundance of 5-HT receptor subtypes within the CLA, finding elevated levels of 5-HT1A, 2A, and 2C receptors. CLA-ACC neurons were then recorded in the presence of 5-HT and antagonists of each of these receptors to observe their contributions to the 5-HT effects. Recordings performed in the presence of the psychedelic 5-HT2AR agonist, DOI, caused increases in sEPSC frequency and amplitude. Next, we observed spike-timing dependent plasticity (STDP) in CLA-ACC neurons, revealing anti-hebbian long-term depression. DOI reversed this LTD into a robust long-term potentiation. Finally, RNA scope combined with confocal imaging was performed to interrogate the colocalization of each of the 5-HT receptor subtypes in the claustrum.

## **Chronic Psychosocial Stress in Female Rats Throughout Age**

Kim Bretland, Ivana Djuricic, Danny Craig, Eric Blalock

Chronic psychosocial stress has deleterious effects on long-term health and exacerbates many age-related conditions (reproduction, body weight, and cognition, etc.). The prevalence of these age-related and stress-exacerbated conditions are projected to increase as the U.S. population ages: the 2020 U.S. Census reports 17% of U.S. population are above age 65, with an expected increase to 25% by 2060. However, despite years of research clearly showing that aspects of aging are accelerated by psychosocial stress, little work has investigated the age-course of the stress response or the impact of the normal brain aging phenotype on the stress response itself.

To address this, our ongoing experiments are characterizing the physiological, behavioral, and molecular effects of chronic stress at four age-points (3, 6, 12, and 18 months) in F344 rats. Currently, we have completed 3 of a planned 5 cohorts of female animals (male subjects will begin in the fall). In this update, body weight, water maze, and estrous cycle data are reported for these first 3 cohorts of animals. As expected, body weight increased, maze performance declined, and there was a marked shift in the estrous cycle with age. Further, the behavioral stress response appears to change with age, showing a greater negative effect in young adult animals. Additional measures will be taken after all samples have been collected (hippocampal immunohistochemistry and transcriptional profiling, longitudinal and terminal blood samples for sex and stress hormone signaling) to identify candidate molecular underpinnings of the age-related change in stress response.



## High-Low training as a rehabilitative therapy for chronic spinal cord injury

Daimen Britsch

**Objectives:** The technique of "living High, training Low" (H-L) is used by athletes to improve exercise performance. H-L involves cycles of exercise performed at normoxic conditions coupled with hypoxic exposure at rest. Rodent models of spinal cord injury (SCI) implementing exercise after injury often show improved recovery of locomotion, while application of hypoxia can induce respiratory motor plasticity. Respiratory disease is a leading cause of morbidity/mortality post-SCI, and <1% of SCI survivors recover full function. Our hypothesis is that H-L training is tolerable and improves functional recovery.

**Methods:** 62 S.D. rats received a C2 hemisection, n=49 surviving. Pair-housed cages were randomly assigned to H-L, hypoxia-only, exercise-only, or control group. Treatment began 6-7 weeks post-injury (WPI) and lasted 8 weeks. Treatments were implemented via voluntary exercise and/or administration of sustained hypoxia. For 5 days a week, H-L and exercise subjects were housed individually in cages with exercise wheels overnight, then returned to pair-housed cages in the morning. Sedentary subjects were individually housed overnight without wheel access. Following return to pair housing, H-L and hypoxia groups received 4 hours of 11% O<sub>2</sub>. Functional assays were performed pre-SCI, 5 WPI, 9 WPI and 13 WPI. At 14 WPI subjects underwent a terminal diaphragm EMG. Splenocytes were collected for cell profiling.

**Conclusions:** Treatment was well-tolerated and has translational potential. The H-L group experienced decreased respiratory frequency ( $p=0.004$ ) and increased tidal volume ( $p=0.005$ ) between pre-treatment and 4 weeks later, while control subjects did not. H-L treatment also appears to prevent development of SCI-associated anxiety.

## **An Exercise-Trained Gut Microbiome Ameliorates Skeletal Muscle Atrophy**

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**Abstract:** Regular exercise yields a multitude of systemic benefits. Due to the ability of the gut microbiome and its associated metabolites to affect host physiology, some of these benefits appear to be mediated by the gut microbiome. Our lab has previously reported that dysbiosis of the gut precludes the proper adaptation of skeletal muscle to exercise-training. The aim of this study was to determine if an exercise-trained gut microbiome (i.e., the gut microbiome of a host that has been subjected to exercise) is able to ameliorate skeletal muscle atrophy in mice undergoing hindlimb immobilization. Our findings demonstrate that hindlimb-immobilized mice receiving cecal microbial transplants (CMTs) from mice that have undergone exercise training have reduced skeletal muscle atrophy compared to those receiving CMTs from sedentary mice. Moreover, top microbial-derived metabolites identified as being associated with an exercise-trained gut microbiome were able to attenuate skeletal muscle atrophy and preserve muscle function upon direct administration to mice undergoing atrophy induced by hindlimb immobilization. Taken together, these findings indicate that an exercise-trained gut microbiome and its associated metabolites are able to alleviate losses in skeletal muscle size and function in hindlimb-immobilized mice.

## **Comparison of differently valued rewards on long-term post-stroke cognitive function after B cell depletion**

Katherine M. Cotter, Thomas A. Ujas, Mary K. Colson, Pavel Yanev, Edric Winford, Jadwiga Turchan-Cholewo, Ann M. Stowe

Background: Many behavioral tests require the use of a food reward to promote the learning of a task but to maintain motivation, the mice often undergo food restriction. However, food restriction is detrimental to post-stroke survival and recovery (PMID:27449604). Additionally, it is known that B cells migrate into the brain after stroke and remain there long-term, but whether their presence is beneficial or detrimental remains to be determined in aged animals.

