

TEXAS A&M UNIVERSITY School of Medicine

# **RESEARCH DAY**

# Summer Research Program July 28, 2023

Medical Research & Education Building 2 Bryan, Texas

# Schedule of Events

9:00 AM - 12:00 PM	<b>Poster Viewing &amp; Judging</b> MREB 2 Lower Level
12:00 - 12:30 PM	<b>Lunch</b> MREB 2, room 1403
12:30 - 1:30 PM	<b>Keynote Speaker</b> "History, Humility and Hunger" J. Scott Wieters, MD, FACEP Associate Campus Dean - Temple MREB 2, room 1403
1:30 - 2:00 PM	<b>Presentation of Certificates</b> Brett Mitchell, PhD, FAHA Director, Summer Research Program MREB 2, room 1403
2:00 PM	Group Photo & Adjourn



## Keynote Speaker



**J. Scott Wieters, MD, FACEP** Associate Campus Dean - Temple School of Medicine, Texas A&M Health

Title: "History, Humility and Hunger"

After growing up on a family farm in South Texas, Dr. Wieters graduated from the Fightin' Texas Aggie Class of 1997. He received his MD from the University of Texas at San Antonio in 2001 and completed his Emergency

Medicine (EM) residency at Scott & White Hospital in 2004. After seven years in private practice in Waco, TX, he jumped into academics in 2011, as the Clerkship Director in Emergency Medicine. He helped develop and launch A&M's required EM Clerkship as well as bootcamp courses for transition to residency. He is a seven-time recipient of the EM teaching award by his students. He has received the Scott and White EM Residency Teaching Award and the Distinguished Teaching Award by A&M as the top clinical educator for all A&M campuses. The Society of Academic Emergency Medicine recognized him as the National Clerkship Director of the Year.

Dr. Wieters, has chaired and led multiple teams with a focus on curriculum development and assessment. He has served nationally on the Clerkship Directors in EM Board of Directors, on the Education Committees of the American College of Emergency Physicians, and the Texas College of Emergency Physicians. He is an internationally recognized speaker in medical education and clinical EM. Currently, he serves as the Temple Campus Dean of the Texas A&M School of Medicine and is a passionate student advocate.

Despite his professional accomplishments, he is most proud of "meeting expectations" as a trophy husband to his wife, "The real Dr. Wieters", and a proud parent of four children who are all "exceeding expectations," although they all agree he "needs improvement" in his role as a youth sports coach.



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#### A SYNERGISTIC EFFECT OF CEFIDEROCOL AND GALLIUM NITRATE AGAINST PSEUDOMONAS AERUGINOSA

Junyoung Ahn, Ricardo Munoz-Medina, Carolyn Cannon Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

Pseudomonas aeruginosa (PA) is a Gram-negative bacterium that has been a serious clinical threat globally. P. aeruginosa infections are frequent in immunocompromised patients and are often associated with hospital-acquired infections (HAI). Our objective is to determine the synergistic effect of Cefiderocol and Gallium(III) on inhibiting the growth of P. aeruginosa and the antimicrobial efficacy in both iron-rich environments and iron-depleted environments. Cefiderocol, a siderophore-conjugated cephalosporin, can be transported into the cell by binding to Fe(III) at its siderophore moiety and moving through the iron transport system, preventing cell wall synthesis by binding with Penicillin Binding Protein. As Ga(III) is chemically similar to Fe(III), Ga(III) can bind to siderophore and get inside the cell, interfering with the iron uptake. Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) of both Cefiderocol and Ga(III) against PA2-72 were collected to determine the lowest concentration of each antibiotic needed to kill the strain. Cation-Adjusted Mueller Hinton Broth (CAMHB) and Iron-Depleted Cation-Adjusted Mueller Hinton Broth (IDCAMHB) were used to make iron-rich and iron-depleted environments. Then, the synergy was tested using a checkerboard assay using both drugs. The MIC of the Cefiderocol and Ga(III) mixture was lower than the MICs of each drug. However, there was not a significant decrease in MIC for the antibiotic mixture to be synergistic. Also, we found that the antibiotics worked better in iron-rich environments, not iron-depleted environments. Further investigation is needed to determine the effect of antibiotics on different strains and the effect of iron on antibiotic processes.

JA was supported by the Texas A&M School of Medicine

#### GLIAL REACTIVITY PROFILE IN A 6-HYDROXYDOPAMINE MODEL OF PARKINSONISM IN NOVEL TRANSGENIC MICE WITH CONSTITUTIVELY UPREGULATED β2\* NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

Andrea A. Alarcon, Gauri Pandey, Sara M. Zarate, Roger Garcia, Rahul Srinivasan Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

Parkinson's disease (PD) is caused by the loss of substantia nigra pars compacta (SNc) dopaminergic (DA) neurons. Current treatments for PD are merely symptomatic, which makes it vital to develop neuroprotective treatments for PD. In this regard, the endoplasmic reticulum (ER) stress response is thought to play a major role in DA loss during PD. We previously showed that the smoking cessation drug, cytisine reduces the ER stress response by chaperoning  $\beta$ 2-subunit containing ( $\beta$ 2\*) neuronal nicotinic acetylcholine receptors (nAChRs) from the ER to the plasma membrane. To assess the role of  $\beta$ 2\* nAChR upregulation in neuroprotection during PD, our lab created novel transgenic mice with constitutively upregulated  $\beta$ 2\* nAChRs. Analysis of ER exit sites needed for protein export showed upregulation and neuroprotection in transgenic female but not male mice. Since astrocytes and microglia play important roles in neurodegeneration, we sought to assess the reactivity profile of these glial cell types in a 6-hydroxydopamine (6-OHDA) model of parkinsonism in our transgenic mice. Female  $\beta$ 2\* wildtype (Wt), heterozygous (Het), and homozygous (Hom) mutant mice were further tested for expression of glial fibrillar acid protein (GFAP) and ionized calcium binding adaptor molecule 1 (IBA1) as markers of reactivity in midbrain astrocytes and microglia, respectively. We observed a significant increase in GFAP expression in the 6-OHDA lesioned side of Wt, Het and Hom mice. In addition, IBA1 showed no increase in expression for all three genotypes. These data suggest a relatively minor role for glial reactivity in the 6-OHDA model of parkinsonism in mice.

This work was supported by NIH R01 NS115809. AA was supported by NIH R25 DK12664. The authors acknowledge the assistance of the Integrated Microscopy and Imaging Laboratory at the Texas A&M School of Medicine. RRID:SCR\_021637.

#### SILVER SALT OF IBUPROFEN USED TO TREAT PSEUDOMONAS AERUGINOSA INFECTIONS

Jacob Boyd, Francesca Agobe, Ricardo Munoz, Carolyn Cannon Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

Pseudomonas aeruginosa is a ubiquitous gram-negative pathogen responsible for healthcare-associated infections, affecting various sites such as blood, lungs, and surgical wounds. It is resistant to multiple antibiotics in multiple different classes, which is significantly dangerous, as it can spread through contaminated hands, equipment, and surfaces, causing approximately 32,000 infections and 2,700 deaths in hospitalized patients. The plan to combat this bacterium is to use silver's bactericidal potential and ibuprofen in unison. We hypothesize that ibuprofen has bactericidal capabilities due to the NSAID improving the lung function in cystic fibrosis patients who possess P. aeruginosa in their lungs. During our study, we performed a Minimum Inhibitory Concentration (MIC) assay, the clinical standard of measuring antimicrobial treatment, with silver acetate before experimenting with silver ibuprofen. The subsequent assay we used was a growth curve assay to record the period of growth over time. We used the MIC assay to test free silver acetate against P. aeruginosa, and the growth curve assay tested three different concentrations of silver acetate against three other P. aeruginosa strains. I checked the concentration of bacteria by measuring the optical density every 2 hours after the initial measurement at 650 nanometers. The final measurement was recorded after 24 hours. The MIC assay resulted in 4 P. aeruginosa strains experiencing growth inhibition and death to low concentrations of silver acetate. Silver acetate managed to cause P. aeruginosa to experience growth inhibition and death at a concentration of 4ug/mL. As for the growth curve, one strain managed to survive one concentration of silver acetate, vet the following strains were killed by each concentration. The assessments of silver acetate show great promise for using silver ibuprofen against P. aeruginosa. The future steps of this project will involve using a delivery method that will allow silver ibuprofen to attack acute wounds infected with this bacteria without damaging this antibiotic in the process.

