**Abstract Program**

*Postdoc Poster Session &*

*Graduate Student Poster Session*

16th Annual Trainee Research Day

Monday March 24, 2025

University of Kentucky Gatton Student Center

Grand Ballrooms



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**Role of glucose-6-phosphate dehydrogenase (G6PD) in platelet function**

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**Introduction:** Glucose-6-phosphate dehydrogenase (G6PD) is a crucial enzyme in the pentose phosphate pathway (PPP), producing NADPH, essential for neutralizing oxidative stress, and pentoses, which contribute to nucleotide synthesis1. G6PD deficiency, the most common human enzymopathy, affects 400 million people globally and is linked to hemolysis due to insufficient NADPH in red blood cells (RBCs)2. Beyond RBCs, emerging evidence suggests G6PD deficiency may impact platelet function, particularly in cardiovascular disease patients over 603. However, its role in platelet activity remains underexplored.

**Aim/Objective:** This study aims to elucidate the effect of G6PD deficiency on platelet function using a G6PD Mediterranean mutation (Med) conditional knock-in mouse model and G6PD knock-out (KO) mice.

**Methods:** We treated mouse platelets with a G6PD inhibitor and evaluated Ca2+ influx. Additionally, we analyzed platelet functionality in G6PD KO mice, measuring platelet count, morphology, Ca2+ influx and clot contraction thrombin stimulation. Tail bleeding times were also assessed. Similar parameters including clot contraction and tail bleeding were evaluated in G6PD Med-mutant mice.

**Results and Discussion:** G6PD inhibition reduced Ca2+ influx in a dose dependent manner while G6PD KO mice showed a reduction in Ca2+ influx, indicating impaired platelet activity. Clot contraction in KO mice was significantly decreased. Tail bleeding time was slightly prolonged in KO mice, indicating reduced platelet activation. Conversely, G6PD Med mutant mice exhibited enhanced platelet activity, with faster clot contraction and reduced bleeding time.

**Conclusion:** G6PD deficiency impairs platelet function, reducing Ca2+ influx and clot contraction, whereas G6PD Med mutation enhances platelet activity, potentially elevating cardiovascular risks.

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**Edaravone protects the hippocampus from brain damage following insulin-induced severe hypoglycemia**

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**Introduction and Objective:** To determine if Edaravone, a free radical scavenger and neuroprotective agent with antioxidant properties, could prevent brain damage following insulin-induced severe hypoglycemia.

**Methods:** 10-week-old Sprague-Dawley rats were divided into three treatment cohorts: 1) euglycemic controls, 2) rats treated with insulin-induced (15U mg/kg) severe hypoglycemia (SH: 10-15mg/dL for 90 minutes), and 3) rats treated with SH followed by once daily with Edaravone (3mg/kg) (SH+EDV). After one week animals were euthanized, perfused and brains extracted. Sections from the hippocampus (40µm) were stained using Fluoro-Jade C (FJC), Iba-1/CD68, Cleaved Caspase 3 (CC3), or 4-Hydroxynonenal (4HNE). Stains were analyzed using ImageJ and one-way ANOVA.

**Results:** As compared to euglycemic controls, severe hypoglycemia increased Iba-1/CD68, CC3, and FJC stained cells as well as 4HNE+ areas (Fig. 1). As compared to SH alone, SH+EDV reduced all stained cells to a level not different from controls.

**Conclusion:** Edaravone protected the brain from severe hypoglycemia induced cell death indicated by FJC and CC3 immunohistochemistry staining. Edaravone also reduced neuronal inflammation indicated by reduced Iba-1/CD68 staining, and reduced oxidative stress as indicated by 4HNE staining. In this rodent model, post-hypoglycemia treatment with Edaravone protected against brain damage.

**O-glycosylation of intrinsically disordered proteins regulates homeostasis of extracytoplasmic proteins in streptococci**

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**Background:** Despite lacking a tertiary structure, intrinsically disordered regions (IDRs) of proteins play a range of functional roles including cell signaling and protein folding in eukaryotes. However, the functions of bacterial IDRs are poorly understood.

**Aims:** To understand the function of IDRs ofextracytoplasmic proteins in the biology of *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Streptococcus mutans*.

**Methods:** In this study, deep learning algorithms were used to predict extracytoplasmic IDRs in the proteome of three human pathogens- *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Streptococcus mutans*. Proteomics analysis, immunoblotting, and biofilm assays were utilized to identify the functions of IDRs.

**Results:** We identify that streptococci possess a subset of proteins harboring long-extracytoplasmic IDRs enriched with serine/threonine residues that are O-glycosylated with N-acetylgalactosamine (GalNAc) by *pgf* operon in *S. mutans*, and α-glucose by GtrB-glycosyltransferase in *S. pyogenes* and *S. pneumoniae*. Peptidyl-prolyl isomerase PrsA and penicillin-binding protein Pbp1A are identified as the major glycoproteins. Furthermore, loss of IDR glycosylation in PrsA resulted in a defect in biofilm formation in *S. mutans*. Biochemical and functional characterization demonstrates that IDR does not affect PrsA stability and is protected with GalNAc from proteolysis by an unknown protease in *S. mutans*. Also, PrsA expression and the degree of glycosylation in *S. mutans* strongly depend on the length of IDR.

**Conclusion:** These data suggest that O-glycosylation of IDRs of extracytoplasmic proteins contributes to streptococcal pathogenesis.

(This work was supported by NIH grants R01 DE028916 from the NIDCR, R21 AI149366 from the NIAID and R35 GM131767 from the NIGMS.)

**Inhibiting human metapneumovirus matrix protein expression with peptide-conjugated phosphorodiamidate morpholino oligomers: impact of reducing the matrix protein on the stages of infection**

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Human metapneumovirus (HMPV) is known to cause severe respiratory tract infections. The HMPV matrix (M) protein is important in infection for its role in virus assembly and budding, which has been well documented. To further characterize the specific roles of HMPV M, we used peptide-conjugated phosphorodiamidate morpholino oligomers (PPMOs) to block translation of M during an infection. We found that varying concentrations of M-targeted PPMOs (PPMOM) led to dose-dependent changes in M expression. Immunofluorescence staining verified that M signal is also reduced in infected cells treated with PPMOM, and demonstrated that loss of M led to a change in nucleoprotein (N) and fusion (F) protein distribution. In the absence of M, F was diffused across the cell periphery with no localization in long filaments extending from the cell body. Fluorescence in-situ hybridization (FISH) probes were used to stain vRNA, which showed that a reduction in M led to an increase in inclusion body (IB) count/cell, but a decrease in average IB volume/cell at 12 and 24 hpi. Surprisingly, qPCR revealed that total and sense-specific vRNA did not change when compared to a negative control PPMO-treated infection. When HMPV-infected cells were treated with PPMOM at 6, 12, and 18 hpi, there was an ~92%, ~49%, and ~21% reduction in viral particle formation by the 24 hpi mark, respectively. Altogether, we show that PPMOs can serve as a tool to examine the importance of M in a viral infection by controlling expression levels and the time of administration.

**Stem Cell mitochondria are transferred to muscle fibers in response to a hypertrophic stimulus**

Jensen Goh

Muscle stem cells (MuSCs) fusion during skeletal muscle hypertrophy has traditionally been studied with an emphasis on nuclear contributions, leaving the fate of other organelles, such as mitochondria, largely unexplored. Recent evidence suggests that activated MuSCs significantly upregulate mitochondrial biogenesis prior to fusion. Thus, this study aimed to determine whether MuSCs transfer their mitochondria to myofibers during hypertrophy.

To address this question, we crossed MuSC-specific CreER mice with inducible mitochondrial Dendra2 reporter mice to label MuSCs mitochondria. Mice were implanted with osmotic pumps for continuous EdU administration, and hypertrophic growth was induced via synergist ablation to apply mechanical overload (MOV) on the plantaris muscle for 3, 7, or 14 days. At the designated time points, the plantaris muscle was carefully excised, sectioned, and analyzed via immunohistochemistry to quantify Dendra2+ myofibers.

Based on the pattern of Dendra2+ fluorescence, we identified three types of Dendra2+ domains within myofibers and observed a progressive increase in Dendra2+ domains over the time course. From the Dendra2+ domain types, we inferred that MuSCs fusion occurred prior to 3 days of MOV and preferentially in type 2A fibers. Dendra2+ labeled mitochondria were consistently localized to MuSCs-derived myonuclei across all Dendra2+ domain types and time points, indicating that fusion is the primary mechanism for mitochondrial transfer.

Quantification of EdU+ myonuclei demonstrated that early fusion events (MOV < 3 days) were division-independent, while proliferating MuSCs contributed primarily to later fusion events. Collectively, this study presents the first evidence that MuSCs mitochondria are transferred to myofibers during fusion in hypertrophy. Moreover, our findings provide a deeper understanding of the temporal dynamics between division-dependent and division-independent MuSCs fusion, highlighting a greater complexity in MuSCs fate than previously recognized.

**The impact of highly effective modulator therapies (HEMTS) on the abcg5 abcg8 sterol transporter**

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**Background**: Cystic Fibrosis (CF) is caused by a genetic mutation in the *CFTR* gene that encodes an ATP-Binding Cassette (ABC) Transporter. CF is treated with HEMTs (Ivacaftor + Tezacaftor + Elexacaftor) to rescue CFTR function. Sitosterolemia is characterized by excess xenosterol accumulation and is caused by mutations in either *ABCG5* or *ABCG8*, an obligate heterodimer that secretes sterols into bile and opposes their absorption in the small intestine. Our goal is to determine if HEMTs can also rescue function of *ABCG5* or *ABCG8* mutants.

**Methods**: Lentiviral Transduction of Human HepG2 hepatocytes creating cells expressing ABCG5 and ABCG8. Cells were treated with HEMTs and levels of each protein determined by immunoblotting. In vivo, mice fed a Western-Type Diet and administered triple HEMTs using allometric dosing by oral gavage for 5 days. Basal bile, feces, plasma, and tissues were collected and analyzed for total G5 and G8, cholesterol, and biliary lipids.

**Results:** HEMTs increased G5 protein levels and induced the formation of an unknown high molecular weight form but had no effect on G8 *in vitro*.Liver weights were increased in the HEMT treated mice compared to controls. Biliary cholesterol, bile acid, and phospholipid concentrations were significantly reduced.

**Conclusion**: HEMTs alter G5 abundance and apparent molecular weight, suggesting a post-translational modification(s), formation of a G5 homodimer, or novel protein-protein interaction. HEMTs interact with hepatic lipid transporters and disrupt biliary lipid secretion.

**Integrin α6β4 Upregulates IDO1 Expression and Decreases IFNγ-Mediated T Cell Growth in ER-Negative Breast Cancer**

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The kynurenine metabolic pathway degrades tryptophan into immunosuppressive metabolites following pro-inflammatory cytokine stimulation and is upregulated in ER-negative (ER(-)) breast cancers, where it is associated with a worse prognosis. The laminin receptor integrin α6β4 is expressed in a majority of hormone receptor negative breast cancers, where it is known to promote an aggressive phenotype by regulating cellular signaling and epigenetics. Here, we seek to identify how integrin α6β4 regulates the IFNγ-mediated induction of the first kynurenine pathway enzyme, IDO1, and its impact on immunosuppression in ER(-) breast cancer. We demonstrate that expression of integrin β4 in ER(-) cells regulates IDO1 expression by altering methylation of the IDO1 promoter and gene body compared to empty vector (EV) control, resulting in increased IDO1 expression determined using high throughput sequencing, qPCR, and immunoblot analyses. Upon IFNγ stimulation, the expression of integrin β4 resulted in a more dramatic upregulation of IDO1 at the mRNA and protein level. Furthermore, integrin α6β4 signaling significantly increased secretion of the immunosuppressive metabolite kynurenine following IFNγ stimulation. Using conditioned media transfer onto Jurkat cells, we show that conditioned media from integrin β4-expressing cells stimulated with IFNγ significantly reduced T cell growth compared to media from IFNγ stimulated EV cells or unstimulated β4 cells. In summary, our data suggest that integrin α6β4 increases IDO1 induction in ER(-) breast cancer cells, amplifies kynurenine secretion, and suppresses T cell growth in the presence of the inflammatory cytokine IFNγ, thus suggesting a novel role of integrin α6β4 in limiting T cell-mediated antitumor immunity.

**Enhancing TIL efficacy in NSCLC through epigenetic reprogramming and computational modeling**

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Funding: T32 CA165990 (DRP), R01 CA237643 (CFB), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources)

Novel therapeutic protocols are desperately needed to treat non-small cell lung cancer (NSCLC), the world’s deadliest cancer. Recently, tumor-infiltrating lymphocyte (TIL) therapy has shown promise as a viable and highly personalized approach. Yet many obstacles remain, including optimizing expansion protocols for better *in vivo* TIL proliferation, enhancing T cell homing and targeting post-infusion, and minimizing IL-2/lymphodepletion side effects. We hypothesize that inhibiting epigenetic enzyme EZH2 will improve TIL expansion and infusion outcomes in NSCLC patients. Additionally, we propose that stochastic modeling of gene signaling can identify and address alternative T cell suppression mechanisms, suggesting novel TIL-combination targets.