Aim: To assess the use of two different rewards – peanut oil (PO) and strawberry milkshake (SM) – without food restriction. We hypothesized that aged female mice would complete more trials with SM the strawberry milkshake reward, but that the behavioral trends due to B cell depletion would not be affected by reward type. We also hypothesized that B cell depletion would result in more severe post-stroke cognitive deficits in the autoshaping (AUTO) operant touchscreen task in aged animals, and that this decline in performance correlates with the loss of neuroprotective B cells in the hippocampus and pre-frontal cortex.

Results: Mice on peanut oil reward averaged 26 trials/session with only 7/20 mice completing session criteria of 40 trials/60 min. Several mice (6/20) did not respond as well to peanut oil but remained within the cohort. When using a strawberry milkshake reward, 7/8 aged female mice consistently reached the criterion of 40 trials/60 min for the AUTO task. B cell-depleted mice exhibited an improvement in their cognitive performance at 4- (PO: 3-way rmANOVA;  $p=0.0228$ ) and 6-weeks post-stroke (SM: 3-way rmANOVA;  $p=0.0306$ ).

## Exploring Isoform Signatures Across Human Brain Regions and in Stimulated CD4+ T-Cells with Long-Read Single Cell RNA-seq (scRNA-seq)

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Single-cell RNA sequencing (scRNA-seq) provides insight into cellular diversity and mechanisms underlying disease, offering novel therapeutic targets obscured by bulk sequencing. Although single-nucleus RNA sequencing (snRNA-seq) is used as an alternative to scRNA-seq for frozen tissue, some cell types do not survive freeze-thaw cycles. Additionally, cytoplasmic signatures that provide crucial information about cell state may be lost in snRNA-seq. scRNA-seq typically uses short-read sequencing, which collapses all measures of single-gene isoform variants into a single gene expression measurement and, due to insufficient depth and/or mapping quality, cannot truly detect isoform-level expression. Long reads provide broad coverage of isoforms that may provide insight into functional variations in the resultant protein. Our understanding of isoform-level expression in cellular populations is limited, partially due to the absence of single-cell approaches for long-read sequencing until recently. Recent studies using long-read scRNA-seq to find isoform-level changes in bacteria, humans, and mice reveal a new technology that will inform novel disease mechanisms and drug targets. Expanding on these studies, we adapted Particle-templated Instant Partition Sequencing (PIP-seq) for long-read sequencing. PIP-seq offers fast, instrument-free cell preparation, a critical advantage over standard 10X Genomics approaches for collecting fresh clinical samples that become available unpredictably. Our pilot's objective is to demonstrate effective use of our novel long-read scRNA-seq preparation with human brain tissue and stimulated CD4+ T-Cells. Overall, we establish innovative utilization of the PIP-seq protocol for long-read single-cell sequencing. Our objective for future studies is to inform novel gene and isoform markers for disease-associated and region-specific cellular phenotypes.

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## Revealing novel functions of putative cytotoxins in *Chlamydia trachomatis* infection

Gracie Eicher

*Chlamydia trachomatis* is the leading cause of bacterial STIs in the US, with treatment being expensive due to prevalent reinfection. *Chlamydia* species display a considerable degree of genome conservation. However, a plasticity zone (PZ) harbors considerable genetic variations among serovars and strains. Within the PZ of *C. muridarum*, is a series of genes encoding three highly similar proteins, TC0437-0439, with homology to a single protein (CT166) found in the urogenital strains of *C. trachomatis*. These proteins are putative cytotoxins that are expected to inhibit host-cell actin polymerization. Initial studies have shown that when CT166 is ectopically expressed, the host-cell actin is disrupted and cell rounding occurs; this supports the hypothesis that this protein is contributing to cytotoxicity of *Chlamydia*. Our research shows that the CT166 and TC0438 have the catalytic motif necessary for glycosyltransferase activity. We used FRAEM mutagenesis to delete the *tc0437-0439* genes in *C. muridarum* effectively creating a toxin deletion mutant. When the mutant was used in infections, our results showed that the toxin does not contribute to immediate toxicity, mediated by collapse of the actin cytoskeleton during infection. Instead, deletion did cause a defect in invasion. Immunofluorescence and trypsin degradation assays provided evidence to support surface localization of CT166 in *C. trachomatis*. Together, this data suggests that CT166 might not have a role in immediate toxicity but is localized to the surface of *Chlamydia* and aids in invasion and/or attachment. Further investigation will need to be done to elucidate the relevant function of these proteins and to identify the specific targets.

## **A Case of Dysphagia Following Carotid-Carotid Bypass**

Logan Elliott, BS. Logan Fluty, MD. Sanford Archer, MD.

The common carotid arteries travel up the neck lateral to the esophagus and trachea and then branch into the internal and external carotid arteries, where they go on to supply blood to the neck, face and brain. A carotid-carotid artery bypass graft is a procedure performed to help restore blood flow to the carotid artery where a biosynthetic graft is placed in the retroesophageal space, as this offers a short and straight route from right to left carotid artery. In the case of a 71-year-old male who underwent this procedure, he presented with hoarseness, dysphagia, and the sensation of food getting stuck in his throat that he stated began after his procedure. Neck CT revealed evidence of the carotid-carotid graft impinging on the patient's hypopharynx and superior cervical esophagus. The modified barium swallow also revealed narrowing at these areas where the hardware was present. On neck examination, there was no adenopathy, thyromegaly, mass or nodule, and a well-healed scar. The patient underwent a rigid esophagoscopy and direct laryngoscopy which revealed that the vascular graft was positioned submucosally along the posterior pharyngeal wall at the level of the cricopharyngeus, impinging the esophageal introitus. Based on imaging and physical exam, it was determined that the symptoms were linked to the hardware from the carotid-carotid bypass. From this case, it can be shown that while carotid-carotid bypass shows significant improvement in overall quality and length of life, there can be significant side effects from this procedure.

