36TH ANNUAL UBRP CONFERENCE
ABSTRACTS & PRESENTERS

(in alphabetical order by presenter's last name)

INCORPORATING STABLE MONOMERIC FLUORESCENT PROTEINS FOR IMPROVED IMAGING IN SYNTHETICALLY MULTICELLULAR ESCHERICHIA COLI

***ADEOLUWA AJAYI*, ETHAN HOWLEY, INGMAR RIEDEL-KRUSE**

Synthetic biologists reconfigure living organisms to improve their utility. Notably, multicellular bacterial systems–with defined morphology, patterning, and environmental responses–are able to streamline complex tasks through division of labor. These systems are applicable to the exciting fields of tissue engineering, biochemical pathway modularization and bioremediation.

Utilizing traditionally unicellular Escherichia coli as a design platform, Riedel-Kruse lab investigators fabricate and assess fluorescent cell assemblies with engineered surface displays. My focus is on the optimization of our established cell constructs; by developing new fluorescent DNA plasmids. Prior work suggests the use of smaller fluorescent proteins (FPs) in engineered cells, as a way to decrease metabolic burden and structural interference. To test this hypothesis, I recombined new FP DNA into our established surface display plasmids–reasoning that the incorporation of truly monomeric FPs into our cell constructs would reduce FP clustering. Proving this hypothesis will improve our microscopic imaging–advancing our bottom-up understanding of multicellular systems.

INTERVENTION SUCCESS AND RETENTION: DOES EARLY PROGRESS PREDICT LONG-TERM SUCCESS?

MOHIMA ALI

MATHEMATICAL MODELS VS EXPERIMENTAL: DISPROOF-BASED MECHANISTIC MODELS IN SCIENCE: THE CASE OF THE CLASSIC LOGISTIC EQUATION VS THE FINKE-WATZKY 2-STEP CHEMICAL MECHANISM AND POPULATION-BALANCE MODELING

NISA ALIYEVA

RELATIONSHIP BETWEEN ADVERSE CHILDHOOD EXPERIENCES IN POSTPARTUM WOMEN WITH OPIOID USE DISORDER AND INFANT SLEEP LOCATION

GAGANA AMENENI

IDENTIFYING AGE-RELATED BRAIN MORPHOLOGY CHANGES IN THE FEMALE FERRET USING MRI

***LEILI ASGHARZADEH FALBINAN*, LAUREL DIECKHAUS, ELIZABETH HUTCHINSON**

Age-related changes impact both cognition and brain structure, yet the specific brain regions most vulnerable to these changes remain unclear. Magnetic resonance imaging (MRI), particularly tensor-based morphometry (TBM), provides an unbiased approach to studying local brain volume changes over time. However, clinical application of TBM is challenging due to the need for longitudinal imaging and the influence of patients' underlying conditions. To address these challenges, we analyzed TBM in a cohort of four co-housed female ferrets imaged at 1–2 years old and again at 3–4 years old. Diffusion MRI was used to capture the random Brownian motion of water molecules, offering indirect insights into brain structures, particularly white matter. Each ferret's 3–4-year scan was co-registered to its 1–2-year scan to generate TBM maps that quantify regional volume changes. Negative TBM values indicate volume reduction, while positive values reflect growth. An average TBM map was calculated for the cohort to identify shared patterns of brain volumetric changes. Our findings revealed significant volume reductions in key regions, including the superior colliculus (25%), inferior colliculus (23%), cingulate cortex (23%), and posterior sigmoid gyrus (20%). The superior and inferior colliculi, critical for sensory integration, suggest that these structural changes may underlie age-related declines in visual and auditory acuity. To explore whether these structural changes are linked to behavioral alterations, we are evaluating functional differences between aged ferrets (3–4 years) and younger ferrets (~1 year) by testing their ability to track moving targets with varying paths and speeds. By integrating behavioral testing with structural MRI observations, we aim to uncover the relationship between age-related structural changes and sensory processing performance.

DEVELOPING LIQUID CRYSTALS TO VALIDATE COMPLEX MAGNETIC RESONANCE IMAGING TECHNIQUES

***MALCOLM BARRIOS*, COURTNEY COMRIE, TED TROUARD, ISABELLA AGUILERA CUENCA, ELIZABETH HUTCHINSON**

Diagnosis of Alzheimer's disease currently requires autopsy to identify Beta-Amyloid Plaques and Neurofibrillary Tangles in the brain. However, Beta-Amyloid Plaques and Neurofibrillary Tangles develop early in life, changing the brain on a microstructural scale. Advanced diffusion magnetic resonance imaging (dMRI) is a promising way to detect Alzheimer's disease early in life because it can detect these microstructural changes in the brain. Before advanced dMRI techniques are applied to clinical studies, they require validation. This is where phantoms become important. Phantoms are physical objects identified or created by researchers for their well-known specific properties and structures. Phantoms are scanned with dMRI, generating data about the phantom. The dMRI data is compared to the known properties of the phantom to determine scan accuracy and shortcomings.

This project developed two dMRI phantoms in the form of liquid crystals. Liquid crystals are a state of matter between solid and liquid where molecules arrange themselves into crystalline structures but still experience liquid-phase diffusion. Phantom 1 was developed to display randomly oriented microstructural anisotropy. Phantom 2 was developed to display predictably oriented macroscale anisotropy. Phantom 1 and Phantom 2 were both imaged with traditional dMRI. Phantom 1 was additionally imaged with cross-polarized bright field microscopy to confirm its structure.