In a murine NSCLC model, the EZH2 inhibitor valemetostat(Val) combined with anti-PD1 led to tumor regression, robust IFN-gamma T cell responses, increased MHC expression, pro-T cell cytokine signaling, and enhanced tumor-eliminating myeloid populations. To translate these findings to TILs, we are securing NSCLC samples from our Biospecimen Core, establishing patient-derived tumoroids, and expanding TILs *ex vivo* with/without Val under “young” protocols. At *ex vivo* endpoint TILs will be assessed for Val-driven differences in viability, differentiation, and reactivity via flow cytometry. Complementary bioinformatic and mathematical approaches using publicly available NSCLC datasets will be used to build signaling networks differentiating immunotherapy responders from non-responders that we will use predict new therapeutic targets through phenotype control theory.

Given the refractory nature of advanced NSCLC, improving precision medicine options like TIL therapy is a crucial goal for the field. Our integration of bench science and computational approaches has the potential to deepen understanding and enhance therapeutic responses.

**Liposomal clodronate causes macrophage depletion following severe high-thoracic spinal cord injury** Sajeev Kaur1, Reena Kumari1, Fernanda S. Franca1, JayLa A. Hudson1, Anna Baur3, Amir M Campbell1, Michael Hash1, Warren J. Alilain2, Samir P. Patel1, and John C. Gensel1

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Spinal cord injury (SCI) leads to an intraspinal inflammatory response including infiltrating blood leucocytes. Some of these subsets of immune cells (monocytes) contribute to ongoing tissue degeneration after SCI. Currently, there are no FDA-approved therapies for SCI. One promising therapy, clodronate liposomes (Formumax), depletes monocyte-derived intraspinal macrophages and several independent laboratories have reported therapeutic effects after lower thoracic SCI. The extent to which clodronate liposomes (CL) are effective after severe SCI or higher thoracic (T3) SCI has not been studied. Here, we determined the effectiveness of CL after T3 SCI after two different injury severities. Adult female Wistar rats were subjected to T3 spinal contusion with two different forces 300 kdyn (5s dwell time) and 400 kdyn (5s dwell time). For each severity, injured rats were randomly divided into two groups, one group received 2 ml Clodronate (7mg/ml) on days 1, 3, and 6 post-injury (once a day) through tail vein injections, and the control group received vehicle (2ml saline). Spinal cords were isolated 7dpi and histological assessment was performed CD-68, IBA-1 and CD-11b. The analysis revealed significant decreases in activated macrophage (CD-68) and macrophage/ microglia (IBA-1) accumulation after T3 injury. Ongoing statistical analysis will determine if macrophage accumulation and the magnitude of CL-mediated depletion are injury severity-specific. Identifying the effectiveness of CL across multiple severities is clinically significant.

**Modeling intra-and intermolecular cooperativity between myosin heads using spatially explicit simulations.**

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As for the skeletal, cardiac muscle contraction arises from the cyclical interaction between sarcomeric proteins actin and myosin. Regulation of muscle contraction is primarily driven by the calcium-dependent activation of the actin-containing thin filament. However, an additional mechanism on the thick filament has been discovered in which myosin controls itself through autoinhibitory interaction between the two heads in a dimer. The structural basis of this inactivated, or OFF state is the interactive-heads motif (IHM). This conformation is stabilized by intra-molecular interaction, with one free head blocking the other, and by the interactions with myosin heads in the adjacent crowns. The transition from the IHM toward an active conformation is regulated through phosphorylation of the regulatory light chain (RLC) located below the myosin head. Despite the increasing interest in this transition as a possible modulator of cardiac output, there are still unanswered questions. The effect of RLC phosphorylation on individual head kinetics and inter-head cooperativity has remained largely elusive. Moreover, the discovery that IHM can interact differently with adjacent crowns and myosin-binding protein C depending on its conformation opens the doors to new hypotheses on how heads with different intermolecular interactions may exhibit a different regulation.

In this work, I will use the myofilament spatially explicit model FiberSim, to explore the dynamics between the two heads of a dimer when the RLC is phosphorylated and if this regulation is affected by the different structures of IHM. FiberSim-base simulations represent an excellent tool to reveal molecular mechanisms hard to investigate in vivo.

**Vamp8-dependent platelet secretion drives aneurysm progression: insights from clinical and experimental models**

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Background and Objective: Platelet activation and cargo secretion influence thrombus formation and vascular remodeling, potentially driving aortic aneurysm progression. However, their precise role remains unclear. This study integrates a retrospective clinical analysis of aspirin therapy with an experimental AngII-induced AAA model to evaluate platelet inhibition and VAMP8-mediated secretion in aneurysm pathogenesis.

Methods and Results: A retrospective study (2010–2023) at the University of Kentucky Healthcare used AI-driven natural language processing (NLP) to extract aortic diameters. Cohort 1 included AAA/TAA patients and matched controls for platelet count evaluation, while Cohort 2 analyzed aneurysm growth in patients with serial imaging. Multivariable regression revealed aspirin use was associated with accelerated AAA progression in females with small aneurysms (<50 mm) but had no significant effect in males or TAA patients. Platelets were lower in aneurysm patients but not thrombocytopenic. In an AngII-infused hypercholesterolemic mouse model, platelets accumulated at sites of elastin degradation. Bulk RNA sequencing of washed platelets and aortic tissue showed transcriptomic changes in ECM regulation, inflammation, and platelet signaling, supporting a "platelet-aorta axis." VAMP8 deficiency impaired platelet secretion, delayed thrombosis, and significantly reduced AAA incidence and rupture. Aortic tissue from VAMP8-deficient mice exhibited decreased expression of genes linked to ECM degradation and inflammation.

Conclusion: These findings reveal a critical role for platelet cargo secretion in aneurysm progression and suggest that VAMP8 inhibition protects against AAA. Aspirin therapy's sex-specific effects highlight the need for tailored antiplatelet strategies in aneurysm management.

**Targeting Lipid Metabolism to Improve Efficacy of Braf-Targeted Therapy in Colorectal Cancer**

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Aberrant lipid metabolism is associated with poor prognosis in CRC. FASN, a key enzyme of lipid synthesis, is overexpressed in CRC. BRAFV600E is the mutation occurring about 10-15% of CRC cases. BRAF-targeted therapy is effective, but resistance develops quickly, which is associated with an increase expression of FASN. Therefore, our central hypothesis is that inhibition of lipid metabolism will delay CRC resistance to PLX8394.

**METHODS.** HT29, PT130, and PT24249pt, parental and PLX8394 resistant, CRC cells were utilized. Cell viability, cell invasion, triglyceride assay, Seahorse XF analysis, western blot, confocal microscopy, synergy studies, and colony formation were used to evaluate differences between parental and resistant cells. RNA-seq and lipid analysis used to evaluate changes in gene expression and lipids levels. Combination of PLX8394 and TVB3664 (FASN inhibitor) was tested on cell viability.

**RESULTS.** PLX8394 resistant cells exhibit an increase in cellular proliferation, invasion, lipid metabolism, FASN expression, oxidative phosphorylation, and triglyceride storage. Parental cells show a synergetic effect of PLX8394 and TVB3664 treatment. FASN-knockdown cells exhibited higher sensitivity to PLX8394 as compared to control cells supporting the role of FASN in resistance to PLX8394. Importantly, addition of TVB3664 to PLX8394 treatment postpones the development of resistance to PLX8394 through suppression of cell cycle.

**CONCLUSION.** Collectively, this data shows that combination of PLX8394 with FASN-targeted therapy at treatment initiation reduces BRAFV600E CRC cell proliferation via inhibition of their cell cycle progression. These findings suggest that targeting FASN could enhance the efficacy and delay the development of resistance to PLX8394 in BRAF-mutant CRC.

**2-Photon Imaging of Astrocyte Metabolic Activity in an Awake Mouse Using the Percevalhr and Peredox Nanosensors**

Sophiya Sims

**Background:** By characterizing brain energy dynamics, metabolic processes integral to maintaining and regulating homeostatic equilibrium can be addressed in healthy and diseased states. Indeed, the disruption of key energetic pathways and vascular function are hallmarks of cognitive decline (i.e. Alzheimer’s disease and related dementias). However, there is still currently a paucity of research dedicated to investigating neurometabolic processes *in vivo*. Therefore, here, we used PerecevalHR (ATP:ADP) and Peredox (NADH:NAD+) to assess metabolic status in astrocytes in 5xFAD mice during rest and movement using 2-photon techniques. Concomitantly, during imaging, a fluorescent dextran was used to visualize the vasculature to measure changes in vessel tone.

**Methods:** Craniotomies were performed on all animals included in the study. Animals were either injected with PercevalHR (1 uL or 2 uL; GFAP promoter, AAV2) or Peredox (1 uL; Gfa104 promoter, AAV2). Awake animals were then imaged across excitation wavelengths (790 nm – 975 nm), as appropriate for the respective biosensors. During imaging, measures of sensor fluorescence intensity and animal velocity were captured.

**Results:** Unexpectedly, across 29 mice treated with PercevalHR and 12 mice treated with Peredox, no significant correlations between ambulation and fluorescence intensity signal were detected on either 5xFAD or control mice. Future work will be conducted with Peredox injections in 5xFAD animals to further characterize the sensor in this mouse model.

**Funding:** This project was supported by P01 AG078116.

**A Preclinical Rodent Model to Explore Sepsis-Induced Effects Post-Spinal Cord Injury**

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Sepsis is a major contributor to poor outcomes and increased mortality in spinal cord injury (SCI) patients, exacerbating secondary complications and worsening overall prognosis. Despite its clinical relevance, no experimental model currently exists to investigate long-term sepsis complications in SCI survivors. This study establishes a clinically relevant rodent model of sepsis survivor to address this gap.

Rats were assigned to four groups: Sham, Sepsis, SCI, and SCI+Sepsis. SCI at T10 was created using an Infinite Horizon impactor (200 kDyn), sepsis was induced via intraperitoneal injection of cecal slurry (3mL) 15-min post-SCI. Supportive care, including fluid resuscitation and antibiotics, was administered 8-hours post-injury and continued twice a day for 5-days. Animals were monitored for survival, body weight, cytokine levels, and functional recovery. Locomotor function was assessed using BBB scoring, horizontal ladder tests, and in vivo muscle strength evaluations. ELISA was used to measure cytokine levels in blood and tissues at acute time points, while spinal cord histological analysis was performed at 12-weeks post-injury.

Results demonstrated significant (p>0.05) bacteremia in the SCI+Sepsis group at 6-hours post-induction prior to antibiotic resuscitation, with lowest survival rates. SCI+Sepsis animals exhibited, greater muscle weakness, impaired locomotor recovery compared to SCI alone, alongside splenomegaly, reduced leg skeletal muscle mass, reduced spinal cord tissue sparring, and elevated cytokine levels in blood and spinal cord tissue were also evident.

This experimental model effectively replicates sepsis-induced complications following SCI, offering a valuable platform for investigating underlying mechanisms and developing targeted therapies to enhance long-term outcomes in SCI patients.

**Acknowledgement:** This project was supported by funding from the National Institutes of Health (NIH), including grant 1R21NS128749-01A1 (SP/HS) from the National Institute of Neurological Disorders and Stroke (NINDS) and grant P20 GM148326 from the National Institute of General Medical Sciences (NIGMS), U.S. Department of Health and Human Services.

**Microglial HIF1a is Necessary to Restrict Demyelination and Neuroinflammatory Sequelae in a Mouse Model of White Matter Degeneration**

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Though white matter (WM) degeneration is commonly associated with multiple sclerosis, it is also present amongst a variety of aging-related neurodegenerative disorders, suggesting convergent mechanisms. Common to white matter degeneration are reactive subsets of microglia enriched for HIF1a expression, a master regulator of glycolytic metabolism. Our goal was to examine whether HIF1a plays a direct role in the outcomes of cuprizone-induced demyelination. We generated microglia-specific conditional knockouts of HIF1a via *Tmem119CreERT2+Hif1*a*FL/FL* (cKO) and their wildtype littermates: *Tmem119CreERT2negHif1*a*F/F*(wildtype; ‘WT’). Mice were placed on two cuprizone paradigms to examine both demyelination and remyelination pathophysiological sequelae. Brain tissue was harvested to examine histopathology, single-cell RNAsequencing, and spatial transcriptomics. Our findings demonstrate that cKO mice exhibited enhanced significant deficits in MBP staining, increased microglial reactivity (CD68, Iba1, Clec7a), which was carried over into our findings in the remyelination paradigm. scRNAseq revealed exacerbated disease-reactive subtypes of microglia in cKO mice, relative to WT. Using a novel 480 gene panel in our Xenium spatial transcriptomic workflow, we demonstrate the accumulation of subsets of microglia demonstrating varying heterogeneity across anatomical locales. We observed significant accumulation of disease-reactive microglia subsets in response to cuprizone, and our cKO mice again showed marked accumulation of two disease-responsive subsets, compared to WT mice. Taken together, our findings are the first to demonstrate that HIF1a is a necessary restraint on microglial over-activation in response to cuprizone-mediated demyelination and its absence drives exacerbated pathological outcomes. These findings point toward targeting microglial glycolytic intermediates as a potential therapeutic to combat against WM degeneration.