## **Role of Gut Microbial Metabolites in Intestinal Wound Healing**

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Abstract: Interactions between intestinal epithelial cells, microbes, and metabolites in the gastrointestinal tract have substantial impacts on human health, including in intestinal wound healing and epithelial barrier repair during the resolution of inflammation induced by inflammatory bowel disease (IBD). Here, we link microbial changes occurring during inflammation to increased intestinal epithelial cell proliferation and migration, two key steps in the wound healing process. Microbiome analysis shows characteristic changes at both the phyla and species level in the gut microbiomes of IBD mouse models, and metabolomics data indicate alterations in microbial metabolites. Further studies using these metabolites show increased intestinal epithelial cell proliferation and migration. The combination of these results suggest that certain bacterial metabolites contribute to intestinal wound healing necessary to repair the damage incurred during active IBD inflammation.

## Co-Exposure to Mono(2-ethylhexyl) Phthalate and Elevated Temperature Inhibits Mouse Antral Follicle Growth and Steroidogenesis *In Vitro*

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Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer found in common consumer goods. DEHP is rapidly metabolized to mono(2-ethylhexyl) phthalate (MEHP), which is bioactive and exerts toxicities in the ovary. Humans are ubiquitously exposed to DEHP/MEHP through these goods while simultaneously exposed to rising temperatures given the progression of climate change. An *in vitro* follicle culture system was used to test the hypothesis that exposure to high temperature (HT) will exacerbate the effects of MEHP exposure on antral follicle growth and steroidogenesis. Antral follicles from CD-1 mice were cultured at a control temperature (CT; 37°C) or high temperature (HT; 42°C; 8hr/day) and treated with vehicle control (DMSO) or MEHP (0.2-20µg/ml) for 96 hours. Follicular growth was measured every 24 hours with follicle and media collections at 96 hours for gene expression and hormone measurements (n=3-7, p ≤ 0.05). Antral follicle growth was decreased by HT and HT+MEHP treatment at 72 and 96 hours compared to DMSO. Estradiol levels were decreased by CT+MEHP, HT, and HT+MEHP treatment. Testosterone levels were increased by CT+MEHP and decreased by HT+MEHP treatment. The mRNA levels of *Hsd3b1* were decreased by CT+MEHP and HT+MEHP treatment. The mRNA levels of *Cyp17a1* and *Hsd17b1* were decreased by HT and HT+MEHP treatment. The mRNA levels of *Cyp19a1* were decreased by CT+MEHP, HT, and HT+MEHP treatment. These findings suggest that exposures to HT and MEHP inhibit antral follicle growth and disrupt steroidogenesis. Thus, combined exposures to ubiquitous chemical and non-chemical stressors may potentiate ovarian dysfunction. Supported by R01ES033767, UL1TR001998, and P30ES026529.



## **Immune Cell Function & Metabolism is Permanently Altered by Obesity and Type 2 Diabetes**

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**Abstract:** Type 2 Diabetes (T2D), one of the top ten causes of death worldwide, is fueled by chronic inflammation. T2D is considered a metabolic disease, and there is a great push to target metabolic and associated inflammatory pathways to ameliorate the disease & its comorbidities i.e., obesity and cardiovascular disease. The driver(s) of inflammation remains unknown; I posit that metabolic abnormalities perpetuate T2D-associated inflammation.

Bariatric surgery has become a standard treatment for obesity that causes significant weight loss, tangentially causing patients' glycemic control to improve. However, weight loss and eventual regain is highly variable among bariatric surgery patients. Bariatric surgery's effects on immune cells' metabolome is also thus far understudied. My project aims to analyze inflammation through measuring immune cell function (i.e., cytokine production) and immune cell metabolism (particularly energy-producing pathways).

## **Advances Towards Saturating Transposon Mutagenesis for *Chlamydia trachomatis***

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*Chlamydia* is an obligate intracellular pathogen with a biphasic development cycle, both of which pose challenges for genetic manipulation. Reverse genetics is possible with targeted gene inactivation, but advances in forward genetics is lagging. In response to this limitation, efforts have been dedicated to developing a system for transposon mutagenesis in the chlamydial species. Previous attempts in *Chlamydia muridarum* were inefficient. Efforts to apply this system in *Chlamydia trachomatis* utilized an expression plasmid to overcome apparent toxicity of the transposase. This resulted in inefficient transposition that did not support purification of isolates from the complex mutant pool. Additionally, runaway transposition, due to a stably maintained shuttle vector, was an issue. Our study describes a novel approach to enhance transposon mutagenesis efficiency in *C. trachomatis* by reengineering the system. We employ the pKW expression plasmid, which leverages inducible control and fluorescence reporting. Unlike previous attempts, our method allows for curing of the plasmid without the need for antibiotic selection. Additionally, we can implement a high-throughput screening approach to efficiently purify and confirm individual mutants. Ongoing efforts to optimize the system aim to enhance mutagenesis efficiency and streamline the isolation process. The refinement of our transposon mutagenesis system is essential to overcome the existing limitations in chlamydial genetics.

## Zebrafish Models Define the Oncogenic Mechanism of Phosphatase of Regenerating Liver 3

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High expression levels of Phosphatase of Regenerating Liver 3 (PRL-3) correlate with increased metastatic potential and a poor patient prognosis in many cancer types. Despite its prominent implications in metastasis, the mechanism by which PRL-3 drives oncogenesis has never been clearly defined. Recently, PRL-3 has been found to use its phosphatase catalytic site to tightly bind to the CBS-pair Domain Divalent Metal Cation Transport Mediators (CNNM) family of proteins. This interaction promotes the accumulation of intracellular magnesium, though the exact implications of this in cancer progression are still unclear.

To determine whether PRL-3's phosphatase or CNNM binding activity drives its oncogenic function, we utilized a set of PRL-3 mutants deficient in one activity or the other and expressed them in a transgenic zebrafish model of Rhabdomyosarcoma (RMS). While both wild-type PRL-3 and PRL-3 capable of binding CNNM significantly increased tumor size ( $p < 0.0001$ ) and promoted invasive phenotypes, the expression of PRL-3 with only phosphatase activity did not significantly enhance the model compared to the control ( $p = 0.9343$ ). When these same mutant constructs were expressed in human cancer cells, we found that PRL-3's CNNM binding properties could enhance cancer cells' survival *in vitro* when exposed to various stress conditions reminiscent of the tumor microenvironment, while phosphatase-only PRL-3 did not enhance survival ( $p < 0.0039$  and  $> 0.7915$  respectively).