Cross-polarized bright field microscopy of Phantom 1 revealed birefringence patterns within the phantom, indicating a randomly oriented crystalline microstructure. However, MRI imaging of Phantom 1 did not detect randomly oriented microscale anisotropy in the phantom. This showcases the limitations of traditional dMRI as being unable to detect microstructural information. DMRI of Phantom 2 displayed ordered macroscale anisotropy as expected, indicating successful creation of the phantom. Imaging these phantoms with advanced dMRI methods in the future will allow for comparison between traditional dMRI results and validation of complex dMRI methods.

OUTCOMES FOR INFANTS WITH CONGENITAL HEART DEFECTS BY INSURANCE TYPE

RYENNE BELT

BONE STROMAL CELL MATRIX ENHANCES OSTEOBLAST DIFFERENTIATION

***ADRIAN BERNAL-RIOS*, HUNAIN KHAWAJA, CYNTHIA MIRANTI**

Background: Prostate cancer, the second most common cancer among men, has an increased 5-year mortality rate dropping from 90% in locally advanced cancers to 30% once it has metastasized. Overwhelmingly, prostate cancer metastasizes to bone, but the reasons and mechanisms behind this are poorly understood. To combat this issue, the development of a Prostate-Bone metastasis model is needed to examine the signaling that occurs between the two organs, which promote bone metastasis. Previously, our lab has developed a Prostate-on-a-Chip (PoC) model which we aim to attach to a Bone-on-a-Chip (BoC) model, allowing for the examination of signaling, cell migration, and the interactions between the bone and prostate that drive prostate-bone cancer metastasis. Bone itself is a living, metabolically active organ whose 3-dimensional structure and function is largely determined by the interactions of osteoblasts, bone building cells, and osteoclasts, bone resorbing cells. Additionally, there are other cells which contribute to the signaling of the bone including Mesenchymal Stem Cells (hMSC), Hematopoietic Stem Cells, Stromal Cells, Adipocytes & many more. The goal of this project is to develop the Bone-on-a-Chip model.

Conclusion: These data demonstrate the importance of including a bone matrix to support Osteoblast differentiation for long term culture. Moreover, Osteoclast differentiation requires a precise initial cell seeding volume and supplementation of RANKL to induce successful osteoclast differentiation. These studies have identified ideal conditions for generating a bone-on-chip model for future prostate cancer bone metastasis modeling.

TARGETING MDM2 DEGRADATION FOR IMPROVING EFFICACY TOWARDS AML TREATMENT

***ARRIANNA BIANES*, ALEXIS CRUICKSHANK, WEI WANG**

Acute Myeloid Leukemia (AML) is a hematological cancer with over 20,000 new diagnoses per year in the United States. After diagnosis of AML, the five-year survival rate is approximately 30%. Therapeutics for the disease such as chemotherapy, radiation, and stem cell transplant are outdated with harsh side effects and low survival rates. Within AML and many other cancers, the MDM2 protein, an E3 ligase, is overexpressed and reduces tumor suppressor p53 activity by actively degrading p53 via the ubiquitin-proteasome degradation pathway. MDM2 is an attractive target for AML since the majority of patients do not acquire mutation of p53. Many small molecule inhibitors have been developed to target MDM2. However, none of these compounds have been FDA approved to date. These inhibitors subsequently cause the compensatory upregulation of MDM2 without resolving issues in the development of toxicities and drug resistance. The development of a molecule which targets and degrades MDM2 could minimize the MDM2 upregulation and offer potential for overcoming these issues observed with MDM2 inhibitors. This project focuses on the development of a drug-like MDM2 degrader for the treatment of AML with unmatched potency and highly favorable pharmacokinetic and pharmacodynamic properties.

FACILITATING LIPID EXCHANGE USING CYCLODEXTRIN

***DAVEENA BISWAS*, TAPASYATANU DASH, ANNIKA SILVERBERG, MICHAEL MARTY**

Lipids constantly migrate from cell to cell, allowing for cell signaling, energy storage, membrane composition, and the function of membrane proteins. Regular lipid dysfunction can lead to several diseases such as type 2 diabetes, Alzheimer's disease, and atherosclerosis making them an important area of study. However, these molecules are especially difficult to investigate because of their small size, wide structural diversity, and a limited number of methods capable of studying their influence on membrane protein structure and functions. We have previously developed a technique to measure the lipids exchange between lipoprotein nanodiscs with and without a membrane protein cargo. The differences observed in the distribution of lipids on a membrane reveal the membrane protein's preference for certain lipids. However, these exchanges depend on monomeric diffusion/movement and require long incubation times to achieve an equilibrium. Among other things, this creates a problem of longer experimental times and hinders any downstream automation in lipidomic analysis. One way to combat this problem is with the use of a cyclic oligosaccharide molecule known as methyl-B-cyclodextrin (MBCD). Due to its cylindrical structure with a hydrophobic interior and a hydrophilic exterior, MBCD can be used to transport lipids to and from lipoprotein nanodiscs. We aim to validate this hypothesis using synthetic lipids and MBCD, by lowering the exchange equilibration time and comparing it against physiological benchmarks.