JB was supported by NIH R25 DK126642

#### EXAMINING HOW AGE AFFECTS STEM CELL GRAFTS AFTER SPINAL CORD INJURY

Lexi Carter, Erika C. Marks, Sarah Ingram, Jennifer N. Dulin, Cédric G. Geoffroy Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

Globally there are about 250,000 to 500,000 patients that suffer from spinal cord injury (SCI) each year. Most patients that suffer from SCI are in the age range from 16-30 years old, though there is an increasingly large population of individuals over the age of 65 being affected by SCI. Neural Stem Cells (NSCs) are multipotent cells that are commonly targeted to improve functional recovery after SCI due to their implications in helping regenerate severed axons, along with increasing connectivity between separated spinal cord segments when injected into the lesion site. The aim of this study is to determine how age will influence stem cell grafts after SCI. We hypothesize that with aging the stem cell graft will be significantly smaller than in the younger test group. Sample spines were acquired from juvenile (about 2-monthsold) or aged (20–24-months-old) mice that had a cervical dorsal transection with a GFP-positive stem cell graft at the time of injury. These spines were sectioned into 20-micron sections and then immunohistochemically stained and analyzed for GFP, a marker only found in the novel stem cells introduced into the injury site. Our results show that there was a significant graft size difference between the two ages. The aged mice had a smaller total graft size and fewer axonal projections than the juvenile mice. We concluded that age does have an effect on the stem cell grafts after spinal cord injury. Thus, even with the stem cell therapy age will contribute to SCI recovery.

LC was supported by NIH R25 DK126642

### THE SPLICING FACTOR HNRNP F PLAYS A CRITICAL ROLE IN REGULATING ANTIMICROBIAL RESPONSE IN MACROPHAGES

Nina M. Castaneda, Kaitlyn S. Armijo, Mackenzie H. Smith, Robert O. Watson, Kristin L. Patrick Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

Macrophages are professional innate immune cells that sense and respond to pathogen- and damage-associated molecular patterns (PAMPs and DAMPs). There are many factors involved in regulating antimicrobial responses in macrophages. Recent literature indicates that RNA binding proteins play key roles in the macrophage innate immune response and that they themselves are regulated, via differential phosphorylation, when macrophages encounter a pathogen. A recent report shows that heterogeneous ribonucleoprotein F (hnRNP F) is phosphorylated at serine 104 upon bacterial infection of macrophages. To explore the relationship between hnRNP F and macrophage antimicrobial responses, hnRNP F knockdown (KD) murine RAW 264.7 macrophage-like cell lines and human U937 cell lines were stimulated with various innate immune agonists. The agonists, including lipopolysaccharide (LPS), interferon stimulatory DNA (ISD), and polyinosinic-polycytidylic acid (poly I:C), were chosen to mimic different types of pathogens (i.e. gram negative bacteria, dsDNA viruses and dsRNA viruses). We isolated RNA from hnRNP F knockdown cells, alongside scramble (SCR) controls, and measured expression of pro-inflammatory cytokines and interferon stimulated genes at 0, 2, 4, 6, and 8 hours post-stimulation. We found that macrophages lacking hnRNP F failed to induce interferon stimulated genes in response to all three agonists, suggesting the target of hnRNP F is a shared component of these three pathogen sensing cascades. Our data also demonstrate that hnRNP F functions similarly in human and mouse macrophages. Taken together, our work identifies hnRNP F as a key player in mounting antimicrobial responses in macrophages.

KP is supported by NIH R35 GM133720. NC was supported by the Texas A&M School of Medicine.

#### LYMPHATIC ENDOTHELIUM-DERIVED MOLECULAR CUES ALTER RESPONSE TO CHEMOTHERAPY IN 3-DIMENSIONAL CHOLANGIOCARCINOMA SPHEROID MODELS

Anabella Cheong, Priyanka Banerjee, Niyanshi Gaddam, Sanjukta Chakraborty Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

Cholangiocarcinoma (CCA) is an aggressive, hepatobiliary cancer with less than 2% survival following metastasis to the draining lymph nodes. Tumor migration to nodes is significantly influenced by lymphatic endothelial cell (LEC)-derived factors that create a pro-metastatic niche currently unknown in the context of CCA. In this study, we first aimed to develop scaffold-free 3-dimensional (3D) cholangiocarcinoma spheroids and then measure response to CCA chemotherapeutic agents. Spheroids were established from MzChA1 and HuCCT1 CCA lines harboring patient-specific mutations then exposed to LEC-secreted factors in presence and absence of CCA chemotherapies pemigatinib, infigratinib, and gemcitabine+cisplatin. Growth of the spheroids were regularly measured over 10-14 days. RNA was isolated and modulations to tumor metabolism in response to LEC-derived factors was determined. Diameter tracking over 2 weeks revealed enhanced proliferation in spheroids grown in the presence of LEC-CM and confirmed that presence of the chemotherapies suppressed spheroid growth. However, spheroids grown in the presence of LEC-CM showed resistance to growth inhibition. Furthermore, we observed decreased guiescence in CM-treated spheroids indicating LEC-CM induces differentiation of quiescent cells towards a proliferative phenotype. Further, gene expression analysis of RNA isolated from these spheroids, showed a marked induction in redox, metabolic, and EMT genes. Our data suggest that tumors show differential response to prevalent CCA chemotherapeutics when exposed to LEC-derived cues and enhance our understanding of lymph node metastasis in CCA. The identified altered pathways will serve as a novel target for future therapies in the management of CCA progression and metastasis through lymphatics.

This work was supported by CPRIT RP210213 and RP230204 to SC. AC was supported by the Texas A&M School of Medicine.

#### TARGETING ADIPOSE TISSUE VOLUME EXPANSION IN LYMPHEDEMA

Sydney Criscitiello, Bailey Duhon, Thien Phan, Andie Reyna, Andy Chiu, Joseph Rutkowski Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

Approximately 20-30% of breast cancer survivors in the United States will develop lymphedema in their lifetimes. Lymphedema is a debilitating condition that results from disrupted lymphatic transport resulting from lymph node dissection during cancer surgery. Due to lymphatic insufficiency, the patient's arm exhibits chronic swelling and is prone to increased inflammation and infection; pain and quality of life are also significant clinical problems. One aspect of the tissue swelling is expansion of adipose tissue (fat) in the affected tissue. Here, we wanted to target adipose tissue reduction and health specifically to remediate limb volume. We utilized a well-established model of lymphedema in the mouse tail by making a circumferential incision that severs all dermal lymphatics while preserving the blood vasculature and underlying tendons of the mouse tail. First, we utilized "AdipoChaser" mice to genetically pulse label fat cells to determine if adipose expansion occurs due to increased fat cell number or increased fat cell size. Four weeks postsurgery tail samples were collected and immunolabeled for the genetic tag to define adipose fatty acid oxidation with the PPARa agonist fenofibrate or (b) improve adipose health with the PPARg agonist rosiglitazone. Fenofibrate had a significant effect on reducing overall lymphedema swelling and significantly reduced fat cell size while rosiglitazone had no impact on lymphedema volume. Pharmacologically targeting adipose tissue could therefore be a potential therapy to reduce lymphedema-associated tissue mass in breast cancer patients.