Our results suggest that PRL-3 exerts its oncogenic activity by binding CNNM proteins rather than dephosphorylating cellular substrates. We are further characterizing this interaction to determine how the PRL-mediated increase in magnesium gives cancer cells a survival advantage

## **Functional characterization of the nucleic acid binding activity of PlzA, the borrelial cyclic-di-GMP binding protein**

Nerina Jusufovic\*, Andrew C. Krusenstjerna, Christina R. Savage, Keira Schinaman, Jessamyn P. Morris, Timothy C. Saylor, and Brian Stevenson

The Lyme disease spirochete, *Borrelia burgdorferi*, must integrate environmental cues to properly regulate gene expression and maintain survival during the enzootic life cycle. *B. burgdorferi* has a two-component signaling system which produces the signaling molecule c-di-GMP. Upon binding of this molecule by PlzA, the only universally encoded c-di-GMP binding protein in *B. burgdorferi*, expression of c-di-GMP responsive genes is modulated. PlzA and c-di-GMP are required for *B. burgdorferi* survival in the tick vector and maintaining the enzootic life cycle. Despite the importance of PlzA, the mechanism of this regulator was previously unknown. One set of genes modulated by PlzA/c-di-GMP is those of the glycerol catabolism operon (*glp*), which is important for *B. burgdorferi* survival in the unfed tick. Through electrophoretic mobility shift assays (EMSAs) using nucleic acid sequences derived from the regulatory region of the *glp* operon, we show that PlzA is a c-di-GMP dependent nucleic acid binding protein. PlzA predominantly interacts with the major groove of DNA and prefers longer and AT-rich sequences. Biochemical characterization coupled with computational analyses identified regions in the N-terminal domain as important for PlzA nucleic acid binding. Mutagenesis of several residues in these regions impacted PlzA-DNA binding affinity. *B. burgdorferi* plzA-mutant strains are currently being produced to determine the consequences of aberrant PlzA-nucleic acid binding function on borrelial physiology. The presented work characterizes PlzA nucleic acid binding properties ultimately to better define the PlzA regulon. Our studies will further inform mechanisms by which the Lyme disease pathogen regulates gene expression for infection.

## **Integrin $\alpha 6\beta 4$ Regulates Tryptophan Metabolism through the Kynurenine Pathway in Triple Negative Breast Cancer**

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Integrin  $\alpha 6\beta 4$  is overexpressed in over 80% of triple negative breast cancer (TNBC) cases and contributes to TNBC aggression. The kynurenine pathway, which degrades tryptophan to  $\text{NAD}^+$ , is often upregulated in TNBC and is associated with poorer outcomes. Here, we identify novel kynurenine pathway regulation by integrin  $\alpha 6\beta 4$  and demonstrate its impact on tumor growth in TNBC. We performed RNA sequencing on BT549 cells that stably expressed integrin  $\beta 4$  and empty vector (control) and found that compared to control cells, integrin  $\beta 4$ -expressing cells have significant upregulation of key kynurenine pathway enzymes. When we knocked down integrin  $\beta 4$  in other TNBC cell lines, we found decreased expression of IDO1, the rate limiting enzyme of tryptophan degradation. The positive correlation of integrin  $\beta 4$  and IDO1 expression in TNBC cells is further supported by analysis of the TCGA Breast Invasive Carcinoma dataset. To test if integrin  $\beta 4$  upregulation of IDO1 impacts tumor growth, we treated cells in 3-dimensional (3D) culture with Epcadostat, an IDO1 inhibitor. Upon treatment with Epcadostat, we demonstrate no basal differences in cell viability between our integrin  $\alpha 6\beta 4$  positive and negative lines; however, Epcadostat treatment in our integrin  $\alpha 6\beta 4$ -expressing model decreases invasive growth in 3D culture. Treatment with  $\text{TNF}\alpha$ , a known stimulator of kynurenine production, increases media kynurenine in  $\beta 4$ -expressing cells compared to control. In summary, our data suggest that integrin  $\alpha 6\beta 4$  promotes the kynurenine pathway through transcriptional regulation of IDO1 to facilitate tumor growth, which has significant implications for TNBC metabolism and disease progression.

## Loss of Exonuclease 1-MSH2 Interaction Results in Alteration of Multiple DNA Repair Pathways

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DNA repair is the process by which cells identify and correct mutations present within the genome which helps maintain genomic stability and prevent the development of cancer. The process by which MMR corrects mispairs includes recognition of the mispair by the MutS complex, recruitment of the MutL complex to the mutation site, excision of the mispair, and gap filling by DNA polymerase. Exonuclease 1 (Exo1) is the protein primarily responsible for the excision step of MMR by binding to both the MutS heteroduplex (MSH2-MSH6) and MutL heteroduplex (MLH1-PMS2). The MutL interaction with Exo1 in budding yeast is facilitated by an Mlh1 interaction peptide (MIP) box. We recently identified a Msh2 interaction peptide (SHIP) box in yeast Exo1. We created point mutations within the predicted binding domains of human Exo1. We observe loss of the MMR-mediated apoptotic response when a subset of Exo1-mutations are expressed in the presence of endogenous wildtype Exo1, indicating a potential for a dominant negative interaction. Alterations in Chk1 phosphorylation suggest the Exo1 mutations alter DNA damage response pathways. Yeast Exo1 mutants that cannot bind to MSH2 are moderately sensitive to methyl methanesulfonate (MMS), indicating that the Exo1-MSH2 interaction may also play a role in post-replicative repair. Our ongoing studies are expected to shed more light upon how these interaction motifs influence cross-talk between MMR and other repair pathways in which Exo1 is involved. This study will have important implications for understanding how genome stability is maintained between multiple pathways with overlapping members.