**A diagnostic rubric to differentiate LATE-NC Stage 3 from FTLD-TDP**

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Limbic-predominant age-related TDP-43 encephalopathy neuropathologic change (LATE-NC) affects >30% of autopsied individuals in advanced old age. Medial temporal lobe structures tend to be particularly vulnerable to TDP-43 proteinopathy in LATE-NC. However, in LATE-NC Stage 3, TDP-43 proteinopathy is present in the middle frontal gyrus (MFG), thus posing a potential diagnostic challenge in distinguishing this entity from frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP). LATE-NC Stage 3 and FTLD-TDP are clinically distinct, yet a diagnostic rubric is required to differentiate between these entities based on pathology.

We examined LATE-NC Stage 3 cases from the University of Kentucky and Mayo Clinic and the University of California Irvine. Pathologic features of LATE-NC Stage 3 were compared with those of FTLD-TDP and other TDP-43 pathologic structures. Digital pathology and computational tools were used to quantify pathology burden and complemented by neuropathologic examinations to evaluate qualitative features such as FTLD-TDP types as well as subtypes of neuronal cytoplasmic inclusions (NCIs).

Focusing on TDP-43 proteinopathy in the MFG and using digital pathology quantification or a previously described manual counting method, most cases could readily be classified as either LATE-NC Stage 3 or FTLD-TDP. However, a minority of brains with pathologic features that were challenging to assign, including a subset of FTLD-TDP Type B cases with relatively subtle MFG TDP-43 pathology and another non-LATE-NC, non-FTLD-TDP pathologic entity with extensive MFG pathology.

Using an updated, data-driven diagnostic rubric, cases of LATE-NC Stage 3 was distinguishable from FTLD-TDP, addressing key diagnostic pitfalls.

**Differential Impact of Closed-Head Injury on CA1 and Dentate Gyrus Synapse 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.

**Acknowledgements:** Support for this study was provided by the NIH T32AG078110, R01NS120882, and R01NS103785.

**Chronic Perfluorooctanesulfonic Acid Exposure Promotes Proliferation of Colorectal Cancer Cells**

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Perfluorooctanesulfonic acid (PFOS) is a “forever chemical” frequently detected in drinking water leading to its high absorption through the gastrointestinal tract.Recent studies demonstrate that PFOS exposures promote intestinal inflammation and gut barrier dysfunction. However, how a long-term PFOS exposure affects colorectal cancer (CRC) progression remains elusive. Therefore, the purpose of this study is to delineate the effect of PFOS exposure on CRC cell proliferation and to test the potential mitigation strategy for the harmful effects of PFOS. SW480 and HCT116 cells have been treated with 1 ug/mL PFOS for 1 month, 2 months, and 3 months followed by a Presto Blue Cell Viability Reagent fluorescence assay to measure proliferation. Proliferation markers were assessed by qPCR and Western blot and a colony formation assay was performed. We show that chronic, low-dose PFOS exposure promotes proliferation of SW480 and HCT116 cell lines starting at 3 months since the first exposure. The increase in proliferation is associated with upregulation of Cyclin D and reduction of DEFA5. We also show that PFOS promotes alterations in lipid metabolism via overexpression of FASN and CD36. Our studies suggest that chronic PFOS exposure promotes proliferation of CRC cells by increasing pro-carcinogenic gene expression and signaling and by reducing expression of DEFA5. Furthermore, our findings suggest that PFOS promotes alterations in lipid metabolism associated with CRC progression and worse disease prognosis. Our studies warrant further investigation of the mechanisms behind the effect of PFOS exposure on cancer progression.

**Recombinant TRIM72/MG53 Protein Enhances Plasma Membrane Repair and Reduces Neurotoxicity in Models of Alzheimer’s Disease**

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Amyloid beta (Aβ) is one of the earliest hallmarks in Alzheimer’s Disease (AD) that has been shown to localize with the plasma membrane, decrease membrane integrity, elevate intracellular calcium concentrations, increase oxidative stress and directly induce membrane damage. Compensation for such damage to the plasma membrane requires a robust repair mechanism to avoid cell death. Our recent published studies demonstrated a plasma membrane repair defect in neurons within the APP/PS1 mouse brain, which overexpress Aβ, and with the application of AD patient cerebrospinal fluid (CSF) samples to primary neurons. *Here we tested if enhancing membrane repair with recombinant TRIM72/MG53 (rhMG53) protein could reduce neurotoxicity and cell death.* To analyze the effect of rhMG53 on membrane repair, we exposed 6-month C57Bl/6 and APP/PS1 mouse brain slices to 1µM rhMG53 and conducted the laser damage assay where a two-photon laser is used to ablate a portion of the plasma membrane in the presence of FM4-64, and dye entry was used as a proxy for repair capacity. We repeated this assay on primary neurons treated with rhMG53 and recombinant Aβ or AD patient CSF. Lastly, we measured cell death by propidium iodide staining, intracellular calcium by Fluo-4 staining, and oxidative stress by CellROX staining in the presence and absence of rhMG53. Our results demonstrated a significant increase in membrane repair capacity in APP/PS1 brain slices treated with rhMG53 compared to untreated slices, and no significant difference between C57Bl/6 control slices indicating a rescue to baseline repair kinetics (**Fig. 1**). Furthermore, we observed a significant increase in membrane repair capacity when primary neurons were treated with rhMG53 in conjunction with Aβ or AD CSF (**Fig. 2**). Lastly, we observed a significant decrease in cell death and neurotoxicity markers when treated with Aβ and rhMG53 compared to Aβ treated cells (**Fig. 3**). These results indicate using rhMG53 to enhance plasma membrane repair capacity can compensate for downstream neurotoxicity in AD neurons induced by Aβ. Our future studies will focus on treating AD mouse models with rhMG53 to determine if the protein can enhance spatial learning and memory and other aspects of AD pathology.

**SETDB1 Promotes Prostate Cancer Progression Through Transcriptionally Silencing RHOB**

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Prostate cancer (PCa) is the most common cancer diagnosed in male and the second cancer-related death in the United States. Although patients response to the androgen deprivation therapy (ADT) initially, here are still a large proportion of patients develop more aggressive PCa. The cause of PCa progression has remained elusive. In addition to genetic alterations, epigenetic aberrations such as histone modification also play a pivotal role. SETDB1 is a well-known protein lysine methyltransferase which can methylate histone H3 at lysine 9 to transcriptionally silence expression of its target gene. SETDB1 is implicated as an oncogene in various cancers including prostate cancer, its overexpression predicts poor prognosis. But the underlying mechanism has not been well explored. To elucidate the mechanism of SETDB1 promoting PCa progression, we performed ChIP-seq and RNA-seq in PCa cell line, results identified Ras homolog family member B (RHOB), a tumor suppressor, is the epigenetic repressed target of SETDB1. Previous studies showed that downregulated expression of RHOB has often been detected in many human cancers, which lead to uncontrolled tumor progression. But the role of RHOB in prostate cancer is still ambiguous and the exact mechanism remains unexplored. Our RNA-seq result showed RHOB negatively regulates epithelia-mesenchymal transition (EMT). Our central hypothesis is SETDB1 silences RHOB expression through its methyltransferase activity to activates EMT process, eventually contributing to PCa progression. The goal of this study is to identify a therapeutic approach to abolish SETDB1 epigenetic function and restore RHOB expression in PCa.

**Keywords:** SETDB1, RHOB, EMT, prostate cancer

**Single-cell RNA sequencing identifies distinct visceral adipose tissue-resident γδ T cell subsets with age-dependent shifts**

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A hallmark of age-associated pathologies includes chronic low-grade inflammation, exacerbated by adipose tissue dysfunction. Recently, we demonstrated that γδ T cells increase in visceral adipose tissue (VAT) due to aging and contribute to local and systematic inflammation. To further understand the subsets of γδ T cells contributing to this phenotype, we isolated VAT-resident γδ T cells from young (4-5 m.o.) and aged (22 m.o.) mice for single-cell RNA sequencing and V(D)J profiling. From unbiased clustering, 8 clusters of γδ T cells were identified, of which one cluster (C) corresponded to γδT1-type cells, expressing *Ifng* and *Cd27*. The other 7 clusters were similar to γδT17-type cells, characterized by *Blk*, *Maf*, *Sox13*, *Rorc*, *Cxcr6*, and *Zbtb16* expression; however, expression of *Il17a* was limited to only 2 clusters (C4 and C1). Further, cells in C4 and C6 decreased in frequency by aging while cells in C0 and C7 increased in frequency compared to young mice. Alongside upregulated genes related to inflammatory pathways, C7 is distinguished by expression of *Stat1*, *Ifi47*, *Igtp*, and *Tgtp1*, yet shared many similarities with C4; thus, we propose that a phenotype shift from C4 to C7 occurs with age. Pathway analyses of genes significantly changed by aging across clusters indicated upregulation of pathways related to stress response, cell differentiation, proliferation, and γδ T cell activation, and downregulation of pathways related to apoptosis. Collectively, these data highlight distinct phenotypic differences among γδ T cells in VAT, providing insight into the role of γδ T cells in age-related adipose tissue dysfunction.

**Asprosin is a hypertensive adipokine**

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Hypertension is a major risk factor for cardiovascular disease and is closely linked to metabolic syndrome (MS) through complex and multifactorial mechanisms. Asprosin, a recently discovered adipokine, is positively correlated with several metabolic disorders, including obesity, type 2 diabetes, fatty liver disease, and cardiovascular conditions such as coronary artery disease and hypertension. Herein, we identified blood pressure (BP) modulation as a novel neural function of asprosin. Previously, we demonstrated that asprosin regulates appetite through Ptprd (Protein Tyrosine Phosphatase Delta) signaling in hypothalamic AgRP neurons and thirst through Ptprd signaling in cerebellar Purkinje neurons. In this study, we revealed that asprosin also engages Ptprd in oxytocinergic neurons to modulate BP. Asprosin-deficient mice (a model of human Neonatal Progeroid Syndrome, NPS) and mice with oxytocin neuron-specific Ptprd deletion exhibited significantly lower BP compared to age- and sex-matched littermate controls. Notably, these mice maintained normal appetite, water intake, energy expenditure, activity levels, and respiratory exchange ratio, indicating that asprosin’s BP-modulatory effects occur independently of its metabolic functions. Furthermore, oxytocin neuron-specific Ptprd knockout mice displayed hyperosmolar urine and increased renin-angiotensin-aldosterone system (RAAS) activity, suggesting a compensatory peripheral response to neurogenic hypotension. Mechanistically, asprosin treatment significantly attenuated oxytocin neuron firing and resting membrane potential, while Ptprd deletion in oxytocin neurons led to increased c-Fos expression, indicative of heightened neuronal activation. Overall, this study establishes asprosin as a key regulator of BP via oxytocinergic Ptprd signaling, providing novel insights into neurogenic hypertension and potential therapeutic strategies for its treatment.

**Uncovering mRNA Modification-Dependent Dysregulation in Alzheimer’s Disease: A Comparative Epitranscriptomic Analysis of Post-Mortem Human Brain Tissue**

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**Abstract (<250 words):**
Alzheimer’s disease (AD) is the most common neurodegenerative disorder in aging, currently affecting approximately 6.9 million Americans. Despite extensive efforts, the transcriptomic mechanisms driving AD progression remain incompletely understood. Although numerous high-risk loci for AD have been identified, most lack well-characterized functional mutations. Moreover, these genes frequently produce multiple isoforms that can encode distinct protein variants, complicating our understanding of their contributions to AD. Emerging evidence suggests that post-transcriptional RNA modifications—particularly pseudouridine (Ψ)—may act as key regulators of neuronal integrity, yet conventional RNA sequencing techniques are ill-suited to detect Ψ reliably, limiting crucial insights into its potential role in AD pathology. To address this gap, we employed long-read direct RNA sequencing on dorsolateral prefrontal cortex samples, allowing direct detection of Ψ and precise mapping of isoform-specific modifications. Preliminary analyses uncovered unique Ψ sites within protein-coding regions of genes implicated in neurological disorders, including AD, suggesting that these modifications could impact neuronal function and protein expression in ways relevant to AD progression. By illuminating a novel layer of transcriptomic regulation, this research underscores the potential of targeting Ψ-related pathways as a new frontier in therapeutic development. Harnessing these insights may ultimately bolster our capacity to combat AD and improve patient outcomes.

**The Impact of Proteolytic Modifications on Lipoprotein Metabolism and Atherosclerosis**

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**Introduction:** LDL (low-density lipoprotein) and HDL (high-density lipoprotein) are clinical biomarkers used for predicting risk of atherosclerotic CVD. These lipoproteins are susceptible to posttranslational modifications that can affect their atherogenicity. For example, neutrophil elastase cleaves isolated LDL and HDL *in vitro*, possibly promoting plaque development. However, the impact of elastase on lipoproteins in plasma has not been reported. Furthermore, in human plasma LDL and HDL bind the elastase inhibitor Alpha-1-antitrypsin (AAT) suggesting a possible physiological importance of protecting lipoproteins from elastolytic modification.