SC was supported by the Texas A&M School of Medicine

#### DEVELOPMENT OF TRANSCRIPTIONAL REPORTERS TO EXAMINE REGULATORY PATHWAYS IN TUBERCULOSIS

Diana Estrada, Lyle Tobin, Kent Koster, Aaron Benjamin, Kevin P. Francis, Jeffrey D. Cirillo Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

Tuberculosis is a respiratory infection that took over one million lives in 2021. Although research on tuberculosis has been ongoing for many years, large gaps in knowledge remain, most notably in the mechanisms of pathogenesis. *Mycobacterium tuberculosis* can adapt to and grow within different environments throughout the body. Although there is no prior evidence that this bacteria participates in quorum sensing, our lab has identified putative autoinducers in *M. tuberculosis*. The effects of these on mycobacterial gene regulation are unknown, yet their interactions could help us understand more about tuberculosis pathogenesis. To explore the effects of these autoinducers on gene expression, we developed a bioluminescence resonance energy transfer (BRET) reporter, composed of NanoLuc luciferase fused to a long Stokes shift fluorescent protein. This NanoBRET reporter will be moved into mycobacteria to further investigate promoters that are differentially regulated in environmental conditions. Once inserted into the *M. tuberculosis* genome, this reporter can be used to identify genes that are affected by the autoinducers. Due to the slow growth of *M. tuberculosis* and resistance to genetic manipulation, movement of this construct into mycobacteria is challenging. We will use a shuttle phasmid for phage transduction of a transposon to insert reporters first into the rapid-growing mycobacteria, *Mycobacterium smegmatis*. Once validated, this method will be applied to the slow-growing and pathogenic *Mycobacterium tuberculosis*.

This work was supported by NIH Al165913, Al149383, and EB032983 to JDC. DE was supported by NIH R25 DK126642.

#### INVESTIGATING GSDMD ASSOCIATION WITH THE MITOCHONDRIA

Stacy L. Hahn, Eduardo L. Martinez, Kristin L. Patrick, Robert O. Watson Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

The way an innate immune cell dies can dictate downstream inflammatory and immune outcomes. Our lab discovered that the pore forming protein and executioner of pyroptosis, gasdermin D (GSDMD), can initiate another cell death called necroptosis. This occurs when GSDMD associates with, and forms pores in, the mitochondrial network, causing mitochondrial damage and release of mitoDNA and mitoROS into the cytosol. Although certain mutations and stresses are associated with enhanced GSDMD-dependent necroptosis, the mechanism by which GSDMD is recruited to mitochondria to trigger necroptosis is unknown. We hypothesized that a cleavage event or a conformational change in GSDMD allows it to associate with the mitochondrial network. I set out to determine how three different GSDMD constructs associated with mitochondria in cells. Using immunofluorescence microscopy, I asked whether full-length GSDMD, an N-terminal fragment, and a full-length version engineered with a Prescission protease site colocalized with the mitochondria protein TOM20. Surprisingly, I found that the Prescission GSDMD, which is similar to the full-length allele except for an artificial cut site in the flexible linker, preferentially localizes to mitochondria. We believe that Prescission GSDMD has increased association with the mitochondria because of a conformational change that causes a special region to be exposed or prevents a repressor from binding. To determine the part of Prescission GSDMD that directly binds to mitochondria, I also cloned a set of GSDMD fragments. Future experiments will test whether these constructs associate with mitochondria and determine if mutating these regions can limit necroptotic cell death driven by GSDMD.

This work is supported by NIH R01 AI155621 and NIH R01 AI145287. SLH was supported by the Texas A&M School of Medicine.

#### PROFICIENCY OF INTERMITTENT FASTING FOR ALLEVIATING COGNITIVE IMPAIRMENT AND NEUROINFLAMMATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

#### Hannah Hernandez, Yogish Somayaji, Leelavathi N. Madhu, Bing Shuai, Maheedhar Kodali, Ashok K. Shetty Dept. of Cell Biology and Genetics Texas A&M School of Medicine College Station, Texas

Alzheimer's disease (AD) is characterized by neuroinflammation, extracellular amyloid plagues, and the hyperphosphorylation of tau within neurons, eventually leading to cognitive impairments. This study examined whether an intermittent fasting (IF) regimen would delay AD-associated cognitive impairment by restraining the progression of neuroinflammation. One-month-old 5xFAD mice, a mouse model of early-onset AD, were subjected to either the 16:8 fasting regimen (AD-IF group) or a standard ad libitum diet (AD alone group) for 11 months. Animals underwent an object location test (OLT), a hippocampus-dependent cognitive test, when they reached ten months of age, following which the extent of neuroinflammation and amyloid plagues were analyzed. Animals in the AD-alone group displayed significant cognitive impairment in the OLT. However, animals in the AD-IF group exhibited proficiency for object location memory, akin to animals in the age-matched naive control group. Better cognitive function in the AD-IF group was linked with reduced microglial clusters in the hippocampus. Moreover, mice in the AD-IF group displayed reductions in the percentage of microglia presenting NLR family pyrin domain containing 3 (NLRP3) inflammasome complexes and the concentration of IL-18 (a product of inflammasome activation) than mice in the AD-alone group. Such restrained neuroinflammatory events in AD-IF mice also reduced the amyloid plaque burden. The results suggest that the 16:8 IF regimen effectively delayed cognitive impairment in AD mice. Notably, such a beneficial effect on cognition was associated with reduced inflammasome activation in microglia leading to reduced amyloid plagues and IL-18, which likely prevented the hyperactivation of downstream inflammatory signaling pathways.

This work was supported by NIH AG074256 to AKS. HH was supported by NIH R25 DK126642.

#### EFFECT OF CIRCADIAN DYSREGULATION ON GUT PERMEABILITY

Lauren LaDuque, Morgan Jackson, David Earnest Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

The systems of the human body are incredibly interconnected and for many of them to function properly, good sleep is a necessity. Sleep is important for two reasons: the maintenance of circadian regulation of body systems and homeostatic regulation of sleep itself. The circadian rhythm allows organisms to regulate the physiological processes and anticipate any shifts. The homeostasis of sleep refers to the body's need to rest. Circadian dysregulation can result from shift work, jetlag, or lifestyle changes; however, shift work is the most prevalent cause in 20–24-year-old adults. Those placed in a shift work environment were expected to have increased gut permeability and dysbiosis. To test these long-term effects of shift work on the gut-brain axis, two groups of mice were placed on a 12:12 hour light/dark cycle. The "shift" group has their light/dark cycle shifted forward 12 hours every 5 days for 80 days, then returned to the original light/dark cycle until they were middle aged at 13 months old; The mice were then sacrificed, and their ileums were collected. To determine gut permeability, the villi and crypts found in the ileum were counted, measured, and compared between the two groups. We would expect the villi of the control group to be long and skinny and the shifted groups' to be shorter and broader with an increase in the number of crypts. This morphology is closely related to hyperpermeability and demonstrates changes to the gut barrier that alters gut-brain axis homeostasis.