## **Altering ApoE in mice expressing human amylin in the pancreas exacerbates brain amyloid pathology and behavior deficits**

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### **Background:**

The *APOE*  $\epsilon$ 4 allele is the most prominent genetic predisposition for sporadic Alzheimer's disease (AD). Amylin, a neuroendocrine hormone co-secreted with insulin from the pancreas, is increased in blood in AD and readily forms neurotoxic homo- and hetero-oligomers with  $\beta$ -amyloid in AD. Here, we investigated whether mice humanized for amylin and ApoE demonstrate ApoE isoform-specific alterations in cerebrovascular amylin deposition and  $\beta$ -amyloid homeostasis.

### **Methods:**

Mice humanized for ApoE3 or ApoE4 and amylin (ApoE3HIP and ApoE4HIP) and amylin without ApoE expression (ApoE-KO-HIP) were tested for behavior deficits before brain microvessel isolation and amylin/ $\beta$ -amyloid quantification. GFAP-amylin colocalization in the brain was quantified using immunohistochemistry (IHC), double-immunofluorescence was used for amylin-ApoE colocalization, and immunoprecipitation experiments were conducted to confirm brain amylin-ApoE binding interactions.

### **Results:**

ApoE4HIP mice demonstrated worsened behavioral deficits vs. E3HIP mice. IHC of ApoE4HIP and ApoE3HIP brains revealed increased deposits of GFAP and amylin in ApoE4HIP mice. Amylin in brain parenchyma was higher in ApoE4HIP vs. ApoE3HIP mice while ApoE-KO-HIP mice demonstrate reduced amylin in both fractions.  $\beta$ -amyloid 40 levels were elevated in ApoE4HIP brain and microvessels.

### **Conclusions:**

Our data suggest ApoE may function as a transporter of amyloid-forming amylin in the brain with amylin binding ApoE4 stronger than ApoE3. The increased affinity of amylin for ApoE4 coincides with worsened brain amyloid pathology, disrupted  $\beta$ -amyloid homeostasis, increased astrogliosis suggesting neurodegenerative insult, and functional impairments due to elevated amylin amyloid burden. These data may implicate the amylin-ApoE interaction as a mechanism underlying ApoE4-specific neuropathology.

## Haploinsufficiency for the Long isoform of Myosin XVA Leads to Heightened Susceptibility to Cochlear Insults.

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**Background:** Auditory hair cells form precise and sensitive staircase-like actin protrusions called stereocilia bundles, which mediate mechanotransduction and thus hearing itself. In mammals, these hair cells do not regenerate or renew thus they need to maintain their stereocilia bundles for up to several decades. Myosin XVA, an unconventional myosin, plays a vital role as a molecular motor in stereocilia elongation and maintenance, with its short and long isoforms serving these functions, respectively (Fang, et al., 2015). Mice lacking one allele of the long isoform (*Myo15<sup>+/ $\Delta$ N</sup>*) exhibit normal hearing thresholds and typical stereocilia bundles. However, we recently found that they display reduced mechanotransduction (MET) amplitudes and increased open channel probability at early postnatal stages compared to wild-type littermates. This led us to investigate if *Myo15<sup>+/ $\Delta$ N</sup>* mice might be more susceptible to cochlear insults, testing their response to noise exposure or ototoxic drugs.

**Methods:** Organ of Corti explants were isolated from *Myo15<sup>+/ $\Delta$ N</sup>* mice and their wildtype control littermates during the first postnatal week to obtain mechanotransducer (MET) currents (via patch-clamp recordings and fluid-jet bundle deflections) or for 24-hour incubation in a medium supplemented with gentamicin or control conditions (cell survival was assessed via immunofluorescence and confocal microscopy). Auditory brainstem responses (ABRs) were measured in 4-week-old mice, before and up to 3 weeks after exposure to broadband noise at 100 dB SPL for 30 min. Temporal bones from mice at ages ~P50 and ~P100 (with or without previous noise exposure) were isolated and either processed for scanning electron microscopy (SEM), or immunostained and imaged with confocal microscopy.

**Results:** Our noise exposure protocol produced hearing threshold shifts that fully recovered at 8 and 16 kHz but not at 24 and 32 kHz in both *Myo15<sup>+/ $\Delta$ N</sup>* and *Myo15<sup>+/+</sup>* mice. However, the permanent threshold shifts (PTS) were significantly larger (~13 dB) in *Myo15<sup>+/ $\Delta$ N</sup>* mice, indicating that mice lacking just one copy of the long isoform of myosin XVA exhibit an increased sensitivity to permanent noise-induced hearing loss. We are currently processing the temporal bones from these noise-exposed mice to evaluate the integrity of stereocilia bundles and ribbon synapses. Lastly, in vitro exposure of early postnatal organ of Corti explants to gentamicin led to higher rate of hair cell death in heterozygous mice when compared to their wild-type littermates.

**Conclusions:** In humans, mutations on the myosin XVA gene cause the autosomal recessive nonsyndromic deafness DFNB3. Therefore, our data suggests that individuals carrying “recessive” mutations affecting the long isoform of myosin XVA could have increased susceptibility to cochlear insults.

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## **A novel CD27+CD138+ B cell subset localizes to the brain in aged mice**

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Subsets of B lymphocytes arise from the skull bone marrow and mature into antibody-producing cells that are distinct from peripheral populations. However, it is unknown if cells from this region contribute to post-stroke neuroinflammation. Therefore, the objective of this study is to identify B cell populations in the brain that are distinct from peripheral populations and that may derive from the skull bone marrow.

Aged C57BL/6 female mice underwent 30-minute transient middle cerebral artery occlusion on the left side and were sacrificed after 3 weeks. Brains and spleens were analyzed using flow cytometry to identify B cell populations. Subset numbers were analyzed in GraphPad Prism with 2-way ANOVA with multiple comparisons (Benjamini;  $\alpha=0.05$ ).