INDIGENOUS DATA SOVEREIGNTY: PROTECTING CULTURAL HERITAGE AND RIGHTS

***JARIAH CALLADO*, CHERIE DE VORE**

Indigenous Data Sovereignty (IDSov) principles can effectively inform engineering research practices, address historical wrongs, promote ethical inquiry and contribute to outcomes that empower Indigenous knowledge. STEM research has historically harmed the environment and Indigenous communities by extracting knowledge, resources and data without consent or collective benefit. This history emphasizes the immediate need for frameworks that respect Indigenous ways of knowing and research practices. Integrating Indigenous Data Sovereignty and Governance principles ensures the protection of cultural knowledge and data, while informing ethical research practices. The Níhí Environmental Engineering research laboratory, for example, is a Native led research community that addresses environmental engineering solutions using an Indigenous centered framework that builds upon IDSov principles. Níhí has operationalized protocols for ecological monitoring, sample collection, navigating place/space, analytical techniques and data repository management, for example. Data governance mechanisms

with in the Níhí Lab and others include Indigenous communities having autonomy over the collection, ownership, and application of data related to their cultures, land and resources. Such practices strengthen not only the ethical foundation of research but are impactful. In summary, recommendations include focusing on creating transparent data governance frameworks co-designed with Indigenous partners, as it ensures that all research processes are consensual and inclusive. Researchers are also encouraged to complete cultural competency training as it helps those develop the skills necessary for fair collaborations.

FUNCTIONAL EVIDENCE FOR AN ANCESTRAL ROLE OF A STRIPE-SPLITTING ENHANCER IN INSECT SEGMENTATION

***CONNOR CARNEY*, VIOLET ROWLAND, LISA NAGY**

Segmentation is the division of an organism's body plan into repeated parts, a process determined during early development. Many organisms, including humans, undergo segmentation, though the mechanisms vary. In *Drosophila melanogaster (*the fruit fly), segments form all at once during development. In contrast, in *Tribolium castaneum*, (the red flour beetle), embryos add segments sequentially, raising the question of how the "all at once" segmentation process evolved. Our research focuses on the role of one gene, even-skipped (eve), involved in a molecular oscillator in *Tribolium,* believed to drive its sequential segmentation. eve is first expressed in one broad stripe, then splits to form two distinct segment specific stripes. We are investigating potential *Tribolium* enhancer regions that regulate eve expression to discover how both the primary and secondary stripes are regulated. One *Tribolium* enhancer I'm investigating is expressed exclusively, and abundantly, in the late arising secondary stripe. This expression pattern is identical to a known *Drosophila* secondary stripe enhancer. Surprisingly though, the fly secondary stripe enhancer has no known function. Why then would its expression pattern be so highly conserved? We hypothesize that this enhancer is responsible for eve-driven stripe splitting in *Tribolium* and that its function in *Drosophila* has been replaced by another gene. We predict knocking out the 4.0 enhancer region prevents stripe splitting, and embryos will develop with 8 segments instead of the full 16. So far, using CRISPR-Cas9 to disrupt the *Tribolium* secondary stripe enhancer hints that the enhancer is necessary for proper segmentation. Injected embryos show two strong phenotypes — a small percentage have been developing slower, with fewer segments than their control counterparts, while a majority have been embryonic lethal. We are now exploring whether disrupting this enhancer only affects the stripe splitting process or it entirely stops segmentation, as well as when exactly lethality occurs .

THE EFFECTS OF SHORT-TERM FASTING ON FOXO TRANSCRIPTION FACTORS INVOLVED IN CANCER TREATMENT

BRANHAM CARPENTER

MICROBIAL TRANSFER COMPARISON ON DRY AND WET LAUNDRY

ABBY CASSIUS

THE ROLE OF VASCULAR DYSFUNCTION IN THE DOCA-SALT RAT MODEL OF HYPERTENSION

***DEANNA CLINCH*, MELISSA DENNIS, MARK MORALES, CHRISTOPHER BANEK**

Nearly half of American adults are diagnosed with hypertension (HTN), or high blood pressure, defined as a systolic pressure over 130mmHg and/or a diastolic pressure over 80mmHg. Vascular dysfunction, characterized by the inability of blood vessels to dilate or relax, is linked to increased damage and shear stress on the endothelial lining. This dysfunction is widely demonstrated to be associated with HTN; however, its causative or responsive role remains debated. The deoxycorticosterone acetate (DOCA)-salt model of hypertension in Sprague Dawley rats is mediated in part by dysfunctional autonomic tone and declining kidney function, however, it is unclear whether vascular dysfunction contributes to the overall progression in parallel to the neural and renal dysfunction. Vascular dysfunction is reported to contribute to and drive the hypertension progression in other preclinical models, but the role in DOCA-salt HTN remains debated with conflicting reports. In this study, we aim to elucidate the role of systemic and renal vascular dysfunction in the DOCA-salt rat. We hypothesized DOCA-salt rats will have deteriorated systemic and renal vascular function compared to normotensive vehicle controls.

To address this hypothesis, eight male SD rats were uni-nephrectomized. Following a two week recovery, animals were administered a subcutaneous silicone implant containing either vehicle (VEH) (n=4) or 100mg DOCA (n=4). DOCA animals also received 0.9% saline replacement for their drinking water for 21 days. Following the 3-week DOCA-salt treatment, vascular function was assessed by Mulvany wire myography. Vessels were pre-constricted using thromboxane mimetic U46619, then dose response curves were generated to acetylcholine (ACh) and sodium nitroprusside (SNP) for endothelial and smooth muscle function respectively. Vascular function was presented as a percentage of relaxation of the blood vessels relative to baseline. Repeat measurements were compared across strains by two-way ANOVA with Bonferroni post hoc test (*p<.05). Endpoint measurements were compared by t-test (*p<.05). Data are presented as Mean±SEM. Regarding vascular function, no difference was detected in ACh mediated relaxation between DOCA and VEH groups for both mesenteric (DOCA 96.62±1.386 ; VEH 89.02±5.802 % relaxation) nor renal arteries (DOCA 95.38±1.178 ; VEH 94.49±8.921 % relaxation). Additionally, no difference in smooth muscle function was noted in SNP mediated relaxation in either treatment groups for both mesenteric (DOCA 97.93±0.9297; VEH97.33±0.7846 % relaxation) nor renal arteries (DOCA 107.5±4.746; VEH 137.8±10.05 % relaxation).