LL was supported by the Texas A&M School of Medicine and the Air Force Academy

#### eEF2K REGULATES T-CELL RESPONSE DURING VIRAL INFECTION

Eunice Lara, Liqing Wang, Jianxun Song Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

Eukaryotic Elongation Factor-2 Kinase (eEF2K) is a structurally distinct member of the alpha-kinase family that contributes to both cell viability and proliferation making it crucial for cell survival under stressful circumstances. eEF2K phosphorylates eukaryotic elongation factor 2 (eEF2) and inhibits it which is essential to the biological system. However, how eEF2K interferes with T cell is barely investigated during viral infection. We hypothesize eEF2K will influence the effector T cell performance during viral infection. Our aim is to find a method to improve effector T cell function. In order to test our hypothesis, we intraperitonially injected wild type (WT) and eEF2K<sup>-/-</sup> mice with Vaccinia Virus (VACV) and analyzed the VACV-specific T cell alteration within 5 weeks in mixed spleens and lymph nodes samples. The tissues were smashed into single cell suspension then stained with CD8 antibody and VACV B8R<sup>+</sup> tetramer. After being stained, flow cytometry was used to view and analyze the difference in T cells between the wild-type and eEF2K<sup>-/-</sup> mice had a higher percentage of CD8<sup>+</sup> B8R on day 14 than the WT mice during viral infection. In the future, we hope to use eEF2 inhibitors to boost the immune response and will see to improve effector T cell killing activity and improve T-based immunotherapy efficiency to treat cancer or viral infection.

EL was supported by NIH R25 DK126642

#### AGE-RELATED ALTERATIONS IN MICROGLIAL NEUROINFLAMMATION IN THE BRAIN

Eshan Manchanda, Madeline Huber, Xin Wu, D. Samba Reddy Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

Microglia are resident cells of the brain that regulate brain development, maintenance of neuronal networks, and injury repair. They are activated for injury & immune response or changes in brain homeostasis. Remarkably, microglia exhibit polarization states, M1 (pro-inflammatory) and M2 (anti-inflammatory), which repair cortical damage by releasing various immune factors. While microglia proliferate early and continuously screen the environment for injury, relatively little research has been conducted on how microglial response varies from pediatric to geriatric age. In this study, we analyzed the distribution pattern and basal density of microglial cells in 4 developmental stages. Rat pups were perfused postnatal at 21 days (p21), 28 days (p28), 90+ days (p90, adult), and aged (12 mo). Coronal brain slices were stained with IBA1(+) to identify and analyze microglia via immunohistochemistry. Area fraction of particles to the total area of the cells was examined using the NIH Image J software. The IBA1(+) expressing microglia are distributed in the hippocampus subfields CA1, CA3 and the dentate gyrus (DG) regions. There was greater IBA1(+) expression in adult and aged as compared to pediatric rats. Histological analysis showed a similar level of expression of IBA1(+) microglia in adult and aged rat brain. These studies demonstrate the age-dependent distribution of microglia in the pediatric, adult and aged rat brain, with implications in neuroinflammatory brain disorders.

This work was supported by NIH U01 NS117209 to DSR

#### FEMALE AND MALE SELF ADMINISTRATION OF THE CANNABANOID CB1 RECEPTOR AGONIST WIN 55,212-2 IN MICE

Mallory McDaniel, Yuhong Guo, Laura N. Smith Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

We have established a baseline mouse model of cannabinoid addiction using both male and female mice, as well as using the CB1 receptor agonist WIN 55, 212-2. While this model has previously been established in male mice, the use of female mice is a novel method. This model allows testing of similar cannabinoid agonists in order to evaluate addictive properties. Male and female C57BL/6J mice were surgically catheterized and trained to intravenously self-administer (IVSA) either WIN 55, 212-2 or a vehicle solution. Each mouse completed a ten-day acquisition period, where comparative frequencies of responses were recorded. Persistence of drug-seeking behavior was then measured during an extinction period. During a progressive ratio program, motivation to receive WIN 55, 212-2 was measured. During each of these programs, significant differences were found between mice receiving WIN 55, 212-2 and vehicle, as well as differences between sexes of mice. Future directions for this project including using this model to investigate addictive properties of experimental compounds that also bind to the CB1 receptor, in the hopes of developing a non-opioid method of relieving pain.

This work was supported by NIH 1UG3 NS128439-01. MM was supported by the Texas A&M School of Medicine.

#### POLARIZING MACROPHAGES TOWARDS AN ANTI-INFLAMMATORY (M2) PHENOTYPE ATTENUATES BLOOD PRESSURE AND TESTICULAR INFLAMMATION IN MICE WITH SALT-SENSITIVE HYPERTENSION

Robert Montalvo, Shobana Navaneethabalakrishnan, Hannah Smith, Bethany Goodlett, Brett Mitchell Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

Hypertension (HTN) affects about half of the U.S. population and leads to end organ damage. We recently reported that HTN is associated with testicular inflammation, inflammation-associated lymphangiogenesis, and dysfunction. However, it is unclear whether the increase in pro-inflammatory (M1) macrophages in the testes causes reproductive dysfunction. We hypothesized that polarizing macrophages towards an anti-inflammatory (M2) phenotype will reduce testicular inflammation and damage. Male mice of 10–14 weeks of age were made hypertensive by providing nitro-L-arginine methyl ester hydrochloride (L-NAME; 0.5 mg/ml), in the drinking water for 2 weeks, followed by a 2-week washout period, and a subsequent 3-week high-salt diet (SSHTN). Another group received AVE0991 (0.58 nmol/g) through daily i.p. injections during the last 3-week high salt diet (AVE). Control mice received a normal diet and tap water *ad libitum*. We observed a significant decrease in systolic blood pressure in the AVE group when compared to the SSHTN group. Flow cytometric analysis showed a significant decrease in testicular M2 macrophages were increased in the AVE treated mice compared to the SSHTN group. Gene expression analysis revealed a significant reduction in inflammation and inflammation-associated lymphangiogenesis in the testes of AVE mice when compared to SSHTN mice. In addition, testicular function was also improved in AVE treated mice. Together, these results support our hypothesis and could be a basis for the development of therapeutic strategies to improve the reproductive health of male patients with SSHTN.

RM was supported by NIH R25 DK126642

#### IMPAIRMENTS OF LYMPHATIC VESSEL STRUCTURE AND FUNCTION ARE ASSOCIATED WITH DUCHENNE MUSCULAR DYSTROPHY

#### Ilse M. Paredes Mares, Bhuvaneshwaran Subramanian, Shedreanna Johnson, Akshaya Narayanan, Peter Nghiem, Mendell Rimer, Mariappan Muthuchamy Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

Duchenne Muscular Dystrophy (DMD) is a genetic disease characterized by progressive muscle degeneration due to lack of functioning dystrophin protein; DMD affects 1 in 5000 boys worldwide. The continued muscle cell damage distinctive of DMD leads to chronic inflammatory pathogenesis because of prolonged exposure to intracellular leakage after muscle contraction. Impairment of the normal lymphatic vessel functions, including the transport of immune cells as part of immune responses, is correlated with the pathogenesis of inflammatory diseases. The importance of lymphatics in the inflammation typical of DMD is least explored. We analyzed the structure and function of lymphatics in a D2.*mdx* mouse model. Quantitative RT/PCR analyses showed an increase in inflammation markers and in lymphatic markers in the 8 weeks old D2.*mdx* mice when compared to DBA controls. Micro-lymphangiography and isolated lymphatic vessel studies exhibited a delay in lymph transport and found that lymphatic function was impaired in the D2.*mdx* mice. Immunohistochemistry staining of skeletal muscles further revealed an increase in Lyve1+ lymphatic vessels, demonstrating inflammatory lymphangiogenesis present in DMD. Thus, our data show first evidence that lymphatic structure and function are compromised in DMD animals.

This work was supported by NIH R01 AR080129 to MM. IPM was supported by NIH R25 DK126642.