**Objectives**: 1) Characterize the effect of elastase-mediated proteolysis on lipoproteins in plasma *in vitro*. 2) Use the recently-developed *Serpina1-/-* (AAT-deficient) mouse model to determine the impact of AATD on lipoprotein metabolism and atherosclerosis risk *in vivo*.

**Results:** Human neutrophil elastase (HNE) treatment of whole plasma *in vitro* revealed dose-dependent degradation of apolipoproteins in plasma from wild type and *Serpina1-/-* mice, with *Serpina1-/-* plasma proteins demonstrating more susceptibility to HNE treatment. HNE was then injected *in vivo* into *Serpina1-/-* mice and a similar cleavage pattern was observed. Lipoprotein profile analysis in *Serpina1-/-* mice revealed an altered lipoprotein profile characterized by increased HDL-cholesterol. Administration of an HDL-targeting AAT-mimetic peptide to *Serpina1-/-* mice partially corrected this dyslipidemia, suggesting that alterations in circulating lipoproteins are caused by elevated circulating elastase activity in this strain. Atherosclerosis studies are currently underway to determine the effect of AATD on atherosclerotic plaque burden. Understanding how proteolytic modifications may influence lipoprotein metabolism and influence CVD risk could offer insight into new biomarkers or therapeutic approaches.

**Exploring Astrocytic Insulin Signaling as a Modulator of Cerebrovascular Integrity in Alzheimer’s Disease**

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Vascular dysfunction is increasingly recognized as a critical feature of Alzheimer’s disease (AD) pathology leading to vascular contributions to cognitive impairment and dementia (VCID). However, the mechanisms underlying dysregulation in vascular elements, including upstream cellular contributors and signaling pathways, remain poorly understood. Emerging evidence suggests that astrocytic insulin signaling may play a key role in modulating vascular function, yet its impact on cerebrovascular integrity has not been fully explored. This project aims to elucidate how astrocytic insulin receptor (IR) overexpression (OE) affects vascular dynamics and contributes to neurovascular coupling by leveraging advanced imaging and proteomic approaches to uncover novel mechanisms. In this study, we overexpressed a truncated human IR beta-subunit (hIR-beta) with constitutive activity, in somatosensory astrocytes of 5XFAD and control mice and assessed the impact on gait performance and vascular coupling. We quantified ambulation and various clinically-relevant gait parameters, including deviation from center, stride time and length deviation, average stride length, and paw placement, comparing hIR-beta OE across age and sex. Our preliminary data revealed significant vascular dysfunction in hIR-beta OE mice, including altered vascular morphology, and reduced vessel and astrocytic density. Longitudinal two-photon imaging demonstrated progressive vascular remodeling following hIR-beta OE, while behavioral analyses indicated improved gait performance in 5xFAD mice. We are conducting ongoing investigations into IR signaling pathways, including pAKT, AKT, IRS-1, and IGF-1R, using western blot analysis in astrocyte-enriched S1 tissue from control and 5xFAD mice following hIR-beta OE. These findings suggest that astrocytic insulin signaling disruption contributes to cerebrovascular dynamic and gait performance. The distinct patterns of astrocytic activation in control versus amyloidosis model highlight a potentially novel mechanism linking insulin dysregulation to neurovascular pathology. To further validate the changes of cerebrovascular trees, we will extend our investigations to Cxcl12-GFP mice and access detailed morphological alterations associated with astrocytic insulin signaling dysfunction.

This project is supported by P01 AG078116.

**Investigating Extracellular Vesicles as Biomarkers for Neuronal Damage in Glioblastoma Patients**

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Extracellular vesicles (EVs) are nanoparticles released by most cells. Their cargo depends on the biochemical characteristics of their cell of origin. We previously demonstrated that EVs can be an early indicator of neuronal injury after radiation therapy (RT) in mice. Glioblastoma (GBM) remains an incurable cancer, with surgery, chemotherapy and RT as the standard treatment. We found that GBM patients exhibit higher numbers of EVs. Given that EVs can function as biomarkers and GBM patients experience severe cognitive impairments, we seek to elucidate the **role of GBM-derived EVs in cognitive decline**. We characterized EVs collected from GBM patients. Size and concentration were assessed with ZetaView Nanoparticle Tracking Analysis (NTA) while morphology was assessed with Transmission electron Microscopy (TEM). Then, we developed a murine orthotopic GBM model. EVs collected from tumor mice were significantly higher than their healthy counterparts. To further understand the function of EVs in cognition, we focused on EVs released by GBM after RT. These EVs contain high levels of 4HNE-adducted proteins as confirmed by western blotting and TEM with immunogold labeling. Then, we injected GBM-derived EVs intracranially in immunocompetent mice, which exhibited cognitive decline, DNA damage in cerebral tissue, reduced neuron markers and increased pro-inflammatory cytokines. *In vitro* studies suggest that GBM-derived EVs can activate microglia and cause neurotoxicity via H2O2 release. We conclude that **GBM-derived EVs induce microglia-mediated neuronal damage and cognitive impairment**.

**Adipocyte-Specific Mineralocorticoid Receptor Deletion Improves Obesity-Induced Glucose Intolerance in a Sex-Specific Manner**

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Obesity is a state of overnutrition characterized by increased body mass index. The mineralocorticoid receptor (MR) is a ubiquitous nuclear receptor that mediates metabolic effects of aldosterone and corticosterone. We developed an inducible adipocyte-specific MR knock-out (MRKO) mouse model to investigate the temporal effects of MR deletion on obesity in vivo. Five-week-old MRKO mice and control littermates, were placed on high-fat diet (HFD, 60% kcal/fat). After 9 weeks, tamoxifen was administered (40mg/kg) to induce MR deletion. Body weight, composition (EchoMRI), and glucose tolerance were assessed before and after induction. Fasted mice were euthanized, plasma collected, and tissues flash frozen. RNA was extracted from adipose tissue, and insulin-related genes were assessed via qRT-PCR. Plasma aldosterone, FGF21, and insulin were measured via ELISA.

Across the study, body weight and composition were similar between MRKO and control mice. After gene deletion, glucose tolerance was improved in male MRKO mice (62688±4028 vs. 47520±1715; p=0.01). In addition, plasma FGF21 was increased while insulin was reduced, showing a significant negative correlation (R2 = 0.91 vs. 0.08, respectively). MR deletion increased the expression of Insulin receptor (1.03±0.12 vs 1.88±0.25 ddCT2; p=0.02), insulin receptor substrate 1 (1.04±0.16 vs. 1.89±0.27 ddCT2; p=0.03), glucose transporter type 4 (1.23±0.37 vs 2.81±0.48 ddCT2; p=0.03), and β-klotho (1.19±0.34 vs 3.64±0.93 ddCT2; p=0.04). In female mice, however, MR deletion did not influence adiposity, glucose homeostasis, or adipose tissue gene expression. Thus, this study is first to report that adipocyte MR could negatively regulate glucose homeostasis by reducing FGF21-dependent insulin signaling in a sex-specific manner.

**Ficolin-2 High Consumption is a Marker of Ischemic Stroke Caused by Large-artery Atherosclerosis**

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Atherosclerotic plaque vulnerability is challenging to identify despite specific morphological features. We hypothesized that ficolin-2, a complement system component, drives plaque vulnerability, leading to erosion, thromboembolic complications, and increased stroke risk. We investigated (1) the relationship between ficolin-2 and asymptomatic plaque morphology and (2) ficolin-2 kinetics in acute ischemic stroke (AIS) patients to identify changes associated with large-artery atherosclerosis (LAA).

A prospective cohort of 68 patients (75.1±7.9 years) undergoing carotid endarterectomy for asymptomatic stenosis was analyzed for ficolin-2 levels in circulation and plaques. A second cohort of 301 AIS patients (68.6±16 years) receiving mechanical thrombectomy (HIBISCUS-STROKE) was assessed for circulating ficolin-2 at admission (H0), H6, H24, H48, and M3 via ELISA.

In the first cohort, patients with histologically vulnerable plaques had higher blood ficolin2 levels (5583±4536 vs. 3895 ± 2017 ng/mL). In plaques, <10% of ficolin-2 pixels colocalized with MASP-2, and C4d presence was unaffected by ficolin-2 levels. In the HIBISCUS-STROKE cohort, ficolin-2 levels decreased from H0 (5620 ± 2006 ng/mL) to H6 (4821 ± 1936 ng/mL), H24 (4928 ± 1811 ng/mL), and H48 (5182 ± 1851 ng/mL), indicating early consumption. LAA patients (14%) had lower ficolin-2 at H6 (3810 ± 1775 ng/mL) versus other stroke subtypes (4971 ± 1917 ng/mL). Low H6 ficolin-2 was independently associated with LAA (OR 5.05 [2.29-11.8]).

Ficolin-2 circulating levels anticipate the risk of stroke in atherosclerotic patients, standing as a new biomarker helping to develop stroke prevention as well as a marker of stroke etiology, able to inform on the tailored treatment of patients.

**Assessing the Performance of Polygenic Risk Score Methodologies in the Alzheimer’s Disease Sequencing Project Datasets**

Hady Sabra, Leah Moylan, Blake Byer, Mark Ebbert, Yuriko Katsumata, David Fardo, Justin Miller

Alzheimer’s disease (AD) is a complex neurodegenerative disorder with a significant genetic component spanning at least 75 distinct genetic loci. However, most clinical genetic tests for AD rely solely on the apolipoprotein E (*APOE*) genotype, the largest known genetic association with AD, despite extensive genome-wide association studies (GWAS) identifying additional common genetic risk factors. While *APOE* ε4 is the strongest known genetic risk factor, it accounts for only a fraction of AD heritability, and many individuals with neuropathologic AD lack the *APOE* ε4 allele. Given that AD is 60-80% heritable, it is an ideal candidate for evaluating Polygenic Risk Scores (PRS)—a method that aggregates the effects of multiple genetic variants—to determine the extent to which PRS can improve genetic risk stratification beyond *APOE* alone.

We assessed the performance of several PRS approaches for AD genetic risk stratification using whole-genome sequencing (WGS) data from over 30,296 individuals (17819 controls and 12477 cases) in the Alzheimer’s Disease Sequencing Project (ADSP) release 4, which includes 37 distinct cohorts. We further evaluated PRS accuracy across 6 population groups and determined how PRS performance varies between populations and cohorts.

To calculate PRS, we employed several GWAS, including those from Bellenguez, Kunkle, and Jansen, in combination with multiple PRS methods: traditional Clumping and Thresholding (C+T) approaches (e.g., PRSice2, PLINK) and advanced Bayesian methods (e.g., PRS-CS, PRS-CSx). We accounted for the *APOE* genotype by including it as a separate covariate in a logistic regression model, assessing its impact on the association between PRS and AD risk.

Among all tested methods, the C+T method using PRSice2 with the Bellenguez GWAS achieved the highest overall predictive accuracy, yielding an average AUC of 0.59 (AUC range: 0.49-0.701). Notably, the PRS exhibited a strong and significant association with AD risk (β coefficient = 0.2, p-value = 6.62 × 10⁻31), confirming that as PRS increases, so does the likelihood of AD. To further investigate the impact of *APOE* on AD risk independent of PRS, we incorporated the *APOE* genotype as a covariate, alongside sex and race, in a logistic regression model. This adjustment significantly increased the overall AUC from 0.59 to 0.74, highlighting that while PRS alone has limited predictive power, PRS using current techniques combined with *APOE* genotyping can account for the majority of genetic predisposition for AD.

Our findings highlight that while *APOE* continues to be the most influential single genetic factor, a higher PRS is associated with increased AD risk and can improve genetic risk modeling beyond *APOE* genotyping alone. We highlight a large range in predictive accuracy across the 37 ADSP datasets, indicating that PRS assessments performed on a single cohort may be misleading and overfit to that dataset. Future work should focus on improving PRS generalizability across cohorts, ultimately paving the way for more inclusive genomic risk assessments for Alzheimer’s disease.