Taken together, these data did not support our original hypothesis, as we failed to detect vascular dysfunction in mesenteric and renal artery samples. These data show that both endothelial and smooth muscle vasodilatory functions remained intact in the DOCA animals when compared to controls. Therefore, we concluded that autonomic dysfunction and renal dysfunction are likely the major contributors of hypertension in DOCA salt rats, where vascular dysfunction may not be a primary driver of HTN at this stage of the disease.

ANALYSIS OF NOVEL INNATE IMMUNE RECEPTORS IN UNGULATES

***JESSICA CRUZ*, FIONA MCCARTHY**

Scavenger Receptor (SCAR) genes are a heterogeneous group of genes involved in innate immunity. In humans, scavenger receptors are further classified into twelve subgroups based on function, however, ruminants have an expanded repertoire of SCAR receptors. Our experimental approach is to identify the SCAR gene repertoire in these species and provide standardized gene nomenclature which reflects what is known about the function of these genes, particularly SCAR genes not found in humans. We used orthology and sequence similarity to identify SCAR genes in cattle, horses, pigs, sheep and goats. Gene synteny and phylogenetic analysis were used to determine relationships between mammalian SCAR genes and standardized gene nomenclature was assigned using VGNC guidelines. A novel group of SCAR genes found in mammalian species but absent

in humans have previously been described in cattle as Workshop Cluster 1 (WC1) genes. Subsequently these genes were identified across multiple ungulate genomes and are expressed on gamma delta T cells as cell-surface receptors involved in pathogen pattern recognition. Gamma delta T cells expressing these WC1 coreceptors have been shown to be involved in response to pathogens. Based upon this functional work, we propose that these WC1 genes be renamed T cell receptor gammadelta (TRGD) to better reflect their function and to standardize these gene names with other assigned T cell receptor nomenclature. We have also identified TRGD/WC1 genes in unplaced contigs from cow, goat and pig genomes, which can be assigned to these clusters in their respective genomes.

DEVELOPMENT OF 3D PRINTED FERRET CRADLE WITH ANESTHESIA AND COMMERCIAL COIL INTEGRATION FOR BRAIN MRI

***ALVARO CRUZ PEREZ*, LAUREL DIECKHAUS, JESUS MENDOZA, LEILI FALBINAN, DIEGO CELDRAN-BONAFONTE, KATY PHILLIP, RHEA CARLSON, JEAN-PHILLIPE GALONS, ELIZABETH HUTCHINSON**

Ferrets are a promising pre-clinical model for brain MRI studies due to their shared anatomical features with the human brain. However, sexual dimorphic differences in ferrets pose a challenge for pre-clinical imaging as they do not fit commercial MRI equipment. In our cohort, we observed a 40% larger body diameter (p<0.05) in males compared to females. One method to address this challenge is three-dimensional (3D) printing, which enables the creation of custom-made designs with MRI compatible material. We addressed this challenge by developing a 3D-printed, MRI-compatible cradle designed to integrate with commercial coils and accommodate ferrets of various sizes. Ferrets' sizes were taken from specimens housed in the lab (males, n=4, females n=8), with the largest one selected as the reference for the cradle body size (Length=49 cm, Diameter=16 cm, Weight=1.8 kg, Head Width= 8 cm). Designs were iterated in SolidWorks, and the final prototype was 3D-printed in polylactic acid (PLA). The design included a slot for the surface coil and veterinary needs such as anesthesia delivery that could be adjusted, and tube feed integrated into the cradle body. To evaluate imaging quality, we built phantoms, ferret skulls and brains using stereolithography resin printing and agar, tissue-similar materials. Phantoms were created from previous T2-weighted MRI scans of a male and female ferret. The phantoms were imaged on the Pre-Clinical 7T MRI scanner using 86 mm cylindrical coil for transmission of signal, and a rat brain surface coil for reception of signal. Phantom testing demonstrated a field of view of 40 x 26 x 14 mm for both the female and male skull, comparable to the in vivo coverage currently achievable for female ferrets. Preliminary results demonstrated strong feasibility for in-vivo imaging male ferrets. Next phase of testing will be to image a male ferret on the new cradle system.

THE IMPORTANCE OF EDUCATING AMERICAN INDIAN YOUTH ABOUT LIVING A HEALTHY LIFESTYLE

TRISTAN CUMMINGS

MECHANISTIC ROLE OF NUTRIENT DEPRIVATION IN NRF1 ACTIVATION FOR CARDIOPROTECTION

***SYDNEY CUPISZ*, KIMIKO DELLA CROCE, BRIDGET GLASS, GUANG YAO**

Nrf1 is a stress-responsive transcription factor essential for maintaining tissue homeostasis by regulating proteostasis and redox balance. Nrf1 is actively expressed in neonatal cardiomyocytes, but not in adult hearts, limiting their regenerative capacity. This inactivity leaves adult hearts more vulnerable to injury from ischemia-reperfusion, chemotherapy, and cardiotoxic agents.