#### **RESTRICTION ENZYME-MEDIATED CLONING OF METTL-14 GENE FRAGMENTS FOR FUTURE MECHANISM STUDIES**

Alejandro Perez, Reed Rivera, Yu Xi, Xu Peng Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

Angiogenesis is the formation of new blood vessels from pre-existing ones and plays a crucial role in embryonic development. Vascular endothelial cells (ECs) line the whole vascular system and play central roles in angiogenesis. Recently, studies have documented that mRNA modification can affect RNA stability, localization, splicing, and protein translation. However, how mRNA modification is involved in angiogenesis remains largely unknown. N6-methyladenosine (m6A) modification is the most prevalent and site-specific modification on mRNA and is controlled by "writer" complex proteins, including Methyltransferase-like 14 (METTL14). To study the role of m6A modification in new blood vessel formation, we have created a vascular endothelial cell specific knockout mouse line by crossing METTL-14 flox mice with Tie2-Cre mice. Our preliminary data showed that the inactivation of METTL14 resulted in embryonic lethality before embryonic day 12.5. Whole-mount staining showed that the inactivation of METTL14 interfered with blood vessel formation and caused hemorrhage. Our immunoprecipitation-Mass spectrometry analysis results suggested that focal adhesion kinase (FAK) is a binding partner of METTL14. We validated the interaction between METTL14 and FAK in cultured Ad193 cells. To further determine how METTL14 and FAK interact, we have cloned different METTL14 will interact in the future.

This research was supported by American Heart Association 19TPA34900011 to XP. AP was supported by NIH R25 DK126642.

#### GENERATING A MOUSE MODEL OF HEART FAILURE WITH PRESERVED EJECTION FRACTION

Calissa Perry, Patrick DeSpain, Lilly McAlister, Ashley Kidwell, Carl Tong Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

Heart failure (HF) occurs when the heart cannot provide sufficient blood flow to the body. HF can occur with preserved left ventricular ejection fraction (LVEF)  $\geq$  50%, named HFpEF. HFpEF now accounts for ~ 50% of all HF diagnoses. Many HFpEF patients have antecedent hypertension and obesity. HFpEF currently lacks effective treatment. Thus, an animal model that mimics human HFpEF is needed to explore potential treatments. We hypothesize that obesity and vascular dysfunction can cause HFpEF. To test this idea, we fed mice with a high fat diet and drinking water with L-NAME (HFDwL), then followed with serial echocardiography every 5 weeks for a total of 15 weeks. Echocardiography e' is tissue Doppler of peak myocardial relaxation velocity during early diastole which slows with worsening diastolic dysfunction and E is Doppler of peak blood inflow velocity during early diastole. At 10 weeks, there was no difference in LVEF between groups; however, HFDwL hearts exhibited increased LV wall thickness and diastolic dysfunction (slower e' and higher E/e'): LV posterior wall thickness (mm) HFDwL 3.4 ± 0.089 vs. Control 3.88 ± 0.12, p=0.009; e' (mm/s) HFDwL - 19.64 ± 1.81 vs. Control -30.31 ± 1.65, p=0.001; E/e' HFDwL -31.14 ± 4.0 vs. Control -19.66 ± 1.93, p=0.039. At 10 weeks, HFDwL mice with severe diastolic dysfunction died to lessen the mean difference between HFDwL and control at 15 weeks. HFDwL induced a phenotype resembling HFpEF. A future direction is to rescue HFDwL HFpEF mice with gene therapy of expressing phosphorylated cardiac myosin binding protein-C.

This work was supported by HL145534 to CT. CP was supported by the Texas A&M School of Medicine.

#### THE EFFECTS OF REAL AND SIMULATED MICROGRAVITY ON MOUSE LIVER CELLS

Marion Posner, Jordan Faust, Lynn Herron, John Schock, David Zawieja, Shannon Glaser Dept. of Medical Physiology Texas A&M School of Medicine Bryan, TX

The liver is one of the human body's most vital organs, supporting metabolic and immune functions. It is comprised of multiple cell types, including hepatocytes and cholangiocytes. Cholangiocytes are heterogeneous epithelial cells lining bile ducts and maintaining liver homeostasis. When homeostasis is disrupted, often following liver damage, ductular reaction (cholangiocyte proliferation) is observed. This reaction contributes to inflammation and fibrosis. The study's aim was to evaluate histological and gene expression changes in mouse livers during spaceflight. Mice were housed in three environments: two control groups, vivarium and ground, and one experimental group, flight. Vivarium and ground mice experienced conditions under standard gravity (9.8 m/s<sup>2</sup>). Ground and flight mice were housed in identical cages, with flight mice being sent to NASA's ISS for 38.5 days. To measure spaceflight's impact on the liver, immunohistochemistry (IHC) for CK-19 (biliary mass), F4/80 (inflammation) and Oil Red O (lipid deposition) was carried out in liver sections and qPCR gene profiles were evaluated in total liver samples. Gene profiles involved adipogenesis (Acac $\alpha$  and Ppar $\gamma$ ) and proliferation markers (Ki67 and PCNA). There was an increase in ductular reaction, fat deposition, and macrophage number in flight mice compared to the control groups, there was an increase in adipogenesis and proliferation gene expression. Therefore, it can be concluded that microgravity and space radiation may play a role in altered liver metabolic and proliferative states during space flight.

MP was supported by the Texas A&M School of Medicine and the Air Force Academy

#### RNA-SEQ ANALYSIS OF EPILEPTOGENIC GENES IN A MOUSE MODEL OF POST-TRAUMATIC EPILEPSY

Richard Rasulis, Sreevidhya Ramakrishnan, D. Samba Reddy Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

Post-traumatic epilepsy (PTE) follows an initial traumatic brain injury (TBI) and can develop years after the initial incident. Because PTE has a diverse and unpredictable prognosis, it can be difficult to treat, and many PTE patients prove to be treatment refractory. This study examines patterns in differential expression ontology between sham and TBI groups of controlled cortical impact (CCI) acute injury phase cortex and hippocampal RNA-seq data, as well as examines the relationship between changes of expression and epileptogenic-related genes. We utilized *in silico* methods to analyze 7-day post-TBI RNA-seq data that had been generated following isolation, creation of a cDNA library, next-generation sequencing, and quantification of transcripts. Across 49,315 genes, normalized transcript counts were analyzed using R, and ontological analysis was done using open-source databases from the Gene Ontology (GO) consortium and the Kyoto Encyclopedia of Genes and Genomes (KEGG). Results indicated that there was a high degree of heterogeneity in expression between TBI and sham groups for both the hippocampus and cortex with respect to the number of transcripts produced, although there were comparatively fewer differences in the types of genes expressed. KEGG enrichment showed an increase in the proportion both nervous system, synapse and action potential related genes, and signal transduction, reception and amplification of signaling molecule related genes. Pro and anti-epileptogenic genes based on previous studies were identified in the hippocampus and cortex. This study indicates that widespread signal transduction gene biomarkers of PTE can occur following TBI.

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#### INTRAPERITONEAL IGF-1 IMPROVES LOCOMOTOR AND WEIGHT RECOVERY, BUT NOT COGNITION, IN MALE SPINALLY CONTUSED RATS

#### Leonel Santoyo Herrera, Alex Stefanov, Glenae Nora, Michelle A. Hook Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

There are approximately 300,000 people living with spinal cord injury (SCI) in the United States alone, and it is estimated that up to 60% of these people suffer cognitive deficits. People living with SCI report impairments in memory, learning, and problem solving. Another prevalent consequence of SCI is gut impairment, and recent research has shown that alterations in gut morphology are associated with cognitive deficits in CNS injury models. We investigated whether administration of insulin-like growth factor 1 (IGF-1), which has been shown to improve pathological gut barrier integrity, could protect cognition after SCI. Male Sprague-Dawley rats received either a moderate T12 spinal contusion injury (SCI), or laminectomy (shams). Half of the rats in each group were treated with IGF-1 and half with vehicle. Locomotor function, weight gain and cognition (spatial learning, working spatial memory, cognitive flexibility, short- and long-term recognition memory, long-term location memory) were assessed for 60 days post injury. We found that IGF-1 reduced body weight loss and increased locomotor recovery after SCI. Interestingly, however, sham and SCI rats administered IGF-1 also had increased systemic expression of both anti- and pro-inflammatory cytokines at 2 days post injury compared to pre-injury baseline. Moreover, IGF-1 administered SCI rats did not show an improvement in cognition compared to the vehicle-treated SCI group. These data indicate that although improving gut morphology had a positive impact on certain aspects of recovery, it might not be the key to solving cognitive dysfunction after spinal injury.