CD27 and CD23 are markers of mature B cells and CD138 is a marker of plasma cells. Uniform manifold approximation and projection (UMAP) clustering showed a CD19+CD27+CD23+CXCR5+CD138+IgM+ cell population that was significantly higher in the left cortex versus the spleen and other brain regions in uninjured animals (all  $p<0.01$ ). Interestingly, this difference disappeared in injured animals.

A distinct population of CD27+CD138+ activated memory B cells is elevated in the left cerebral cortex at baseline and decreases after ipsilateral tMCAo. It is possible that these cells release antibodies and contribute to neuroinflammation associated with aging. It is unknown if the post-stroke decrease is due to phenotype change or migration out of the brain. Ongoing studies aim to characterize the origins and roles of this unique B cell population.

## Human Myocardium with ATTR Amyloidosis Exhibit Decreased Force Generation and Increased Fibrosis with No Change in Titin Isoform Ratios

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Cardiac amyloidosis is a restrictive cardiomyopathy characterized by the infiltration of abnormally folded protein into the extracellular matrix. The clinical condition is thought to reflect ventricular stiffening and is characterized by diastolic and late-stage systolic dysfunction. Wild type ATTR is an age-related form of amyloidosis that can be diagnosed via a septal myocardial biopsy or non-invasive tc-99 PYP scan. Using samples from five patients with ATTR amyloidosis and five non-failing donors, we measured muscle force production, fibrosis, and titin isoform ratios, both regulators of myocardial stiffness. Additionally, we quantified amyloid deposition and stained for calcium in the myocardium.

Compared to non-failing myocardium, amyloid hearts had significantly decreased force production, increased fibrosis, and microcalcifications which colocalized with amyloid deposits. Titin isoform ratios were unchanged in amyloid myocardium compared to controls and no differences were measured between the right ventricle, septum, and left ventricle in both amyloid and non-failing myocardium. These results provide tissue-level data showing that both end-stage cardiac amyloidosis and non-failing myocardium titin isoform ratios are homogeneous across regions of the heart. Additionally, colocalization of calcium and amyloid deposits provides a potential mechanism underlying the affinity of tc-99 PYP, a bone tracer, for ATTR myocardium. While amyloidosis is often discussed with emphasis on the extracellular matrix, cardiomyocyte death and dysregulation of sarcomeric proteins may result in decreased force production and contribute to disease progression. Additional work on how amyloid deposition impacts the intra- and extracellular space are required to fully understand amyloidosis pathogenesis.

## Glia-selective replacement of *APOE4* with *APOE2*: response to age and inflammation

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### Background

Apolipoprotein E (*APOE*) is the strongest genetic risk factor for late-onset Alzheimer's Disease (AD) and encodes three common isoforms: E2, E3, E4. Compared with E3, E4 dramatically increases while E2 strongly decreases AD risk. In the brain, ApoE is primarily synthesized by astrocytes and microglia, making these two cell types promising targets for ApoE-directed therapeutic approaches. Our lab has generated an inducible *APOE4* to *APOE2* allelic "switch" model (*APOE4s2*) in which we can conditionally replace the risk-associated *APOE4* with the protective *APOE2* allele in a cell-specific manner. To elucidate potential mechanisms by which astrocytes and microglia expressing *APOE2* or *APOE4* may modulate AD risk independent of amyloid or tau pathology, we characterized the inflammatory response of *APOE* glial-cell-specific "switch" mice to an inflammatory challenge.

### Methods

Aged *APOE4s2* mice were administered tamoxifen to induce an in vivo transition from expression of *APOE4* to *APOE2* selectively in astrocytes (*Aldh1l1-CreERT2*) or microglia (*Tmem119-CreERT2*). A separate cohort of astrocyte-specific or microglia-specific "switch" mice received LPS to induce an inflammatory response 24 hours prior to cytokine measurements and immunohistochemical analysis of gliosis (GFAP, IBA1).

### Results

Astrocyte reactivity and cytokine levels are not affected by a cell-specific replacement of *APOE4* with *APOE2*. Interestingly, astrocyte (but *not* microglia) selective replacement with *APOE2* results in differential IBA1+ microglia expression in response to age and LPS exposure compared to mice expressing *APOE4* in all cell types.

### Conclusions

The ability of astrocyte-derived E2 to regulate microglia expression in response to age and inflammation may be a potential mechanism whereby *APOE2* modulates AD risk.

## Assessing Polygenic Risk Score Accuracy for Dementia Risk Stratification in the Alzheimer's Disease Neuroimaging Initiative Dataset

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### Background:

Polygenic Risk Scores (PRS) quantify genetic predisposition to Alzheimer's disease. Their accuracy can be influenced by the choice of genome-wide association studies (GWAS), but the extent of this effect is not well understood. We calculated PRS for 808 individuals using the Polygenic Risk Score Knowledge Base and dementia GWAS from the GWAS Catalog, normalizing results with the UK Biobank and focusing on 17 significant GWAS.

### Results:

The Mann-Whitney U test showed 11 of 17 PRS significantly differentiated cognitive statuses in ADNI ( $P=3.242 \times 10^{-8} - 0.0454$ ; Cohen's  $d=0.002 - 0.422$ ), with averaging PRS matching the best single-study prediction (GWAS ID: GCST009496;  $P=7.50 \times 10^{-8}$ ; Cohen's  $d=0.422$ ). The chi-squared test confirmed 9 of 17 PRS effectively distinguished top quintile cognitive impairment ( $\chi^2 = 4.5290 - 23.9381$ ,  $P=9.949 \times 10^{-7} - 0.03333$ ), with averaged PRS outperforming the best single-study predictions ( $\chi^2 = 8.1154$ ,  $P=0.004389$ ).