Our long-term objective is to determine whether nutrient deprivation can reactivate the Nrf1 pathway in adult cardiomyocytes to enhance cardiac protection and repair. This investigation was motivated by our finding in an RNA-seq analysis of embryonic fibroblasts (REF/E23 cells) that Nrf1 expression continuously increased with prolonged serum nutrient starvation. This suggests a potential link between starvation-induced stress and the activation of the Nrf1 pathway.

In the current study, we first examined whether starvation increases Nrf1 expression at the protein level by Western blot. Preliminary results showed an increased expression of Nrf1 46 kDa isoform under serum starvation in REF/E23T cells. With this information, we tested nutrient deprivation in a human heart cell line, AC16, which showed an increase in expression of the smaller 25 kDa isoform. To determine if Nrf1 isoforms are present in the nucleus, we isolated nuclei from REF/E23T cells starved over periods ranging from two to sixteen days. Preliminary findings suggest a pattern of increased Nrf1 isoform expression during starvation, though the variability in expression patterns and the isoforms detected -- and their consequences on the target gene expression and potential cardioprotection -- require further investigation.

THE EFFECT OF CSNX1.3 ON MMTV-PYMT TUMOR GROWTH AND METASTASIS

***DELINA DENOGEAN*, BARBARA SANDS, JOYCE SCHROEDER**

The Epidermal Growth Factor Receptor (EGFR) belongs to a group of Receptor Tyrosine Kinases (RTKs) that are a part of the HER family. EGFR mediates many processes in the cell including proliferation, migration, and survival however its overexpression is correlated with poor prognosis in Triple Negative Breast Cancer. Usually, EGF binds to EGFR, dimerizes, and undergoes transphosphorylation where it is then intracellularly imported and taken to the early endosome for ubiquitination. This signals readiness to be taken to the lysosome for degradation or recyclization. In cancerous cells, however, ubiquitination gets inhibited due to their loss of polarity. This results in failure to be taken to the lysosome and allows EGFR to evade degradation and undergo nuclear localization. This process is initiated by the binding of Sorting Nexin 1 (SNX1), a protein responsible for the regulation of trafficking, to EGFR.

Considering the interaction between SNXs and RTKs causes oncogenic activity, we hypothesized that a therapeutic that can modify this interaction could alter tumor-specific retrotranslocation. To target these interactions, cSNX1.3 was developed, a cell-penetrating peptide that allows for the delivery of proteins across the cell membrane. cSNX1.3 interacts with EGFR in the long-lived endosome and binds in competition with SNX1. When cSNX1.3 is bound to EGFR, it inhibits nuclear localization and thus its oncogenic activity.

To fully evaluate the tumor-regressing abilities of cSNX1.3, we used the WAP-TGFα strain of transgenic mice, whose mammary gland tumors are EGFR dependent. These mice were treated with cSNX1.3 or the control drug, cPTD4, for 4 weeks. After treatment, we saw that the tumors of the cPTD4-treated mice continued to grow whereas the tumors of the cSNX1.3-treated mice regressed. Subsequently, we constructed an additional study using a mouse model that would demonstrate how cancer

metastasis reacted to cSNX1.3 treatment, given that metastasis is typically what makes human breast cancer fatal. For these reasons, we decided to use the MMTV-PyMT transgenic mice. Using this model that closely resembles human metastatic breast cancer to evaluate cSNX1.3, will allow us to better understand the future of cSNX1.3 as a therapeutic for human applications.

INVESTIGATING DICTYOSTELIUM DISCOIDEUM CAR1 PHOSPHORYLATION STATUS ON CHEMOTAXIS-DRIVEN DEVELOPMENT

***MEGAN DOWD*, *SHRUTHI SADHASIVAM*, HELENA WORONIECKA, PASCALE CHAREST**

Dictyostelium discoideum is a species of soil-dwelling amoeba and a eukaryote that transforms from unicellular to multicellular during its growth cycle. When starved, Dictyostelium cells transition from their unicellular growth phase to the differentiation phase, where chemotactic cAMP signaling begins a developmental cycle that results in fruiting bodies. When cAMP binds to the cAR1 receptor, a GPCR, this signals the cells to aggregate and continue signaling to other cells, leading to multicellular forms.

This study builds upon previous work in the Charest Lab, where three serine clusters on the tail of the cAR1 GPCR protein are identified as separate phosphorylation sites. The purpose of this study is to determine whether mutations at the Cluster 1 (C1) site impact cell signaling. These mutations include serines to alanines (C1A; phospho-null) and to glutamate (C1E; phosphomimetic).

The methods used to determine the impacts of the mutations include culturing wild-type cells, cAR1 null cells, and cells expressing C1A or C1E before performing developmental assays, which are tracked at 24-hour and 48-hour intervals. Results of the developmental assays show a small level of cell aggregation with a few fruiting bodies produced from the C1A mutant and low levels of cell aggregation with no fruiting bodies created by C1E. As expected, the wild type developed into many fruiting bodies and the cAR1 null cells did not produce multicellular forms. This indicates that there does exist some aggregation in the cells of the C1A mutant, indicating that the phospho-null mutant of the cAR1 component does not entirely inhibit cAR1-mediated cell signaling and aggregation. The lack of fruiting bodies produced by the C1E mutant indicates that the phosphomimetic mutant of the cAR1 component may inhibit this instead.