This work was supported by a generous gift from the Woodnext Foundation. LSH was supported by NIH R25 DK126642.

#### AGE-DEPENDENT ANALYSIS OF BEHAVIORAL DEFICITS AND DOPAMINERGIC NEURODEGENERATION IN A PARKINSONIAN RAT MODEL OF 6-HYDROXYDOPAMINE

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc). As of 2023, PD affects approximately 500,000 individuals in the United States alone and is generally thought to be a disease of aging. However, we do not know if 6-hydroxydopamine (6-OHDA), a commonly used neurotoxin for PD animal models has similar neurodegenerative effects in young and older rats. To address this question, young adult (5-7m) and middle-aged (9-11m), male Sprague Dawley rats were stereotaxically administered 6-OHDA to the dorsolateral striatum. Subsequently, we quantified the number of contralateral rotations in these rats for 15 minutes following intraperitoneal administration of 1mg/kg apomorphine. Rotations were recorded at pre, 7 days post (DP), 14DP, 21DP, and 28DP. At 30DP, animals were sacrificed and tyrosine hydroxylase (TH), a marker for DA neurons, ionized calcium-binding adapter molecule 1 (Iba-1), and glial fibrillary acidic protein (GFAP) were quantified using immunohistochemistry in the midbrain SNc to assess dopaminergic cell loss, microglial presence, and astrocytic activity, respectively. We observed a progressive increase in the number of apomorphine-induced contralateral rotations in both age groups. Additionally, in both age groups of rats, we observed similar levels of DA cell loss, increased microglial activation, and enhanced astrocytic reactivity in both age groups following 6-OHDA. These findings demonstrate that 6-OHDA causes similar levels of DA neurodegeneration in young adult and middle-aged male rats.

This work was supported by the John and Maurine Cox Endowed Chair grant to FS and TAMU funds to RS. AS was supported by the Texas A&M School of Medicine.

#### CORONAVIRUS NSP-15 IMMUNE EVASION VIA RETINOBLASTOMA BINDING MOTIF

Emma Shoemaker, Daniela Hoyos Cardona, Julian Leibowitz Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

STING protein induces an interferon response after cytosolic DNA detection by cyclic GMP-AMP synthase (cGAS) which is inhibited by two distinguished oncogenic DNA viruses, human papillomavirus (HPV) and human adenovirus (Had). The HPV E7 and Had E1A proteins bind the Retinoblastoma protein (pRb) and antagonize STING by their respective LXCXE/D domains. However, RNA viruses, such as Dengue Virus (DENV), have demonstrated STING-dependent induction of interferons and pathway antagonization by STING protein cleavage. Furthermore, the conserved coronavirus NSP-15 protein, an endonuclease that contributes to evasion of dsRNA detection by MDA-5 and RIG-I, also contains a similar LXCXE/D domain (LWCKD). Therefore, the pRb binding domain in NSP-15 may bind and antagonize STING comparably to the DNA virus proteins. A previously designed NSP-15 mutant with a compromised pRb binding domain of LXCXA (LC9) was utilized alongside a wild type NSP-15 (WT) Mouse Hepatitis Virus (MHV) to construct a growth curve with infected STING (+) and STING (-) macrophages. The STING (-) cells retained a 100-fold greater MHV titer than the STING (+) samples, with the LC9 and WT titers exhibiting similar growth and kinetics in each of the cell lines. A subsequent co-immunoprecipitation of lysates of 293T-HEK cells transfected with WT, LC9, and STING expression vectors revealed NSP-15 WT and LC9 proteins bind to STING, consistent with the growth curve. Future work entails the construction of a non-binding NSP-15 mutant, transfection experiments to demonstrate *in vivo* co-localization, and a follow-up macrophage experiment to determine if NSP-15/STING interaction elimination results in viral attenuation.

ES was supported by the Texas A&M School of Medicine

#### KEY ANTIVIRAL INTERFERON STIMULATED GENE (ISG) PROTEINS ARE INEFFECTIVE AGAINST HERPESVIRUS INFECTION OF PLACENTAL TROPHOBLASTS COMPARED TO LOW LEVEL CONSTITUTIVE IFN-III EXPRESSION

Kaitlyn Skeen, K. Yeon Choi, Alistair McGregor Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX

The guinea pig represents an important model for congenital viral infection, but antiviral innate immunity and interferons (IFNs) are poorly evaluated for this animal. Trophoblast cells can respond to both IFN-I and IFN-III, but fibroblasts lack an IFN-III receptor and are only stimulated by IFN-I. A core set of ISGs are upregulated by both IFN-I and IFN-III and a subset of ISGs have direct antiviral activity. Microarray studies defined the core IFN-I response for guinea pig cells. Subsequently, highly upregulated (50-200-fold) key antiviral ISGs were generated as codon optimized synthetic genes in expression plasmids for evaluation of antiviral activity against herpes simplex virus (HSV-1). ISGs included guinea pig MX1, MX2, RSAD2, ISG15, IFI27, IFIT3 and IFIT5. Western blots of plasmid transfected guinea pig cells verified expression of ISGs. Guinea pig trophoblast and fibroblast cells were transfected with ISG expression plasmids in various combinations and subsequently infected with HSV-1 (moi 0.5 pfu/ml). At 48 hours post infection wells were harvested for virus titration. Additionally, a trophoblast cell line was transduced to permanently express IFN-III (Tepi-IFNL). Individual ISGs had potent antiviral activity against HSV on fibroblasts (3-4 log reduction) but individually or in combination were less effective on trophoblasts (0-1 log reduction). In contrast, Tepi-IFNL cells were refractile to virus infection as were trophoblasts treated with low level IFN-I (100 IU/mI). Overall, constitutive expression of low level IFN-III is an effective antiviral environment for the placenta, but trophoblast antiviral activity is not dependent upon antiviral ISG proteins that are effective in fibroblasts.

This work was supported by NIH R01 HD090065 and NIAID R01 AI155561. KS was supported by the Texas A&M School of Medicine

#### HIGH FREQUENCY DMs STIMULATION TRIGGERS LOCAL DOPAMINE RELEASE VIA CHOLINERGIC INTERNEURONS

Harish Sontam, Himanshu Gangal, Ruifeng Chen, Jun Wang Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

The neural mechanisms underlying repetitive behaviors in the absence of a reward are not well understood. In rodentbased behavioral studies, it has been shown that stimulation of striatal direct pathway medium spiny neurons (dMSNs) is reinforcing; however, the reason why the animal chooses to progressively self-administer dMSN stimulation is unclear. This study is aimed to investigate whether the dMSN self-stimulation behavior, which is a reward free behavior, utilizes the dopaminergic system. We hypothesize that dMSN stimulation can trigger local dopamine release by the transient excitation of cholinergic interneurons. Additionally, we hypothesize that this local dopamine release supports the dMSN self-stimulation behavior. Firstly, we found that D1-Cre rats, infused with AAV-FLEX-Chrimson in the dorsomedial striatum, exhibited robust optogenetic self-stimulation of striatal dMSNs. Slice recordings from D1-Cr;Ai32;ChAT-eGFP mice revealed that burst stimulation of dMSNs causes a picrotoxin-dependent pause rebound firing in cholinergic interneurons. Additionally, dMSN stimulation also caused a substance P dependent increase in cholinergic interneuron activity. Separately, in ChAT-Cre;Ai32 mice, we found that excitation of cholinergic interneurons leads to local dopamine (DA) release via nicotinic receptors on the dopaminergic terminals. This suggests that dMSN excitation can trigger local dopamine release via potentiating the activity of cholinergic interneurons, which facilitates the dMSN self-stimulation behavior.