**Establishing a Zebrafish Patient-Derived Xenograft Model for Pediatric Sarcomas**

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**Abstract:**

Rhabdomyosarcoma (RMS) is a high-risk pediatric sarcoma with poor prognosis, especially in metastatic or recurrent cases. Patient-derived xenograft (PDX) models in zebrafish offer rapid and cost-effective alternatives for preclinical drug screening. This study evaluates the feasibility of RMS xenografts in zebrafish larvae and assesses chemotherapy responses in vitro at 34°C, a temperature tolerated by zebrafish, before transitioning to in vivo testing. To establish the model, xenotransplantation methods were optimized using cultured RD cells, identifying the dorsal perivitelline space (PVS) as optimal due to its avascular nature, improved tumor retention, and injection ease. RD cells injected into zebrafish larvae at 48 hours post-fertilization demonstrated an engraftment rate of 61% at 24 hours post-injection, with larvae viable for up to four days post-injection. Chemotherapeutic agents tested included dactinomycin (DAC), vincristine (VIN), and cyclophosphamide (CYC) at 34°C compared to standard 37°C conditions. DAC showed a dose-response curve with IC50 values of 4.3 nM (37°C) and 3.5 nM (34°C), though with greater variability at lower temperatures. VIN displayed considerable variability between temperatures, whereas CYC showed no cytotoxic effects at any tested concentration. Zebrafish toxicity assays confirmed resistance to CYC and significant lethality with DAC and VIN only above 300 nM. These results support zebrafish RMS xenografts as a functional in vivo model. Future studies will expand this approach using patient-derived primary tumor cells, assessing drug responsiveness in vivo to enhance the clinical relevance of this preclinical testing model.

**Leveraging Mitochondrial Metabolic and Energetic Differences to Target Prostate Cancer Regrowth Post Radiotherapy**

 Kahleel Guerrier

Prostate Cancer (PCa) is the second leading carcinoma in men with targeted radiation treatment (RT) being one of the primary treatment options. However, post-radiotherapy recurrence and resistance (radioresistant PCa, RR-PCa) remain significant challenges. Our human biopsy data suggests that PCa may increase mitochondrial quantity through mitochondrial biogenesis (mito-biogenesis) to facilitate re-population after RT. To investigate this, we developed RR-PCa cells (RR-RM-1) from a mouse PCa line (RM-1), capable of forming allograph tumors in immunocompetent mice. RR-RM-1 cells demonstrated lower sensitivity to RT, altered morphology, increased glucose dependency, and higher mitochondrial quantity and mass compared to parental RM-1 cells. We also developed human RR-PCa (RR-PC3) and conducted Stable Isotope Resolved Metabolomics (SIRM) analysis. The results revealed that RR-PC3 cells utilize 13C-glucose through the Krebs cycle, excrete 13C-lactate, with a potential activation of glutaminogenesis, suggesting altered metabolic pathways associated with upregulated mito-biogenesis. Notably, RR-PC3- and RR-RM-1-derived 3D spheroids showed enhanced growth under 1% O2, highlighting the need for future SIRM analysis to clarify metabolite utilization in hypoxia. To overcome RR-PCa, we propose targeting their mitochondrial function and impairing mito-biogenesis by overloading mitochondrial reactive oxygen species (mtROS). The FDA approved antibiotic, Azithromycin (AZM) has been shown to increase mtROS and inhibit mitochondrial protein translation. Using the Seahorse Mito-Stress Test, AZM significantly inhibited basal, maximal, and ATP-linked respiration in RR-PC3 and RR-RM-1 cells, correlating with decreased in their cell viability. AZM also reduced the size of 3D spheroids by inducing cell death and dissociating 3D spheroids, suggesting a novel mechanism for mtROS-induced cell death. These effects were further enhanced when combined with RT, even under 1% O 2. Another mtROS producing antibiotic that acts as peroxiredoxin-3 inhibitor, Thiostrepton, also demonstrates a similar effect. Overall, our findings indicate that modulating mtROS and metabolism can disturb RR-PCa’s ability to regrow after RT, even under hypoxic conditions.

**Elevated Glycosuria Triggers Metabolic Stress Response in Renal *Glut2* Knockout Mice Through the Activator Protein-1 Family of Transcription Factors Involved in Maintaining Glucose Homeostasis**

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Renal *Glut2* deficiency, like SGLT2 inhibition, induces massive glycosuria in mice. Yet, their fasting blood glucose levels remain normal indicating a compensatory production of endogenous glucose. To investigate this, we performed snRNA sequencing combined with ATAC sequencing on the mouse kidney samples and identified the genes that may contribute to molecular pathways involved in glucose production. We used 13C6- glucose to determine the changes in metabolites in the liver, kidney and skeletal muscle tissues using NMR coupled with ion-chromatography-MS. RNA sequencing data revealed an increased expression of genes involved in oxidative phosphorylation and inner mitochondrial membrane proteins whereas transcripts for ribosomal subunits and amino acid metabolism were found to be downregulated in kidneys of renal *Glut2* KO mice. The transcription factors involved in cellular stress response such as the Activator Protein-1 family members (JUN, JUNB/D, FOS, FOSL1/2/D) and their mutual regulators CREB5, SMAD2/3, and BATF were enriched in multiple cell types of kidneys in the KO mice, indicating reprogramming of glucose metabolism through upregulation of glycolysis and enhanced gluconeogenesis. The 13C6-glucose tracer analysis revealed an increase in isotopologue concentration of metabolites of glycolysis (G-6P, F-6P, F-1,6BP, 1,3-BPG, pyruvate), lactate, TCA cycle (citrate, isocitrate, cis-aconitate, α-ketoglutarate) and mannose pathway (mannose-6P, GDP-mannose) in kidneys of the KO mice. Altogether, our data shows that the transcription factors and metabolic pathways involved in cellular stress-response may contribute to the compensatory glucose production observed in the KO mice. Targeting these molecular pathways may help improve the efficiency of SGLT2 inhibitors to treat type 2 diabetes.

**TP53 mutant cooperates with H3K27M to drive radioresistance and tumor progression in DIPG**

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**Background:** Diffuse Intrinsic Pontine Glioma (DIPG) is an aggressive pediatric brainstem tumor, characterized by the presence of H3K27M mutation, which disrupts histone methylation and leads to widespread epigenetic dysregulation, and recurrent TP53 mutations, which contribute to radioresistance. Analysis DIPG patients’ samples identified several TP53 mutations with a high recurrence, most frequently occurring in the DNA binding domain of p53. *The overall objective of this study is to determine how TP53mut and H3K27M collaborate to drive DIPG survival after treatment.* As a first step, we assessed whether p53 mutants retain DNA binding capacity or exhibit gain-of-*function* under DNA damage conditions. **Methods:** We generated expression constructs of TP53 mutants for protein expression analysis in HEK293 cells. These constructs were also co-transfected with histone H3.3 or H3.3K27M to study changes in TP53 RNA expression levels by RT-PCR. We performed ChIP-qPCR to determine the highest TP53wt response under DNA damage by irradiating transfected HEK293 cells. The TP53-DNA binding capacity was then tested for all the mutants by isolating nuclear fraction from transfected HEK293 cells and assessed by EMSA assay. Lastly, we used ChIP-qPCR to determine TP53mut-DNA binding capacity under DNA damage. **Results:** TP53wt and mutants have a different protein expression profile, and RNA levels vary in the presence of histone mutant H3.3K27M compared to H3.3. TP53wt binds to its target genes p21 and MDM2 under DNA damage after 6hs post irradiation using 8Gy dose. EMSA assay shows a significant binding of TP53wt and mutants (R248W, R273C, V157F), while R175H, R342X, S241F have either low or no DNA binding capacity. Binding activity for some mutants varies under DNA damage, such as S241F, which increases the binding activity when compared to R175H which does not bind DNA under normal or DNA-damage conditions. **Conclusions:** DIPG patients have recurrent TP53 mutations, which influence expression and DNA binding activity. TP53wt and mutant proteins have a different expression profiles. The strongest TP53wt response after DNA damage is achieved after 6hs post irradiation at an 8Gy dose. EMSA assays shows strong binding capacity for TP53wt, and R248W, R273C, a low binding capacity for R342X, S241F, and non-binding for R175H. Under DNA damage conditions, TP53wt and the mutants R248W, R273C, S241F bind to DNA as a canonical response, while this interaction decreases significantly when compared with non-stressed HEK293 cells. The TP53 mutant R175H does not bind DNA under DNA damage conditions in HEK293 cells, but its function is still under study to elucidate its cooperation with H3K27M in radioresistant patients.

**Acknowledgments:** Kentucky Pediatric Cancer Research Trust Fund

**L-serine prevents 1-deoxysphingolipids neurotoxicity**

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Taxane-induced peripheral neuropathy (TIPN) is a major side effect of taxane (paclitaxel and docetaxel) chemotherapy treatment. Our laboratory has shown that increased levels of atypical sphingolipids, the 1-deoxysphingolipids (deoxySLs), correlate with the incidence and severity of neuropathy in paclitaxel-treated patients. DeoxySLs are generated when the enzyme, serine-palmitoyltransferase, utilizes L-alanine instead of its preferred L-serine substrate, the precursor of the canonical sphingolipids. Due to their slow degradation, deoxySLs accumulate when produced in excess, leading to neurotoxicity as in the case of TIPN. The mechanisms underlying their toxicity remain unclear, including whether structural differences of individual deoxySL species, such as positional isomers 4E and 14Z, influence toxicity levels.

To address this, we tested the neurotoxic effects of individual deoxySL species in two neuroblastoma cell lines. In differentiated Neuro-2a (N2a), we observed neurite swellings, retraction, and degradation, while in KCNR cells, retraction of the cell processes, resulting in rounding of the cells indicated toxicity. Our preliminary results suggest that all individual deoxySL species induce neurite damage in a dose and time dependent manner.

In a proof of principle experiment, we showed that addition of L-serine decreased deoxySL production in RSC96 cells treated with paclitaxel. Next, we tested an L-serine-enriched diet in paclitaxel-treated mice, finding reduced deoxySL levels in plasma and dorsal root ganglia neurons and rescued neuropathic behaviors. These findings provide a foundation for future studies assessing whether lowering deoxySL levels can alleviate TIPN symptoms in patients.

**Impact of High Dietary Phosphate Intake on Anxiety-Related Behaviors and Brain Gene Expression in Mice**

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Background: Recent research has highlighted the potential impact of dietary phosphate intake on mental health, particularly in relation to anxiety. Inorganic phosphate (Pi), prevalent in the Western diet, may alter brain chemistry and neurotransmitter activity, however, the long-term consequences of dietary Pi consumption remain unclear.

Methods: We investigated the effects of a high-phosphate (HP) diet on behavioral functions in male C57BL6 mice over 12 weeks. Mice were divided into two groups: fed a diet with 2.0% inorganic Pi (HP, n=11) and the other with 0.6% inorganic Pi (normal phosphate, NP, n=13). Behavioral tests included fear conditioning, open field, and elevated plus maze tests. Flow cytometry of brain tissue used a general immunophenotyping panel, and B-cell and T-cell populations were assessed. Immunohistochemical analyses and RNA sequencing of the hippocampus were performed to identify underlying mechanisms. Statistical analyses employed a linear mixed model and t-tests with appropriate corrections.

Preliminary results: HP mice exhibited significantly increased freezing behavior in the fear conditioning test and spent more time in the periphery during the open field test, indicating heightened anxiety (both p = 0.03 for group). RNA sequencing revealed differential gene expression in the hippocampus, with notable changes in genes related to cell-cell junctions, stress response pathways (Rapgef2), and cognitive impairment (Igf2). Flow data showed a trend towards an increased B-cells and a decreased T-cells percentage with HP diet.

Conclusions: Preliminary findings suggest that HP diet may exacerbate anxiety-like behaviors in mice, potentially through alterations in specific gene expressions in the brain.

**Age-Dependent B and T Cell Populations in the Skull and Dura After Ischemic Stroke**

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Abstract

Introduction B cells are found in the brain after stroke and may contribute to chronic neuroinflammation. The skull and dura are hubs of B cell development in healthy animals, but whether these processes are altered after stroke is unknown. The aim of this study is to determine if ischemic stroke induces B cell maturation in the skull and dura in an age-dependent manner.

Methods Young (<14mos.) and aged (>16mos.) C57Bl/6J mice (n=5-11/sex) underwent 30-min or 60- min. MCA occlusion. At 3d or 3wk post-stroke, skull and dura were processed and analyzed with flow cytometry. Cell counts were determined in FlowJo and three-way ANOVA for sex, age, and time since injury were performed (GraphPad). Immunohistochemistry of dura is ongoing.

Results In both young and aged animals, B cell proportion decreased in the skull and dura at 3d, but at 3wk, only young animals returned to naïve levels. Proportion of CD4 T cells decreased in the skull and increased in the dura at 3d and 3wk in aged animals only. No significant sex differences were observed.

Conclusion These results suggest that inflammatory changes remain in the skull and dura in aged animals at 3wk after stroke but largely resolve in young animals. The decrease in CD4 T cells in the skull and increase in the dura, may suggest that these cells travel from the skull to the dura in aged animals after stroke.