NEW CHEMICAL-PROTEOMIC PLATFORMS FOR THE EXPLORATION OF MITOCHONDRIAL DYNAMICS

SAMUEL ELLIS, HIEU PHAM, MICHAEL TAYLOR

IDENTIFYING THE FUNCTIONS OF SOLUTE CARRIER PROTEINS

NANCY ELNADY

LIFESTYLE RISKS AND CANCER SCREENING IN FIREFIGHTERS

***KODI EMERSON*, JAMES HOLLISTER, SHAWN BEITEL, JEFF BURGESS**

CANNABINOL (CBN) IS ANTINOCICEPTIVE IN A MOUSE MODEL OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY VIA THE CANNABINOID RECEPTOR TYPE 1

***CHRIS FAN*, ABIGAIL SCHWARZ, JOHN STREICHER**

Chronic pain affects approximately 20% of the global population, and while current treatments such as opioids are effective, they frequently cause undesirable side effects, including addiction, nausea, and respiratory depression. Alternatives to opioids for chronic pain management are a critical need. Cannabinoid drugs such as CBD and Δ9-THC are well known to have pain-relieving effects but are also highly psychoactive, so our lab has chosen to study other compounds in *Cannabis sativa*, such as the minor cannabinoids. Previous studies in our lab have identified Cannabinol (CBN) as the only effective compound among the five minor cannabinoids tested (CBN, CBDV, CBG, THCV, and Δ8-THC) in having the potential to alleviate chronic neuropathic pain. However, the specific mechanism of CBN remains underexplored. In this study, we aim to investigate the mechanism by which CBN alleviates allodynia caused by chemotherapy-induced peripheral neuropathy (CIPN), focusing on its interactions with the Cannabinoid receptor type 1 (CB1) and Adenosine A_{2a} receptors ($A_{2a}R$). Using a CIPN pain model in both male and female CD-1 mice, we evaluated CBN's cannabimimetic and analgesic effects with and without CB1 (Rimonabant, 10 mg/kg) or $A_{2a}R$ (Istradefylline, 3.2 mg/kg) antagonist treatment. Mechanical pain thresholds were measured using von Frey filaments in mice both before and after CIPN induction, as well as following the administration of antagonists and CBN (200 mg/kg; intraperitoneal). Our results indicate that CBN exerts its analgesic effects through CB1 receptor activation since Rimonabant, the CB1 inverse agonist, blocks CBN's pain-relieving effects. In contrast, Istradefylline, the $A_{2a}R$ antagonist, has no effect, suggesting that the $A_{2a}R$ is not involved. These findings provide valuable insights into CBN's mechanism of action and its potential as a therapeutic option for chronic neuropathic pain.

MODELING EFFECTS OF PROGRESSIVE CORTICAL ATROPHY AND STROKE LESIONS ON THE INDUCED CURRENT FLOW IN TRANSCRANIAL DIRECT CURRENT STIMULATION (TDCS)

***NOAH FRAZIER*, KATLYN NICKELS, ANETA KIELAR**

Direction and intensity of the current flow in transcranial direct current stimulation (tDCS) varies for different tissue types and can be affected by structural changes that result from atrophy and stroke. However, the exact impact of these changes on the transcranial current flow is not well understood. The main hypothesis is that cerebrospinal fluid accumulation in the stroke lesions will redirect current flow and modify the magnitude of the induced current, while cortical atrophy will weaken the induced current magnitude without significantly affecting its direction. SimNIBS is a free and open-source software that provides tools used to simulate and visualize tDCS current flow on individual head meshes, as well as obtain quantitative information about the electric field in regions of interest (ROIs). Because this approach is cost-effective and has a user interface that reduces the need for extensive programming skills, it is easier to implement in a clinical setting to inform decisions about optimal tDCS electrode placement for neurorehabilitation. Data used in this study is derived from three groups of participants who participated in studies conducted in the lab; individuals with post-stroke aphasia, logopenic variant of primary progressive aphasia (lvPPA), and neurotypical age- and education-matched controls. Each participant completed structural MRI scans, which are subsequently used to build individual head meshes in SimNIBS and run simulations of tDCS current flow with SimNIBS. The current flow will be assessed qualitatively and quantitatively, by performing an analysis of specified ROIs to calculate mean electric fields in those areas. Results will be compared to findings in the larger body of literature on the topic.

MUTATIONS IN THE NUCLEAR EXPORT SEQUENCE OF EGFR INFLUENCES CELL PROLIFERATION AND MORPHOLOGY

***MARC GARCIA*, RYAN HECKSEL, JOYCE SCHROEDER**

The epidermal growth factor receptor (EGFR) is known to play a role in several cellular functions such as proliferation, survival, and migration. It is often overexpressed in triple-negative breast cancer, making it a promising druggable target. EGFR can enter and exit the nucleus via the nuclear localization and export sequences (NLS/NES). Once inside, EGFR interacts with transcription factors to regulate pro-oncogenic genes. A peptide drug named cSNX1.3, developed in the Schroeder lab, reduces tumor size in mice and blocks nuclear EGFR in vitro and in vivo. As a result, we hypothesize that using CRISPR/Cas9 to create a mutation in the NES will prevent EGFR from leaving the nucleus and therefore modify cellular activity. We explored cell viability, proliferation, and morphology within multiple cell lines using MTT assays, live cell imaging, and Matrigel cultures. Combing our data allowed us to discover that cells with the NES mutation had an overall decreased rate of growth, while also showing impacted cell viability when treated with cSNX1.3. The Matrigel experiments revealed that the 468 and 231 cell lines became more “mesenchymal-like” rather than epithelial which could account for the decreased growth rate seen in our live cell imaging. Future research will focus on mutating the NLS of EGFR to determine its impact on cellular phenotypes, identifying genes regulated by nuclear EGFR, and examining the effects of NES mutations in non-transformed cell lines. This work will enhance our understanding of the nuclear functions of EGFR across various cell types.