This work was supported by NIAAA R01 AA021505 and U01 AA025932 to JW. HS was supported by the Texas A&M School of Medicine.

#### DESIGN AND VALIDATION OF PRIMERS FOR QRT-PCR-BASED QUANTIFICATION AND COMPARISON OF TRANSCRIPT LEVELS OF GENES THAT ORCHESTRATE EPITHELIAL TO MESENCHYMAL AND/OR MESENCHYMAL TO EPITHELIAL TRANSITION

Felix Tabe, Deepa Upreti, and Rajesh Miranda Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, Texas

Prenatal alcohol exposure (PAE) impairs the growth of developing embryos and fetuses, resulting in a wide range of adverse outcomes, including craniofacial deformities, growth deficiencies, central nervous system dysfunctions, and neurobehavioral impairments, collectively termed fetal alcohol spectrum disorders (FASD). MicroRNAs (miRNAs) comprise a subset of 21-23nt non-protein coding RNAs and are known to coordinate appropriate embryonic development. Our lab has previously identified maternal plasma miRNAs that are altered with PAE, which interfere with placental development and predict future growth deficits and/or neurodevelopmental delay in newborn children and mice. Our overarching objective is to identify mechanisms of maternal miRNA action on placental trophoblast maturation and function. An immediate goal was to design and validate a set of primers for qRT-PCR based quantification and comparison of transcript levels of genes that orchestrate Epithelial Mesenchymal Transition (EMT) and/or Mesenchymal to Epithelial transition (MET). For validation of primers, thermal stability analysis of amplicons generated by each primer pair were assessed for evidence of a single amplicon, and the amplicon was size-fractionated by gel electrophoresis and sequenced to confirm amplicon length and identity. We have designed 17 sets of primers and validated 10 of these (7 of them are currently being tested). We also used an established human trophoblast cell culture model to characterize the effects of miRNAs and alcohol on trophoblast growth, and EMT/MET-like behaviors. Our findings will contribute to the understanding of how maternal circulating miRNAs affect placental development resulting in FASD and could yield novel therapeutic targets to alleviate PAE's impact.

This work was supported by NIH R01 AA029594 to RCM. FT was supported by NIH R25 DK126642.

#### A SENSOR BIOASSAY FOR THE INTERROGATION OF PUTATIVE AUTOINDUCERS IN MYCOBACTERIUM TUBERCULOSIS

#### Lyle Tobin<sup>1</sup>, Kent Koster<sup>1</sup>, Aaron Benjamin<sup>1</sup>, Ken Hull<sup>2</sup>, Dan Romo<sup>2</sup>, Jeffrey Cirillo<sup>1</sup> <sup>1</sup>Dept. of Microbial Pathogenesis and Immunology Texas A&M School of Medicine Bryan, TX <sup>2</sup>Department of Chemistry and Biochemistry Baylor University Waco, TX

Two putative novel autoinducers have been identified in *Mycobacterium tuberculosis*. Their structures have not been fully identified and validated. Ongoing studies seek to isolate the compounds from *Mycobacterium tuberculosis* culture supernatant using liquid chromatography and sensor bioassay-guided fractionation. Our bioassay utilizes the  $\gamma$ -butyrolactone quorum sensing pathway in *Streptomyces coelicolor*, an actinomycete closely related to the genus *Mycobacterium*. Here, we present work on purifying these samples via reverse-phase HPLC. Peaks believed to correspond to the novel autoinducers were collected separately in fractions and analyzed for quorum sensing activity with our sensor bioassay. Further analysis is required to interrogate which peaks possess compounds that can activate quorum sensing pathways. This will enable us to purify sufficient quantities of the compounds to obtain the complete structures. Once identified, these compounds can be artificially synthesized for investigation of their effect on mycobacterial quorum sensing.

This work was supported by NIH Al165913, Al149383, and EB032983. LT was supported by the Texas A&M School of Medicine.

#### SPINAL CORD INJURY AFFECTS HIPPOCAMPAL NEUROGENESIS IN MICE

Aiden Varnell, Erika C. Marks, Ashley J. Douthitt, Cédric G. Geoffroy Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

As of 2023 there are 302,000 people living with spinal cord injuries (SCI) in the United States. Between 40 to 50% of patients with SCI experience cognitive impairment, with a significantly higher risk of developing dementia in the future when compared to their healthy counterparts. The mechanism in which SCI affects cognition is currently unknown, however we hypothesize that SCI reduces adult neurogenesis leading to the cognitive defects previously observed. The objective of this study is to identify if there is a change in neurogenesis after SCI, and whether treatments of naturally occurring gut metabolites Indole and IPA mitigate these differences. Brains were collected 6 weeks post-surgery after a sham injury, or a moderate T8 contusion compression injury with daily administration of indole, IPA, or vehicle control. The histology of the hippocampus was analyzed, with specific focus on doublecortin (DCX), a microtubule associated protein known for staining immature neurons. Total DCX positive neurite length was quantified using the Imaris filament tracer, and the number of immature neurons was manually counted. We expect to see a decrease in average neurite length and total number of cells after SCI compared to the sham and a rescue of these effects in the indole and IPA treated groups.

AV was supported by the Texas A&M School of Medicine

#### AGE-RELATED ALTERATIONS IN GABAERGIC INHIBITORY INTERNEURON PATTERNS IN THE BRAIN

Neo Zhu, Madeline Huber, Xin Wu, D. Samba Reddy Dept. of Neuroscience and Experimental Therapeutics Texas A&M School of Medicine Bryan, TX

GABAergic inhibitory interneurons play a crucial role in regulating neuronal network oscillations, synchronizing principal neuron networks, and ensuring precise synaptic transmission in both the hippocampus and other brain regions. Nevertheless, the plasticity of developmental neurogenesis from pediatric to aged stage remains poorly understood. This research aimed to investigate the impact of age on the distribution of parvalbumin-positive (PV+) GABAergic interneurons in the brains of male rats, spanning from pediatric (P21), adult (3 month) to aged (12 month) stages. The distribution of interneurons in the hippocampal and extrahippocampal regions were studied by using unbiased neurostereology and counting techniques. There was a significant (~35%) loss of interneuron distribution in hippocampal subfields CA1, CA3, and dentate gyrus among the adult and aged rats when compared to the p21 age group. In contrast, the total volumes of the hippocampus showed a ~34% increase in adult and aged rats compared to P21. Furthermore, there was a significant (~40%) decrease in interneurons within the extrahippocampal regions, observed in both adult and aged rats when compared to the P21 group. Additionally, in the aged group compared to the adult, there was a markedly lower number of inhibitory interneurons than the pediatric brain. These findings have significant implications for age-related diseases, such as epilepsy, ADHD, and other developmental brain diseases.

This work was supported by NIH U01 NS117209 to DSR

#### Acknowledgements

The Texas A&M School of Medicine's Summer Research Program (SRP) continues to attract the top students from the best colleges and universities across the country. This year we had 27 participants who completed the ten-week program and two Air Force Academy Cadets who participated for five weeks. These students were selected from a large pool of applicants based on their research experience, desire to attend graduate/medical school, grades, and recommendations. I would like to thank the selection committee who dedicated their time.