**Fkbp5 links HPA Axis dysregulation to elevated glycosuria in Renal Glut2 KO Mice: Implications for SGLT2 Inhibitors**

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We recently showed that kidney-specific glucose transporter 2 knockout (Ksp-*Glut2* KO) mice exhibit glycosuria and have an overactive hypothalamic-pituitary-adrenal (HPA) axis, indicating a potential crosstalk between elevated glycosuria and stress-related comorbidities. To investigate the underlying molecular mechanisms connecting these two conditions, we performed single-nucleus RNA sequencing on renal tissue, exposed mice to elevated plus maze (EPM) and hot plate tests, induced acute and chronic stress using a physical restraint and measured plasma corticosterone levels in male ksp-*Glut2* KO mice. RNA sequencing revealed that the stress-associated gene, *Fkbp5*, was differentially expressed in the renal tissues of ksp-*Glut2* KO mice. Specifically, it was significantly upregulated in the endothelial cells and downregulated in the proximal tubules of the kidneys. Its expression was decreased in the skeletal muscle (100.0±12.2 vs 36.7±5.1, CON vs EXP, p<0.05) and increased in the liver (100.0±20.5 vs 226.7±11.2, p<0.05), suggesting tissue-specific alterations in glucocorticoid signaling. Moreover, ksp-*Glut2* KO mice had higher levels of plasma corticosterone at baseline (36.6±7.0 vs 48.7±8.7 ng/ml), after acute stress (153.7±18.4 vs 243.3±12.0 ng/ml) and following chronic stress (199.5±9.7 vs 262.0±14.4 ng/ml). Experimental mice spent more time in the closed arms (167.0±19.5 vs 236.0±15.2, p<0.05) of the EPM, suggesting increased anxiety-like behavior. Finally, KO mice showed hypersensitivity to pain (10.7±0.9 vs 7.8±0.3, p<0.05). Altogether, our findings identify *Fkbp5* as a critical mediator of HPA axis dysregulation in renal *Glut2* KO mice, linking renal glycosuria to stress-associated behaviors. These results have important implications for the use of SGLT2 inhibitors, particularly in individuals with preexisting neurological disorders.

**The glucose receptor Adgrl1 is a Novel Regulator of Leptin and Insulin Function**

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**Abstract**

We recently identified adhesion G-Protein Coupled Receptor L 1 (Adgrl1) as a novel glucose receptor that is highly expressed in the ventromedial nucleus of the hypothalamus (VMH) and regulates glucose and energy homeostasis. Mice lacking Adgrl1 develop insulin resistance and obesity, followed by fasting hyperglycemia. These findings were recently confirmed in humans. Here, we investigated the role of hypothalamic Adgrl1 in mediating leptin and insulin function. We first measured the effects of leptin on food intake and body weight in mice lacking Adgrl1, specifically in VMH. Using in situ RNA hybridization, we then assessed the expression of *Adgrl1* and leptin receptor (*LepR*) in the VMH. In addition, we selectively activated Adgrl1-expressing neurons in the VMH in Adgrl1Cre mice using chemogenetics and measured insulin sensitivity and glucose tolerance. Finally, we measured the effects of exogenous leptin and insulin on hypothalamic *Adgrl1* expression in different mouse models, including high-fat diet (HFD)-fed and leptin-deficient (*ob/ob*) mice. Unexpectedly, we found that Adgrl1VMH deficient mice had reduced food intake in response to exogenous leptin despite obesity. We also observed that *Adgrl1* co-expresses with the *LepR* in the VMH. In contrast, activation of the Adgrl1VMH neurons improved insulin sensitivity. These results indicate the differential effects of Adgrl1 on leptin sensitivity and glucose homeostasis. Furthermore, exogenous leptin administration decreased hypothalamic *Adgrl1* expression in wild-type (WT) mice. Interestingly, hypothalamic *Adgrl1* was elevated in HFD-fed obese mice compared to normal chow-fed controls but decreased with leptin administration. Conversely, leptin-deficient (*ob/ob*) mice exhibited lower hypothalamic *Adgrl1* levels compared to their WT counterparts. Moreover, exogenous insulin significantly increased hypothalamic *Adgrl1* levels, even in streptozotocin-induced diabetic mice. These findings reveal a novel role of hypothalamic Adgrl1 in mediating leptin and insulin actions to regulate glucose and energy metabolism. This information may be useful in establishing the molecular basis of leptin and insulin resistance in obesity and type 2 diabetes.

**Perfluorooctanesulfonic acid promotes cellular proliferation and activates EGFR signaling in primary colorectal cancer cells**

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Perfluorooctanesulfonic acid (PFOS), a subset of per-and polyfluoroalkyl substances (PFAS), has been recognized as an emerging ecological contaminant due to its widespread environmental persistence, being detected in 45% of USA drinking water. The gastrointestinal tract is directly exposed to environmental pollutants via contaminated drinking water and food. Given its resistance to natural degradation, PFOS can accumulate in intestinal tissues, potentially influencing homeostasis under both physiological and pathological conditions. Despite extensive research on PFOS's impact on various health conditions, including its potential role in tumor promotion, its contribution to colorectal cancer (CRC) progression remains poorly understood. Therefore, this study aims to investigate the effects of long-term exposure to PFOS *in vitro*. To access the effects of PFOS exposure on CRC cell proliferation, a primary CRC cell line, PT130, was exposed to 1µg/mL of PFOS for 3 months. We performed a PrestoBlue assay to evaluate cell proliferation and conducted western blot analysis to assess changes in protein expression. We observed an increase in cell proliferation in PFOS-treated PT130 cells compared to control cells. Additionally, PFOS exposure led to the activation of EGFR, STAT3, and ERK along with increased protein levels of Cyclin D and Survivin, key markers associated with cell proliferation. These findings suggest that PFOS exposure increases CRC cell proliferation, possibly through EGFR pathway activation. Ongoing studies will explore the effects of PFOS on additional downstream proteins involved in CRC progression. Further studies are essential to investigate PFOS's impacts and to develop strategies to mitigate its harmful effects on CRC.

**Amylin vasculopathy impairs cerebral Aβ efflux through altering cerebral vasodilation**

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**Background**

Impaired interstitial fluid drainage in the brain is indicated by the presence of perivascular β-amyloid (Aβ) deposits and is attributed to alterations in contractility and relaxation of vascular-smooth-muscle-cells (SMCs). The brain microvasculature in Alzheimer disease (AD) accumulates amyloid-forming-amylin secreted from the pancreas. Here, we tested the hypothesis that cerebrovascular amylin deposits perturbs cerebral Aβ-efflux by impairing cerebral vasodilation.

**Methods**

Using transgenic rats expressing amyloid-forming human-amylin in the pancreas (HIP-rats) (16-months) and wild-type (WT) littermates, express non-amyloidogenic rat-amylin, we conducted comparative analyses of cerebral blood flow (CBF), pressure myography in isolated pial arteries and vascular SMC oxidative stress experiments.

**Results**

Longitudinal-brain-MRI measurements revealed consistent structural alterations that progressed more rapidly with aging in HIP vs. WT, leading to reduction in CBF in HIP-rats. Plasma nitrite and nitrate, stable nitric oxide (NO) end products, were increased in HIP vs. WT. Pressure myography (pial-arteries) showed significant elevations in arterial tone in HIP than WT. Consistent with these results, vascular SMCs from HIP-rats showed elevated lipid peroxidation (LPO), which was replicated in SMCs incubated with exogenous human-amylin. Increased LPO contributes to oxidative stress in the vascular wall and reduces NO bioavailability, altering vasodilatory function. Both arginase activity and expression (of Arginase 1 and 2) were increased in brain microvascular lysates from HIP-rats than WT, suggesting arginase-NO dysregulation.

**Conclusion**

Perivascular Aβ deposits in the setting of AD are potentially linked to amylin vasculopathy and altered spontaneous contraction/relaxation of cerebrovascular-SMCs. Future experiments will focus on delineating molecular markers of amylin-induced alterations of SMC contractile phenotype.

**Cardiac remodeling, recognition memory deficits and accelerated aging in female rats with prior gestational diabetes**

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**Aims/hypothesis**: Women with prior gestational diabetes mellitus (GDM) have a higher incidence of age-associated diseases, including type 2 diabetes, cardiovascular disease, and cognitive impairment. Human studies cannot readily determine whether GDM causes these conditions and the underlying mechanisms. Here we used a well-validated rat model of GDM to address these questions.

**Methods/Results**: Rats with beta cell-specific expression of human amylin, a pancreatic hormone, were used as a GDM model. Five-month-old rat females were randomly assigned to no-pregnancy, one-pregnancy, and two-pregnancies experimental groups. Glucose tolerance tests and transthoracic echocardiography were performed at baseline and during the postpartum period. At 18 months of age, rats were administered the novel object recognition test, followed by euthanasia and organ collection. All females developed glucose intolerance, cardiac remodeling, and impaired left-ventricular relaxation with aging. Females with two GDM-complicated pregnancies had increased left-ventricular mass compared to the other groups following the second pregnancy and till the end of the study. At 18 months of age, females with prior GDM pregnancies presented aggravated demyelination, particularly in the hippocampus and mid-brain region, oxidative stress, and neuroinflammation, and had a lower recognition index in the novel object recognition test compared to nulliparous females. Higher parity exacerbated these effects. Shorter telomeres and reduced mitochondrial DNA content, two hallmarks of biological aging, were found in the brain, heart and pancreas of rats with prior GDM.

**Conclusions**: These findings support the concept that GDM is a sex-specific risk factor for aging-associated diseases and point to accelerated cellular aging as a contributing mechanism.

***In vivo* hypoxia may alter immune cell populations and cortical mitochondrial energetics**

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**Background**: Previous work in the Stowe lab established a repetitive hypoxic preconditioning stimulus (RHP) regimen that confers neuroprotection to mice who received a stroke 8 wks after conditioning. A recent project investigated whether hypoxia (with or without exercise) improves recovery in rats 6wks after experimental spinal cord injury, where significant improvement was seen in respiratory function. Additionally, peripheral immune cell populations in hypoxia-treated rats were different than controls. Follow up studies are ongoing to determine whether single or repeated administration of hypoxia alters immune cells in naïve mice, with brain regions collected for metabolomic profiling.

**Aim**: These pilot experiments investigate hypoxia-induced modulation of immune cells and mitochondrial function.

**Method**: Acute (8% O2, 4 hr) or repeated hypoxia (8-11%O2, 2-4 hr, 2wks) was administered to mice (CD-1, M+F, 4-6mo.) in group-housed cages. 24 hrs following the last exposure to hypoxia, mice were rapidly euthanized to collect spleen, hippocampus, and pre-motor/motor cortex. Spleen was processed into a suspension of peripheral immune cells and stained for profiling via flow cytometry. Mitochondria was isolated from brain tissues and used for Seahorse Flux Analyzer ATP assays.

**Result:** Hypoxia increased the ratio of rat splenic CD4+:CD8+ T cells (p=0.04), suggesting long-term immune modulation. Preliminary analysis of ongoing metabolomic studies in mice demonstrates increased cortical cell State III OCR (p=0.04) in females after repeated hypoxic exposures.

**Conclusions:** Repeated hypoxia is well-tolerated and shows therapeutic promise. Ongoing mouse studies will elucidate alterations in immune cells and cortical mitochondrial energetics following *in vivo* hypoxia treatment.

**B cell depletion reduces chronic cognitive deficits but increases infarct volume after prefrontal stroke**

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B cells migrate into the hippocampus after MCA stroke, but recovery benefits are unclear in aged animals. Autoshaping (AUTO), a Pavlovian learning task, utilizes an unconditioned reward to build associations with a conditioned stimulus (CS+) on a touchscreen platform not previously used to track cognitive decline in aged mice.

We hypothesized B cell-depleted (BCD) aged mice would have increased post-stroke cognitive deficits correlating with the loss of neuroprotective B cells in the hippocampus and prefrontal cortex.

Female (11-24 month-old, n=34) hCD20tamCRE(+)/fBDNF(+/+)­ mice and littermate controls were trained on the PAL task to confirm motivation for the reward, followed by the AUTO training and Rituximab-induced B cell-depletion. Following baseline, mice underwent a bilateral prefrontal photothrombotic stroke. Mice completed bi-weekly AUTO acquisition, and splenic B cell depletion confirmed WT/BCD designation. Primary measures were #trials/session, #approaches, approach difference, and approach latency.

Rituximab induced long-term depletion of splenic B cells by 56.1% (p=0.001). BCD mice exhibited deficits in the #approaches to CS+ (2-way rmANOVA; F(1,12)=3.689; p= 0.0789) and tray latency after CS- trial (2-way rmANOVA; F(1,12)=4.396; p=0.0579). Analysis of the replicate cohort is ongoing (n=9 final survival). Ongoing infarct volume quantification for n=3 WT and n=4 BCD shows a 29.7% (p=0.0358) increase for BCD mice.

We expected cognitive deficits in BCD-aged females. An opposite protective effect was shown in the chronic phase, with BCD animals improving cognitive performance at 4 weeks post-stroke (p=0.0089) independent of infarct volume, confirming previous studies showing a detrimental effect of delayed B cells on cognition in young mice (PMID:25653369).

**Sex- and Age-Dependent Regulation of the Glucose Receptor Adgrl1 in Mice**

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**Abstract**

**Introduction:** We recently identified adhesion G-protein coupled receptor L1(Adgrl1) as a glucose receptor that is essential for controlling glucose and energy balance. Deletion of Adgrl1 in the hypothalamus causes obesity in a sex-specific manner in mice. While hypothalamus-specific knockout (KO) of *Adgrl1* in male mice causes obesity, ovary-intact female mice have normal body weight even after the hypothalamic Adgrl1 deficiency. Interestingly, ovariectomy unmasks the effects of hypothalamus-specific Adgrl1 deficiency in female mice, indicating sex-dependent regulation by hypothalamic Adgrl1. Therefore, in this study, we determined the role of estrogens on Adgrl1 expression, specifically in the hypothalamus in young and relatively older female mice.