### Discussion:

We show that PRS accuracy can vary based on GWAS, potentially leading to data overfitting and misreporting disease risk. To address this, we demonstrate averaging PRS maintains accuracy for AD risk prediction while limiting GWAS bias effects. Our analyses highlight the need for standardizing PRS methodologies and ensuring accuracy assessment on target populations before broad implementation. With PRS increasingly used clinically, ensuring early screening models are accurate and robust to specific deviations is imperative. Averaging scores across studies improves generalizability and maintains accuracy. Refining PRS inclusion criteria could optimize risk prediction.

**Data Availability:** [https://github.com/jmillerlab/PRS\\_Combinations](https://github.com/jmillerlab/PRS_Combinations)

## Addressing sensitivity to insulin and glucose in neurons and astrocytes using PercevalHR

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### Abstract

Brain homeostatic equilibrium is a well-maintained metabolic process. Loss of this homeostasis is linked to brain aging and is often detected as hypometabolism in Alzheimer's Disease (AD). Recently, brain insulin has been identified as an essential component in regulating cognitive function, particularly in the hippocampus, where it has been shown to ameliorate spatial memory recall deficits. Here, we investigated the energetic status of neurons and astrocytes in mixed primary hippocampal cultures using the ATP:ADP nanosensor PercevalHR. Embryonic rat hippocampi (E18) were extracted and maintained for 12-16 days *in vitro*. Cultures were exposed to a PercevalHR lentivirus (Human Ubiquitin C promoter) for determination of ATP:ADP. To correct PercevalHR's pH bias, some experiments were conducted concomitantly with the intracellular pH sensor pHrodo. To normalize glucose transporter function following ~12 days in high glucose concentration (30 mM), we returned the cells to a serum-free 5.5 mM glucose media ~24 h prior to imaging. After imaging an initial baseline, cells were treated with one of several compounds (0.5 mM, 5.5 mM, and 10 mM glucose; 50 mM KCl; 20  $\mu$ M glutamate; 10 nM insulin). Glutamate and KCl exposures resulted in rapid decreases in ATP:ADP. Surprisingly, insulin exposures nor glucose excursions altered ATP:ADP. To validate our findings, ECAR and OCAR assays were performed and do indeed corroborate the lack of insulin response found during imaging. These data evaluate the bioenergetic status in two closely associated cell types that are known to share metabolic intermediates. Ongoing studies are investigating the ATP:ADP of astrocytes *in vivo* using 2P imaging.

## Impact of intestinal microbiota on *Campylobacter jejuni* pathogenesis

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The interactions between *Campylobacter jejuni*, a critical foodborne cause of gastroenteritis, and the intestinal microbiota during infection are not completely understood. The cross-talk between *C. jejuni* and its host is impacted by the gut microbiota, through mechanisms of competitive exclusion, or microbial metabolites. To investigate the gut microbiota's role in *C. jejuni* infection, we utilized a High Protein Diet (HPD) mouse model to increase the relative abundance of intestinal *E. coli*, abrogating C57Bl/6 mouse model's colonization resistance to *C. jejuni*. Upon investigation of microbially produced metabolites, we found Trimethylamine N-Oxide (TMAO) significantly increased in the colonic lumens of HPD mice. To elucidate the effects of TMAO on *C. jejuni* virulence, we looked at chemotaxis, and invasion of *C. jejuni* in Caco-2 cells. We also investigated the responses of Caco-2 monolayers to TMAO, in the context of *C. jejuni* infection. We determined not only that *C. jejuni* senses TMAO as a chemoattractant, but the presence of TMAO increases *C. jejuni* invasion into Caco-2 monolayers. TMAO treatment of Caco-2 cells caused a significant increase of tight junctional proteins occludin and zonula occludens-2, with no changes to the transcriptional levels. These results establish that *C. jejuni* utilizes microbial metabolite TMAO for increased virulence during infection.

## Regulation of Protein Tyrosine Phosphatase Type F by NEDD4L E3 Ligase

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The phosphorylation state of target proteins is tightly regulated by protein kinases and phosphatases. The vast majority of cellular signaling depends on this dynamic relationship for the propagation or termination of signaling cascades and thus maintenance of homeostasis. PTPRF belongs to a family of receptor type protein tyrosine phosphatases. Our previous studies have been shown that PTPRF acts as an oncogenic phosphatase through the upregulation of Wnt signaling in colon cancer. However, the regulation of PTPRF is not well studied. In this study, we show that NEDD4L, a HECT E3 ligase, regulates the stability of PTPRF protein. Specifically, overexpression of NEDD4L decreases the half-life of PTPRF whereas knockdown of NEDD4L has the opposite effect. In addition, wild-type NEDD4L, but not the E3 ligase activity deficient mutant, increases the ubiquitination of PTPRF, indicating that PTPRF is a substrate of NEDD4L. Interestingly, the protein stability of a catalytically inactive mutant PTPRF is higher compared to wild-type PTPRF, suggesting that the phosphatase activity promotes PTPRF degradation. Moreover, by utilizing modified ubiquitin, we demonstrate that NEDD4L preferentially utilizes K29 ubiquitin linkage to modify PTPRF. Functionally, NEDD4L-mediated degradation of PTPRF blocks the ability of PTPRF to enhance Wnt activation. Taken together, this study identifies a previously unidentified regulatory mechanism of PTPRF through NEDD4L mediated ubiquitination.