CHARACTERIZING THE EFFECTS OF AGE ON CIRCUITS INVOLVED IN SPATIAL WORKING MEMORY

***TORI GARZA*, SAHANA SRIVATHSA, CAROL BARNES**

While many cognitive processes are susceptible to age-related decline, the most commonly affected are episodic memory, information about prior experiences, and working memory, the temporary storage of information for further use. (Nyberg et. al, 2012). As a result, spatial working memory (SWM) capabilities, which arise from the integration of episodic and working memory processes, are especially affected by age (Kapellusch et. al, 2018). Key brain regions implicated in SWM are the medial prefrontal cortex (mPFC) and the hippocampus (HC). These regions are connected by unidirectional projections from the ventral CA1 subregion of the HC to the infralimbic (IL) and prelimbic (PL) subregions of the mPFC (Jay and Witter, 1991). Notably the inputs coming from the vHC do not evenly distribute throughout the different layers of the mPFC, preferentially activating neurons in the infralimbic PFC region (Liu et. al 2018). It is still unclear how vHC activity differentially recruits neurons along the dorso-ventral axis of the mPFC and specifically how this process changes with age. To study this, we stimulated the ventral and intermediate HC regions of anesthetized young (10 mo) and old (24-26 mo) male fischer 344 rats while recording neural responses generated along the dorsoventral axis of the mPFC. Biphasic electrical pulses from 100-600µA were randomized and delivered to the HC while Neuropixels 2.0 probes were used to record from multiple mPFC subregions simultaneously. We observed increased neural responses in IL mPFC compared to PL mPFC. The amplitudes of responses were also reduced in older animals across both regions. This could suggest changes to white matter connectivity within the circuit responsible for SWM in aged animals. By identifying intrinsic age-related changes to mPFC neural recruitment by vHC stimulation, we can begin to identify target areas for treatments that aim to alleviate the symptoms of age-related cognitive decline.

AN UNCHARACTERIZED TRANSCRIPTIONAL REGULATOR CONTROLS C. DIFFICILE VIRULENCE

BHARGAV GHOSH

EXPLORING EVOLUTIONARY DYNAMICS IN POLYPLOID POPULATIONS

***SAM GIBBON*, JUSTIN CONOVER, MICHAEL BARKER, RYAN GUTENKUNST**

Most organisms, including humans, are diploid, meaning they have two copies of each chromosome. For example, there are normally two copies of the sex chromosome in humans which can be in either an XX or XY pair. However, a species can also have more than two complete chromosome sets in their genome. This is called polyploidy. Polyploid species include many economically important crops such as wheat, cotton, strawberries, coffee, and potatoes. Polyploid genetics are, imaginably, more complex than in diploids and are not well understood. For example, gene regulation and cell division are complicated by having more than two copies of a chromosome. Our specific focus is on population genetics and understanding longer-term evolutionary forces such as natural selection and mutation. To investigate these dynamics in polyploids, we introduce a new mathematical framework borrowed from nonlinear differential equations. Our findings suggest that while polyploidy can enhance or deter evolutionary adaptation, its exact effects depend on context.

INVESTIGATING THE POTENTIAL INFLUENCE OF ATATI AND TAK1 ON INSULIN RESISTANCE

***ANNIKA GIPHART*, EMILIE LU, MAC MCGRAW, JUSTIN MONTOYA, SANIYA BARBOUR, NAM LEE, PAUL LANGLAIS**

The United States is currently grappling with an epidemic of Type II Diabetes, a condition characterized by insulin resistance in liver, muscle, and fat cells. Insulin signaling is crucial for the uptake of glucose into cells, where it is used for energy, or in the liver, converted to stored glycogen. The Langlais Lab seeks to explore the roles of alpha tubulin acetyltransferase 1 (ATAT1) and transforming growth factor-β-activated kinase 1 (TAK1) within this signaling pathway to gain insights into the mechanisms behind insulin resistance. TAK1 is known to activate ATAT1, which plays a key role in stabilizing microtubules. Since microtubule stabilization is critical for the translocation of GLUT4—an essential step in glucose uptake—we hypothesize that ATAT1 and/or TAK1 are involved in GLUT4-mediated microtubule stabilization, thus contributing to insulin-stimulated glucose uptake. This research may offer valuable insights into improving our understanding of insulin resistance and Type II Diabetes.

WIRELESS BRAIN-CONTROLLED SPINAL STIMULATION FOR RESTORATION OF MOTION

***IAN GOLD*, REY MENDOZA, DHRUBO AHMAD, ZHONG WANG, JORDAN HOFFMAN, ALEX BURTON, DAN SONG, SAM TRAN, JESSICA HANNA, JAKOB BAKALL, DAVID CLAUSEN, JERRY ANDERSON, ROBERTO PERALTA, KIRTANA SANDEPUDI, ALEX BENEDETTO, ETHAN YANG, DIYA BASRAI, LEE MILLER, MATTHEW TRESCH, PHILIPP GUTRUF**

Brain-controlled spinal stimulation offers a pathway for restoring movement and supporting rehabilitation in individuals with spinal cord injuries or stroke. Current technology available for human subjects are bulky external hardware that is not conducive to everyday use. This project aims to explore hardware that enables a seamless fully implantable technological approach that supports 24/7 operation without restrictions on activity. Specifically, this project develops a wireless network of implantable devices powered by near field communication and are networked with Bluetooth Low Energy (BLE) to enable low-latency, real-time communication between cortical sensor nodes and spinal stimulator nodes. The network consists of flexible, biocompatible sensor and stimulator nodes, alongside a computational node.