**Methods:** We first determined hypothalamic *Adgrl1* expression in 8- and 24-week-old male and female C57BL/6J mice. We also measured *Adgrl1* expression in the ovaries of 8-week-old female mice and compared the expression with that observed in the hypothalamus. Then, we treated a separate cohort of 8-week-old female mice with 17b-estradiol (E2, 1 mg/Kg, intraperitoneally) and measured their hypothalamic *Adgrl1*. In addition, we excised ovaries from another group of 8-week-old female mice (OVX-group), and three weeks later, we collected their hypothalamus to measure *Adgrl1* expression by qPCR. Finally, to determine whether Adgrl1 and estrogen receptors are co-expressed in the mouse hypothalamus, we used RNA fluorescence in situ hybridization to detect their RNA in the ventromedial nucleus of the hypothalamus (VMH).

**Result:** The qPCR analysis confirmed the expression of *Adgrl1* in the mouse ovary, which was about 60% of that present in the hypothalamus. Moreover, female mice had higher Adgrl*1* expression in the hypothalamus than male mice at 8 weeks of age. Hypothalamic *Adgrl1* was reduced with age in both male and female mice. E2 administration decreased hypothalamic *Adgrl1* expression in 8-week-old female mice. In contrast, ovariectomy (reduced circulating E2 levels) up regulated the *Adgrl1* expression. The RNA in situ hybridization demonstrated that *Adgrl1* is co-localized with about 46 ±9% estrogen receptor 1 (α) expression in the VMH.

**Discussion and conclusion:** Our findings suggest that age, estrogen levels, and sex affect *Adgrl1* expression. In conclusion, changes in Adgrl1 expression may explain sex and age differences in the pathogenesis of metabolic disorders such as type 2 diabetes and obesity, and their differential responses to a given treatment.

**TBI does not impact the numbers of new hippocampal granule cells generated six weeks after injury in mice, despite potential shifts development progression.**

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Traumatic brain injury (TBI) induces acute cell death in the hippocampal dentate gyrus, a structure critical for learning and memory. A compensatory increase in proliferation within the dentate gyrus subgranular zone has been linked to neurogenesis and cognitive recovery following injury. However, studies suggest increased proliferation in the first days after TBI could compromise long-term neurogenic capacity, contributing to cognitive decline. To investigate chronic post-traumatic neurogenesis, an inducible reporter mouse model (Ascl1CreERT2/+xR26RtdTom/tdTom) was used to label and trace the long-term fate of Ascl1+ cells born 6 weeks after injury. Ascl1+ cells include type-2a neural progenitor cells (NPCs) and radial glial-like cells (RGLCs), stem cells which adopt a neuronal or astrocytic fate. Mice received a controlled cortical impact (n=10/sex) or sham (n=5/sex) injury 6 weeks prior to induction of TdTomato expression. At 12 weeks post-injury, ample time for labeled NPCs to reach neuronal maturity, tdTomato+ cells were manually counted and classified based on morphology. Injury did not alter the number of tdTomato+ neurons, NPCs or total cells in the granule cell layer. In injured females, however, the subset of new mature neurons (tdTomato+/ NeuN+) was reduced, which could indicate a delay in neuronal maturation. Numbers of RGLCs labeled at 6 weeks that remained at 12 weeks were decreased following TBI for both sexes. These results indicate that early increases in proliferation do not compromise neurogenic production at a late timepoint after TBI. However, altered RGLC dynamics may point to chronic changes in the hippocampal stem cell pool which require further investigation.

Funding: This work was supported, in part, by NIH 5T32 NS077889 and Kentucky Spinal Cord and Head Injury Research Trust grants 19-5A and 23-12.

Key Words: hippocampus, neural stem cells, neurogenesis, plasticity, TBI

**Overexpression of fatty acid synthase increases exosome secretion in colorectal cancer**

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Over 50% of colorectal cancer (CRC) patients develop metastasis. Fatty acid synthase (FASN) overexpression is strongly associated with metastasis and poor prognosis. FASN catalyzes the biosynthesis of palmitate, a key component of structural lipids. Exosomes, lipid bilayer vesicles containing bioactive molecules, play a critical role in establishing a pre-metastatic niche (PMN) that facilitates metastasis. Hepatic stellate cells (HSCs) can be activated by tumor-derived exosomes and transformed into cancer-associated fibroblast, contributing to PMN formation. This study aims to investigate the contribution of FASN in exosomes formation and HSCs activation in CRC. To explore the contribution of FASN in exosomes formation, we utilized FASN-knockdown (FASN-KD), FASN-overexpression (FASN-OE), and pharmacological inhibition of FASN in CRC cells. Proteomics analysis was conducted to assess the impact of FASN on exosomal cargo. To examine the functional effects of CRC-derived exosomes, HSCs LX-2 were used. Exosome secretion was significantly reduced in FASN-KD cells and FASN-inhibitor treated cells compared to control cells, while secretion was significantly increased in FASN-OE cells. Proteomic analysis revealed that FASN modulates exosomal cargo, with changes in inflammatory response, metabolism, exocytosis, and adhesion pathways. Exposure to exosomes from control CRC cells robustly activated of LX-2 cells, as indicated by FAP and α-SMA expression. However, activation was significantly reduced when LX-2 cells were exposed to exosomes derived from FASN-KD cells. In summary, FASN promotes exosomes secretion and modules their protein cargo. The association between FASN expression in CRC cells and LX-2 activation by secreted exosomes suggests a potential role for FASN in PMN formation.

**Hepatic Aster-C Deficiency Protects Against Diet-induced Hepatic Steatosis in Mice**

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**Objective**

Aster family of proteins (Aster-A, -B, -C), which are involved in non-vesicular cholesterol transport from the plasma membrane (PM) to the endoplasmic reticulum (ER). Disruption of Aster protein function impairs PM-to-ER cholesterol transport in the liver, ovary and adrenal gland. Notably, Aster-C is expressed selectively in hepatocytes, the primary cell type responsible for lipid accumulation in metabolic dysfunction-associated steatotic liver disease (MASLD). In this study, we investigated the effects of Aster-C on lipid accumulation in hepatocytes in a mouse model of diet-induced hepatic steatosis.

**Approach and Result**

AAV8-Cre and AAV8-Control were injected in Aster-CFlox/Flox (F/F) mice, then fed 3 weeks of Western diet. Result shows that hepatic Aster-C gene expression was significantly abolished in AAV-Cre injected mice. Perform liver lipid assay, we found that liver cholesterol and triglyceride levels were significantly decreased in AAV-Cre injected mice compared to AAV-Control group (liver cholesterol, 81.86 ± 4.0 vs. 177.38 ± 24.1 µg/mg, P<0.01; liver triglyceride, 149.18 ± 1.9 vs. 292.28 ± 32.6, P<0.01). We also generated Aster-C hepatocytes specific knockout mice (LKO) by crossing Aster-CFlox/Flox mice with albumin-Cre mice. There is no significant difference on the levels of liver cholesterol and triglyceride when mice were fed Chow diet (LKO vs. F/F: liver cholesterol, 12.21 ± 1.6 vs. 10.96 ± 1.4 µg/mg, P=0.562; liver triglyceride, 386.06 ± 21.8 vs. 432.48 ± 36.6 µg/mg, P=0.301). However, with 10 weeks of Western diet feeding, hepatic Aster-C deficient mice show significant decrease in liver cholesterol (31.7 ± 4.1vs. 70.4 ± 10.1µg/mg, P<0.01) and triglyceride (1407.0 ± 433.7vs. 2913.2 ± 404.2 µg/mg, P<0.05) compared to control mice. Liver histology images show less lipid drops and macrophages accumulation in hepatic Aster-C deficient mice. What’s more RNA sequencing result show that ablation of Aster-C improves nonalcoholic steatohepatitis (NASH) relative gene expression in liver.

**Conclusion**

Loss of Aster-C in hepatocytes protects against steatosis by reducing hepatic cholesterol and triglyceride accumulation, ameliorating liver damage, and decreasing expression of inflammatory genes following Western diet feeding.

**Platelet Endocytosis And α-Granule Cargo Packaging Are Essential for Normal Skin Wound Healing**

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The high prevalence of chronic wounds, i.e., 2.5-3% of the US population, causes a large social and financial burden. Physiological wound healing is a multi-step process that involves different cell types and growth factors. Platelet-rich plasma or platelet derived factors have been used to accelerate wound repair, but their use has been controversial with mixed results. Thus, a detailed functional understanding of platelet functions in wound healing beyond hemostasis is needed. This study investigated the importance of platelet α-granule cargo packaging and endocytosis in a dorsal full thickness excisional skin wound model using mice with defects in α-granule cargo packaging (Nbeal2-/- mice) and endocytosis (platelet-specific Arf6-/- and VAMP2/3∆ mice). We found that proper kinetic and morphological healing of dorsal skin wounds in mice requires both de novo as well as endocytosed platelet α-granule cargo. Histological and morphometric analyses of cross-sectional wound sections illustrated that mice with defects in α-granule cargo packaging or platelet endocytosis had delayed (epi)dermal regeneration in both earlier and advanced healing. This was reflected by reductions in wound collagen and muscle/keratin content, delayed scab formation and/or resolution, re-epithelialization, and cell migration and proliferation. Molecular profiling analysis of wound extracts showed that the impact of platelet function extends beyond hemostasis to the inflammation, proliferation, and tissue remodeling phases via altered expression of several bioactive molecules, including IL1β, VEGF, MMP-9, and TIMP-1. These findings provide a basis for advances in clinical wound care through a better understanding of key mechanistic processes and cellular interactions in (patho)physiological wound healing.

Supported by the AHA (1020159), NIH/NHLBI (HL150818), and the VA

**Temporal and Regional Dynamics of B Cell Infiltration after Contusive Traumatic Brain Injury in Mice**

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 Traumatic brain injury (TBI) survivors often face persistent cognitive and neurobehavioral deficits. Unsuccessful clinical trials targeting neuronal injury mechanisms have motivated investigations of other cell types. Despite advances in understanding the roles of immune cells in the secondary injury cascade, little is known of the B-cell response, including the timing and regional extent of B-cell infiltration. We hypothesize that contusion TBI triggers delayed B-cell diapedesis into the cortex. Adult male mice received a lateral controlled cortical impact (CCI) (n = 6-8/timepoint) or sham (n = 4/time point) injury. At 1, 3, 7, 14 or 28 days postinjury, coronal brain sections were immunolabeled with the B-cell antibody B220. Regional cell counts were performed, excluding B-cells within hemorrhagic regions. At 1 and 3 days after CCI, B-cell numbers increased relative to sham only in the cortical contusion and ipsilateral hippocampus respectively. By 7 days, elevated B-cell counts were observed in the contused cortex, pericontusional cortex, and contralateral hippocampus, where numbers declined by 14 or 28 days. In the corpus callosum, B-cell numbers increased in a delayed manner, at 28 days after CCI compared to sham. Higher numbers were also observed in the meninges and ventricles at later time points. Interestingly, atypical B-cell clusters were commonly observed at 14 days in CCI injured animals. These data suggest that delayed B-cell diapedesis after TBI is region specific and B-cells may adopt pathogenic phenotypes. Future studies will characterize B-cell phenotypes within the injured brain to gain insight to their function and impact on injury pathology.

Funding: This work supported by Kentucky Spinal Cord and Head Injury Research Trust grant 22-4, the University of Kentucky Neuroscience Research Priority Area, and the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

**Key Words:** TBI, Inflammation, Adaptive immunity

**Does TRPA1 Signaling Change the Operating Point of the Cochlear Transducer?**

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Our laboratory found that transient receptor potential ankyrin 1 (TRPA1) plays a role in the noise-induced temporary threshold shift through a mechanism likely involving the cochlear supporting cells (Velez-Ortega *et al*., Nat Commun, 2023). We hypothesize the TRPA1-mediated tissue displacements affect the geometry of the organ of Corti thus modifying its operating point.

We used TRPA1-deficient (Kwan *et al*., Neuron, 2006) and wild-type littermates maintained in C57Bl/6 background of roughly 50:50 male:female. ABR to click and tone-burst stimuli were recorded before and after the exposure to broadband noise at 100 dB SPL for 30 minutes. We subtracted the 0° and 180° phase ABR recordings to extract CM data.

Our results show significant differences in the amplitude of the summating potential (SP) in click-evoked ABR; however, five days after noise exposure, the SP differences were no longer observed. Mice showed a direct current (DC) shift in the CM elicited by an 8 kHz tone burst as the sound intensity increased, which was delayed in TRPA1-deficientmice. We observed an overall reduction in CM amplitudes in the TRPA1-deficient mice which was not seen in wild-type littermates. In addition, we observed a larger amplitude of the ABR wave I in response to high intensity stimuli (>90 dB SPL) exclusively in the female TRPA1-deficientmice.