I would also like to thank the faculty that gave their time as mentors. You have provided each of these students with a valuable experience that will undoubtedly help them achieve their career goals.

The SRP was made possible by the following who provided funding and programmatic support:

- Amy Waer, M.D. Dean
- Paula Shireman, M.D., M.S., M.B.A. Executive Associate Dean
- Allison Rice-Ficht, Ph.D. Interim Senior Associate Dean of Research
- National Institute of Diabetes and Digestive and Kidney Diseases (R25DK126642)
- Fernando Vasquez, M.A. Assistant Dean of Admissions
- Samba Reddy, Ph.D. Director, Institute of Pharmacology and Neurotherapeutics
- Air Force Academy Cadet Summer Research Program

The following faculty selected eligible students to represent their school in the NIDDK sponsored **Developing and Readying Underrepresented Minority Researchers (DRUMR)** SRP:

- Laura Weiser-Erlandson, Ph.D. Texas A&M University Central Texas
- Venu Cheriyath, Ph.D. Texas A&M University Commerce
- Riccardo Mozzachiodi, Ph.D. Texas A&M University Corpus Christi
- Richard Laughlin, Ph.D. Texas A&M University Kingsville
- Dennis Daniels, M.P.H, Dr. PH Prairie View A&M University
- Malin Lilly, Ph.D. & Chris Mares, Ph.D. Texas A&M University San Antonio
- Michael Kidd, Ph.D. Texas A&M University International
- Mike Huggins, Ph.D. Tarleton State University
- Nurul Alam, Ph.D. & David Allard, Ph.D. Texas A&M University Texarkana
- Neil Terry, Ph.D. West Texas A&M University

Each week, we had Roundtable Discussions in which participants got to individually engage with faculty. Also, thank you to **Dr. Dianne Kraft**, **Dr. Patricia Watson**, and **Dr. Rajesh Miranda** for leading the weekly Diversity in Biomedical Research Roundtable Discussions.

Finally, I would like to thank the SRP Coordinator **Stacy De Leon**, **Caroline Emery**, and **Meray Lewis** who did a lot of work arranging the arrival, housing, registration, processing, and the weekly meetings. Thank you to our poster judges who had an extremely difficult task of picking the best out of the best. Thank you, students, for your hard work and for a memorable summer – Gig 'em!

Zatt & Lichel

Brett Mitchell, Ph.D., F.A.H.A. Director, Texas A&M School of Medicine Summer Research Program

#### 2023 Texas A&M School of Medicine Summer Research Program Participants

Junyoung Ahn	Texas A&M University	Dr. Carolyn Cannon
Andrea Alarcon	Texas A&M University – International	Dr. Rahul Srinivasan
Jacob Boyd	Prairie View A&M University	Dr. Carolyn Cannon
Alexis Carter	Tarleton State University	Dr. Cedric Geoffroy
Nina Castaneda	University of Texas – Austin	Dr. Kristin Patrick
Anabella Cheong	Williams College	Dr. Sanjukta Chakraborty
Sydney Criscitiello	Case Western Reserve University	Dr. Joseph Rutkowski
Diana Estrada	Tarleton State University	Dr. Jeffrey Cirillo
Stacy Hahn	Wake Forest University	Dr. Robert Watson
Hannah Hernandez	Texas A&M University – San Antonio	Dr. Ashok Shetty
Lauren LaDuque	Air Force Academy	Dr. David Earnest
Eunice Lara	Texas A&M University – Kingsville	Dr. Jim Song
Eshan Manchanda	Texas A&M University	Dr. Samba Reddy
Mallory McDaniel	Harvard College	Dr. Laura Smith
Robert Montalvo	Texas A&M University – Corpus Christi	Dr. Brett Mitchell
Ilse Paredes Mares	West Texas A&M University	Dr. Mari Muthuchamy
Alejandro Perez	Texas A&M University – San Antonio	Dr. Xu Peng
Calissa Perry	University of Arizona	Dr. Carl Tong
Marion Posner	Air Force Academy	Dr. Shannon Glaser
Richard Rasulis	Texas A&M University	Dr. Samba Reddy
Leonel Santoyo Herrera	Texas A&M University – Commerce	Dr. Michelle Hook
Aryan Shaik	Texas A&M University	Dr. Rahul Srinivasan
Emma Shoemaker	Baylor University	Dr. Julian Leibowitz
Kaitlyn Skeen	University of Mississippi	Dr. Alistair McGregor
Harish Sontam	Texas A&M University	Dr. Jun Wang
Felix Tabe	Texas A&M University – Commerce	Dr. Rajesh Miranda
Lyle Tobin	University of Mississippi	Dr. Jeffrey Cirillo
Aiden Varnell	Austin College	Dr. Cedric Geoffroy
Neo Zhu	Texas A&M University	Dr. Samba Reddy

#### 2023 Texas A&M School of Medicine Summer Research Program Seminar Series

Date	Time	Торіс	Presenter	
5/22	9:00 AM	Orientation and Laborato	ory Safety Training	
5/25	9:00 AM	URM Roundtable Discussion	Dr. Rajesh Miranda	
5/26	9:00 AM	TAMU School of Medicine MS and PhD	Dr. Brett Mitchell	
5/30	12:00 PM	TAMU School of Medicine MD Program	Mr. Fernando Vasquez	
6/1	9:00 AM	URM Roundtable Discussion	Dr. Patricia Watson	
6/2	9:00 AM	RCR – Data Management	Dr. Brett Mitchell	
6/6	12:00 PM	CST*R Grand Rounds	MD/PhD Students	
6/8	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
6/9	9:00 AM	RCR – Transparency & Reproducibility	Dr. Brett Mitchell	
6/13	12:00 PM	RCR – Animal Subjects	Dr. John Bova, Dr. Keith Lewy, Kelsey Johnson	
6/15	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
6/16	9:00 AM	Roundtable – Writing an Abstract	Dr. Brett Mitchell	
6/20	12:00 PM	A&M Rural and Community Health Institute	Dr. Nancy Dickey	
6/22	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
6/23	9:00 AM	Roundtable – Organizing Your Poster	Dr. Brett Mitchell	
6/27	12:00 PM	Roundtable – Marketing and Interviewing	Dr. Brett Mitchell	
6/29	9:00 AM	TAMU School of Medicine MD/PhD Program Air Force Academy Poster Session	Dr. Carolyn Cannon Cadets	
6/30	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
7/4		Holiday		
7/6	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
7/7	9:00 AM	Roundtable – Giving a 10 Minute Talk	Dr. Brett Mitchell	
7/11	12:00 PM	RCR – Scientific Misconduct	Dr. Brett Mitchell	
7/13	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
7/14	9:00 AM	Roundtable – Presenting at Conferences	Dr. Brett Mitchell	
7/18	12:00 PM	Southwest Rural Health Research Center	Dr. Jane Bolin	
7/20	9:00 AM	URM Roundtable Discussion	Dr. Dianne Kraft	
7/21	9:00 AM	Roundtable – Applying to School	Dr. Brett Mitchell	
7/24	9:00 AM	Student Presentations		
7/25	9:00 AM	Student Presentations		
7/26	9:00 AM	Student Presentations		
7/27	9:00 AM	Student Presentations, Texas A&M University Walking Tour		
7/28	9:00 AM- 2:00 PM	Research Day Poster Presentations, Reception, Keynote Speaker, Awards Ceremony, and Pictures		

### **Program Director**



#### Brett Mitchell, PhD, FAHA

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Please keep us updated with your contact information & career or school decisions after graduation.

Thank you for your hard work this summer!