## **Astrocyte Ca<sup>2+</sup> in the dorsal striatum suppresses neuronal activity to oppose cue-induced reinstatement of cocaine seeking**

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### **Affiliations**

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### **Abstract**

Recent literature supports a prominent role for astrocytes in regulation of drug-seeking behaviors. The dorsal striatum, specifically, is known to play a role in reward processing with neuronal activity that can be influenced by astrocyte Ca<sup>2+</sup>. However, the manner in which Ca<sup>2+</sup> in dorsal striatum astrocytes impacts neuronal signaling after exposure to self-administered cocaine remains unclear. We addressed this question following over-expression of the Ca<sup>2+</sup> extrusion pump, hPMCA2w/b, in dorsal striatum astrocytes and the Ca<sup>2+</sup> indicator, GCaMP6f, in dorsal striatum neurons of rats that were trained to self-administer cocaine. Suppression of astrocyte Ca<sup>2+</sup> by hPMCA2w/b increased acquisition of cocaine seeking. Following extinction of cocaine-seeking behavior, the rats over-expressing hMPCA2w/b also showed a significant increase in cue-induced reinstatement of cocaine seeking. Suppression of astrocyte Ca<sup>2+</sup> increased the amplitude of neuronal Ca<sup>2+</sup> transients in brain slices, but only after cocaine self-administration. This was accompanied by decreased duration of neuronal Ca<sup>2+</sup> events in the cocaine group and no changes in Ca<sup>2+</sup> event frequency. Acute administration of cocaine to brain slices decreased amplitude of neuronal Ca<sup>2+</sup> in both the control and cocaine self-administration groups regardless of hPMCA2w/b expression. These results indicated that astrocyte Ca<sup>2+</sup> control over neuronal Ca<sup>2+</sup> transients was enhanced by cocaine self-administration experience, although sensitivity to acutely applied cocaine remained comparable across all groups. To explore this further, we found that neither the hMPCA2w/b expression nor the cocaine self-administration experience altered regulation of neuronal Ca<sup>2+</sup> events by NPS-2143, a Ca<sup>2+</sup> sensing receptor (CaSR) antagonist, suggesting that plasticity of neuronal signaling after hPMCA2w/b over-expression was unlikely to result from elevated extracellular Ca<sup>2+</sup>. We conclude that astrocyte Ca<sup>2+</sup> in the dorsal striatum impacts neurons via cell-intrinsic mechanisms (e.g. gliotransmission, metabolic coupling, etc.) and that long-term cellular plasticity after cocaine self-administration is expressed, at least partially, as elevation of neuronal Ca<sup>2+</sup> signals. Therefore, one aspect of astrocyte Ca<sup>2+</sup> in the dorsal striatum may be to suppress neuronal activity and promote resistance to cue-induced reinstatement of cocaine seeking.



## Differential Impact of Closed-Head Injury on CA1 and Dentate Gyrus Neuronal Functions in Mice

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**Objective:** This study was designed to evaluate hippocampal neuronal functions, specifically targeting basal synaptic strength, synaptic efficiency, and presynaptic excitability, at 1 week, 3 weeks, and 6 weeks after a closed-head injury (CHI) mouse model of traumatic brain injury (TBI).

**Method:** 4 month old wild-type (C57BL/6) male mice underwent either a sham procedure or CHI to model TBI. We assessed neuronal function within the CA1 and dentate gyrus (DG) regions of the hippocampus at 1 week, 3 weeks, and 6 weeks post-injury using extracellular field potential recordings. Our evaluations focused on measuring basal synaptic strength, synaptic efficiency, and presynaptic excitability via input-output curves.

**Results:** Preliminary findings indicate that CHI mice exhibited alterations in hippocampal neuronal functions compared to sham controls at 6 weeks post-injury. In the CA1, CHI mice demonstrated a decrease in basal synaptic strength and synaptic efficiency with an increased presynaptic excitability. In the DG, while basal synaptic strength and synaptic efficiency remained unchanged, a decrease in presynaptic excitability was observed.

**Conclusion:** The differential effects observed between the CA1 and DG highlight the nuanced vulnerability of hippocampal circuits to injury, suggesting that TBI induces a multifaceted disruption of synaptic homeostasis. These findings not only deepen our understanding of the pathophysiological consequences of TBI but also emphasize the critical need for targeted therapeutic strategies that address the specific neuronal dysfunctions associated with different hippocampal regions.

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## Ovulatory Angiogenic Factors and Receptors are Altered by an Environmentally Relevant Phthalate Mixture in Mouse Granulosa and Endothelial Cells *In Vitro*

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People are ubiquitously exposed to phthalates, which are endocrine-disrupting chemicals known to target the ovary. Phthalate exposure could impair ovulation by altering necessary processes, including ovulatory angiogenesis. Ovulatory angiogenesis involves the granulosa cell production of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>), and members of the vascular endothelial growth factor (VEGF) family, while endothelial cells express prostaglandin E receptors (PTGER1-4), prostaglandin F receptor (PTGFR), and vascular endothelial growth factor receptors (FLT1, KDR, FLT4). We hypothesized that phthalates alter production of angiogenic factors and expression of angiogenic receptors. Mouse granulosa cells were treated with DMSO or an environmentally relevant phthalate mixture (MPTmix). Following one hour of exposure, they received human chorionic gonadotropin (hCG; ovulatory stimulus) before collection at 11hr (n=5, p≤0.05). Mouse ovarian endothelial cells were treated with DMSO or MPTmix (1-500μg/mL) ±VEGFA before collection at 24hr (n=4, p≤0.05). Exposure to hCG+MPTmix decreased granulosa cell production of PGE<sub>2</sub> at doses of 1μg/mL, 10μg/mL, 100μg/mL, and 500μg/mL; PGF<sub>2α</sub> at 10μg/mL, 100μg/mL, and 500μg/mL; increased VEGFA at 500μg/mL; and decreased *Vegfd* expression at 100μg/mL and 500μg/mL compared to hCG controls. Of the receptors, MPTmix treatment increased *Ptger4* expression at 500μg/mL; decreased *Ptgfr* at 10μg/mL; decreased *Flt1* at 1μg/mL and 100μg/mL; decreased *Kdr* at 1μg/mL and 10μg/mL; and decreased *Flt4* at 10μg/mL and 100μg/mL when compared to DMSO. VEGFA restored the MPTmix-induced changes in the expression of angiogenic receptors to control levels. These data suggest a phthalate-induced dysregulation in the communication between granulosa cells and endothelial cells necessary for ovulatory angiogenesis. Supported by R01ES033767.