TRPA1 activation after noise exposure changes the operating point of the organ of Corti, which could serve as a protective mechanism against noise-induced hearing loss. Additionally, we discovered a previously unknown sex difference in TRPA1-mediated regulation of hearing sensitivity.

**Calpastatin overexpression in mice attenuates posttraumatic cognitive and motor behavioral deficits without neuronal rescue**

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Calpains are a family of calcium-dependent cysteine proteases that are activated within minutes after traumatic brain injury (TBI). Sustained calpain activation contributes to the secondary injury cascade of TBI and has been linked to axonal degeneration, blood–brain barrier dysregulation and neuron death. Calpastatin is an endogenous protein encoded by the CAST gene and a potent inhibitor of calpains. This study investigates the potential of overexpressing human calpastatin (hCAST) in a mouse model of TBI to alleviate TBI-induced brain damage and neurobehavioral dysfunction.

Transgenic mice overexpressing hCAST and wild-type controls (WT) were subjected to controlled cortical impact (CCI) to induce TBI (n=19 - 20 per genotype) or to sham injury (n=12 per genotype). Motor deficits quantified using a neurological severity score were observed in WT mice over the first week and were significantly attenuated in hCAST transgenic mice. Spatial learning ability assessed in a Morris water maze on days 6 through 9 and novel object recognition (days -1 and 10) were reduced following CCI in WT mice but significantly mitigated in hCAST overexpressing mice. At 10 days postinjury brains were collected and cut coronally into 40-micron sections for analysis. Upon Nissl staining a subset of animals (n=12 per genotype), we observed no significant differences in contusion volume between hCAST and WT animals. NeuN immunolabelling (CCI n=7 per genotype) revealed no effect of hCAST overexpression on posttraumatic neuron loss.

These findings suggest that inhibition of calcium-dependent proteolysis aids in restoration of neurobehavioral function following TBI without protecting against cortical or hippocampal neuron death.

**Brain Digital Slide Archive Hub: Organizing Slide Images and Metadata to Support ML Model Training**

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The development of AI and machine learning (ML) models for neuropathology is hindered by the limited availability of high-quality, annotated training data. The Brain Digital Slide Archive (BDSA) project addresses this challenge by creating a federated system that enables research institutions to share neuropathology slide images and annotations securely. Built on the Digital Slide Archive (DSA) platform, BDSA enhances data accessibility while ensuring compliance with institutional policies and regulatory requirements.

A key component of this system is the BDSA Hub Site, which centralizes metadata management and access control. Metadata is organized into a structured hierarchy, allowing researchers to filter and curate datasets efficiently. Access control mechanisms ensure that slide images remain restricted to authorized users, with Data Use Agreements (DUAs) governing institutional sharing. To streamline authentication, BDSA integrates CILogon and OAuth 2.0 on both the Hub site and individual DSA nodes, enabling Single Sign-On (SSO) for seamless user access across institutions.

BDSA Hub also introduces a project-based cohort management system, allowing researchers to collaboratively curate datasets while enforcing strict access policies. Slides can only be added to a project if all members have existing access, preserving data security and compliance. By combining federated data sharing, structured metadata management, and robust access control, BDSA Hub facilitates the development of more generalizable AI models for neuropathology, accelerating research and innovation in the field.

**B cell depletion alters spontaneous neuronal firing in the hippocampus post-stroke**

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Background: Calcium imaging has proven a key tool that provides an indirect but accurate measure of action-potential generation within neurons. The present study focuses on how B cell depletion, in addition to age and sex, influences spontaneous neuronal firing in the hippocampus of the transgenic C57BL/6 synapsin-Cre/GCaMP6S+/- mouse strain.

Methods: C57BL/6 synapsin-Cre/GCaMP6S+/- mice were used in the study. Non-injuredmice (n=21 total) were used (11 males; 10 females) in adult (4-8 mos.; n=10) and aged (11-18 mos.; n=11) groups. 9 mice were B cell depleted over a three week time period, while 12 had an IgG control injections. In the injured groups, we used a tMCAo stroke Injury model (n=20 total), (12 males; 8 females), adult (4-9mos; n=12), aged adult (11-20mos; n=8), 10 B cell depleted, 10 non-depleted IgG animals. Brains were extracted and sectioned into 300µm slices, oxygenated, and kept at 37ºC in aCSF, using an NMDG (N-methyl-D-glucamine) solution to improve cell health. Spontaneous neuronal activity in the CA1 and Dentate Gyrus (DG) of the hippocampus was recorded using wide-field calcium imaging.

Results: In the non-injured groups we found that the aged males had significantly higher DG amplitudes over other groups (Kruskal-Wallis Test, p = 0.0119). Additionally, we saw more significance differences in amplitude in the DG versus the CA1. Aged adults showed higher amplitudes. 3 weeks after stroke, in the CA1, B Cell depletion significantly reduced amplitudes in aged (p=0.0035) and adult male groups (p=0.0003). However, in the DG after tMCAO, data suggests that male B cell depleted cohorts have increased amplitudes. In post-stroke animals, B cell depletion did not affect amplitudes in female mice.

Conclusions: Data suggests that B cells may have a direct or indirect impact on hippocampal cell signaling. Although it appears that younger, adult animals are more unaffected by B cell depletion and stroke in terms of hippocampal signaling.

**The role of conserved promoter elements in the circadian regulation of ion channel-related genes in the heart**

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**Abstract:**

The circadian clock plays a fundamental role in regulating cardiovascular function. RNA sequencing of mouse ventricles identified a select few cardiac ion channel-related genes (*Kcnh2*, *Gja1*, *Rrad*) which oscillate for 24 hours.

We hypothesize the core circadian clock interacts with conserved elements in these promoters to regulate circadian and overall expression.

Using real-time bioluminescence, we analyzed conserved promoter-luciferase reporter constructs of circadian genes (*BMAL1*, *PER1*) expressed in inducible pluripotent stem cell-derived cardiomyocytes (iPSC-CM) and C2C12 myotubes. Full-length and promoter deletion constructs of human cardiac ion channel genes (*KCNH2*, *GJA1*, *RRAD*) were expressed in C2C12 myoblasts, along with variants in the *KCNH2* promoter generated via site-directed mutagenesis. Bioluminescence was measured at 10-minute intervals for 7-10 days, and data were analyzed for circadian characteristics (period, phase and amplitude).

*BMAL1* and *PER1* promoters oscillated in iPSC-CMs, but not in antiphase. In C2C12s, *BMAL1* and *PER1* promoters exhibited antiphase oscillations. *KCNH2*, *GJA1*, and *RRAD* promoter activity oscillated, though *GJA1* showed low~~er~~ overall activity. Deletion analyses identified a highly conserved tandem E-box as critical for circadian and overall *KCNH2* promoter activity. SNPs within this element produced variant-specific effects on promoter activity. A 300bp deletion in the *GJA1* promoter enhanced both oscillating and overall activity, indicating a suppressive function of this region. A 1500bp deletion in the *RRAD* promoter enhanced overall activity but not the oscillating amplitude, indicating this region may function as a suppressor with no effect on oscillation.

C2C12 myoblasts demonstrate a functioning circadian clock not reflected in iPSC-CMs. The effects of promoter deletions vary between constructs, with deletions and mutations both suppressing and enhancing promoter activity. These findings reflect RNA sequencing data and highlight promoter regions important for regulating circadian and overall expression of ion channel-related genes.

**Region-Specific Role of Astrocyte Calcium Dynamics in the Dorsal Striatum in Modulating Cocaine-Seeking Behaviors**

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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 Ca2+. However, the way Ca2+ in dorsal striatum astrocytes impacts neuronal signaling after exposure to self-administered cocaine remains unclear and how regional differences within the dorsal striatum affect astrocytic regulation of cocaine-induced neuronal signaling and behavior. We addressed these questions by over-expressing the Ca2+ extrusion pump, hPMCA2w/b, in dorsal striatum astrocytes and the Ca2+ indicator, GCamp6f, in dorsal striatum neurons of rats that were trained to self-administer cocaine. We demonstrated that suppressing astrocyte Ca2+ signaling in the dorsal striatum increased the acquisition and cue-induced reinstatement of cocaine seeking. This astrocyte-specific suppression also increased neuronal Ca2+ transient amplitude, indicating enhanced neuronal excitability. Next, we focused specifically on the dorsomedial striatum (DMS), utilizing fiber photometry to investigate neuronal Ca2+ dynamics in freely moving animals. We introduced an additional cohort expressing GqDREADD to selectively activate astrocyte Ca2+ signaling. Our results indicated region-specific differences; suppression of astrocyte Ca2+ in the DMS (hPMCA2w/b group) significantly elevated neuronal Ca2+ transients compared to both the control (tdTomato) and astrocyte-activated (GqDREADD) groups. Behaviorally, the hPMCA2w/b animals exhibited increased neuronal Ca2+ signaling but did not show further increases in cocaine-seeking behavior during cue-induced reinstatement, differing from observations in the overall dorsal striatum. Together, these findings suggest that astrocyte Ca2+ signaling distinctly regulates neuronal activity and cocaine-related behaviors in subregions of the dorsal striatum, highlighting the complexity of astrocyte-neuron interactions and emphasizing the need for region-specific therapeutic strategies for cocaine addiction.

**Targeting Pgk1 to overcome ferroptosis Resistance in Breast Cancer**

Felix Oyelami

Therapeutic resistance and recurrence are among the major contributors to poor outcome for patients with breast cancer. Induction of ferroptosis, a form of cellular death characterized by toxic lipid peroxide overload, has emerged as a promising therapeutic strategy against breast cancers including triple-negative breast cancer (TNBC). Nevertheless, certain types of cancer are impervious to induction of ferroptosis and the underlying mechanisms remain incompletely clear. In this study, we show that phosphoglycerate kinase 1 (PGK1), an important enzyme in glycolysis, is highly expressed in breast tumors, and the elevated levels of PKG expression correlate with advanced tumor stages, poor prognosis and ferroptosis insensitivity, particularly in TNBCs. Using genetic or pharmacological inhibition, we demonstrate that knockdown or inhibition of PGK1 enhances ferroptosis sensitivity in both TNBC and luminal breast cancer cell lines. We further demonstrate that depletion of PGK1 destabilizes glutathione peroxidase 4 (GPX4), an anti-ferroptotic defense peroxidase, thereby disturbing cellular redox homeostasis and promoting lipid peroxidation. Moreover, targeting PGK1 disrupts glycolytic metabolism and sensitizes breast cancer cells to ferroptosis induction in tumor cells subjected to glucose deprivation or treated with glycolytic inhibitors. In orthotopic TNBC models, loss of tumoral PGK1 augments the action of the ferroptosis inducer, imidazole ketone erastin (IKE), in inhibiting tumor growth and metastasis, and enhances CD8+ T cell-mediated anti-tumor immunity. These results indicate that PGK1 has a critical role in modulating breast cancer invulnerability to induction of ferroptosis, implying that this kinase may be exploited as a therapeutic target to sensitize breast cancers, especially, TNBC, to ferroptosis inducers.

**Cardiovascular Parameters Lead Core Body Temperature Changes**

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**Objective:** The autonomic nervous system regulates homeostatic variables including mean arterial blood pressure (MAP) and core body temperature (Tb). We examined whether their temporal relationship persists during circadian disruption using dim light at night (DLAN).

**Hypothesis:** Temperature fluctuations around a set point are preceded by cardiovascular changes, with MAP and heart rate (HR) acting as early signals in thermoregulation.

**Methodology:** SV129 mice were implanted with telemetry devices to continuously monitor MAP, HR, and Tb under thermoneutral conditions (30±2°C). After baseline recordings under standard light-dark cycles (200 lux:0 lux), male mice were subjected to DLAN (200 lux:5 lux). Data was analyzed using LOESS regression to distinguish direct physiological coupling from shared circadian rhythmicity.

**Results:** Cardiovascular parameters consistently preceded temperature fluctuations in both sexes (p<0.0001). Detrended data showed robust MAP-HR correlations (males: 0.80±0.02, females: 0.73±0.07) and moderate temperature correlations (MAP-Tb males: 0.68±0.05, females: 0.64±0.06; HR-Tb males: 0.69±0.05, females: 0.75±0.04). MAP led Tb by 6.97±1.93 minutes in males and 8.22±1.61 minutes in females, while HR preceded Tb by 5.64±1.36 and 5.94±1.06 minutes respectively. DLAN exposure in males preserved this temporal hierarchy (MAP-Tb: 7.39±1.53; HR-Tb: 5.83±1.48 minutes) with maintained MAP-Tb correlation and slightly reduced HR-Tb correlation (p<0.05).

**Conclusion:** The preserved temporal sequence reveals autonomic regulation prioritizes cardiovascular adjustments before temperature changes. This sequence persists during circadian disruption, suggesting a hardwired regulatory mechanism rather than one dependent on intact rhythms. These findings provide insight into autonomic coordination of homeostatic variables and may inform understanding of autonomic dysfunction in conditions with circadian disruption.