The sensor node is designed to record cortical activity from sixteen electrodes with a 7 kHz sampling rate, ensuring precise neural signal acquisition. The stimulator node delivers charge-balanced, adjustable pulses to eight electrodes, with parameter updates possible every 50 ms. Both nodes are encapsulated in biocompatible materials and powered wirelessly. Initial evaluations focus on network performance, including power harvesting efficiency, communication latency, and packet loss over extended periods. Ongoing preclinical studies assess the system's ability to restore gait by comparing locomotion under uninjured, injured, and stimulated conditions. Functional restoration is further validated using goal-directed tasks to confirm brain-driven control of movement.

Insight and technologies generated by the project also have direct applicability to multimodal therapies across organ systems, including electrical, optogenetic, and microfluidic interventions, enabling closed loop digital medicine for personalized treatment of chronic disease.

AMINO ACID EXCHANGEABILITIES DIFFER MORE BETWEEN SURFACE AND BURIED SITES THAN AMONG SPECIES

***PETER GOODMAN*, ANDREW WHEELER, JOANNA MASEL**

Evolutionary Biologists capture the process of molecular evolution using substitution models, which represent the likelihood or rate of change at specific sites in the DNA sequence. These models are essential components of maximum likelihood and Bayesian methods for phylogenetic inference, as improved substitution models lead to more accurate phylogenies and branch length estimates. The primary substitution model, Q, the rate matrix, comprises exchangeability values, an abstract parameter representing the relative substitution rate between two sequence elements, and the amino acid frequencies, which are fixed based on a given alignment. Previous work has shown improvements in substitution models that account for protein structural features, but whether this improvement is driven by unique structural exchangeability values or different amino acid frequencies across structural regions is unclear. With recent developments in computational tools for substitution model inference and data availability for protein structure, we investigated the impact of protein structure on amino acid exchangeability values. Using predicted protein structures from a machine learning model, we partitioned orthologous genes from four taxa into surface and buried residue sections and trained Q matrices on each taxon-structure combination. The exchangeability values derived from these partitions differed more between accessible surface area than between taxon, highlighting the greater impact of protein structure on substitution patterns. These results underscore the potential for structure-aware substitution models to enhance phylogenetic inference.

LINAGLIPTIN-BASED DEEP EUTECTIC SYSTEM TO IMPROVE TRANSDERMAL DELIVERY

***ARPITA GULATI*, YOGESH SUTAR, ABHIJIT DATE**

Linagliptin (LIN) is a potent dipeptidyl peptidase-4 inhibitor approved to treat type II diabetes. Low permeability, rapid metabolism, fluctuating plasma levels, and low oral bioavailability limit LIN's utility. Transdermal delivery bypasses first-pass metabolism and offers steady drug release to improve bioavailability. LIN's hydrophilic nature limits transdermal permeation. Deep eutectic solvents (DES) are a promising option to enhance drug solubility and permeability. We hypothesize the transformation of crystalline, hydrophilic LIN into an amorphous, lipophilic DES through pairing with pharmaceutically acceptable, permeation enhancing, lipophilic counterions will improve physicochemical properties and transdermal permeation of LIN.

LIN was combined with oleic acid, linoleic acid, caprylic acid (CA), decanoic acid (DA), undecylenic acid, geranic acid, 4-phenylbutyric acid (PBA), salcaprozic acid, or α-tocopherol succinate in 1:1-1:4 molar ratios. The DESs were characterized using Fourier transform infrared (FT-IR) spectroscopy, nuclear magnetic resonance (^{1}H-NMR), and differential scanning calorimetry (DSC). In vitro permeation test (IVPT) studies using a Franz diffusion apparatus assessed the DESs' ability to enhance LIN transport across Strat-M artificial membrane over 24 hours.

Our studies showed counterions transforming LIN into DESs in a concentration-dependent manner. FTIR and ^{1}H-NMR analysis confirmed the presence of ionic and hydrogen bond interactions between LIN and counterions. All LIN-DESs showed distinct thermal stability compared to pure LIN. Absent/shifting melting peaks of LIN in the DSCs of DESs confirmed amorphous behavior. IVPT studies demonstrated all DESs yielded higher transdermal permeation of LIN compared to pure LIN. LIN-CA, LIN-PBA, and LIN-DA DESs reported a 5-fold, 3-fold, and 2-fold increase in LIN permeation, respectively. Saturated fatty acids and PBA improved LIN permeation compared to unsaturated fatty acids, suggesting counterion structure influences LIN permeation.

This preliminary study demonstrated that pharmaceutically acceptable, lipophilic counterions and LIN formed DESs that successfully modified physicochemical and biopharmaceutical properties of LIN, which is an effective strategy to improve transdermal delivery.

BOOLEAN NETWORK MODELING OF LINEAGE PLASTICITY IN MERKEL CELL CARCINOMA

WALDO GUZMAN BARRIENTOS

THE EFFECT OF CROSSING PATHS ON PATH INTEGRATION

***SARA HARADER*, MATT WATSON, ARNE EKSTROM**

In the field of spatial navigation there is controversy regarding the effect of crossing one's path on keeping track of one's position and direction relative to a starting position (termed "path integration"). This is an important question because when one "zeros out" one's position can have a big impact on knowing where one is. In my project, I am investigating this question by comparing paths that cross themselves compared to those that do not. Previous research (Yamamoto et al., 2014) that suggested no effect of crossing paths may have been confounded by inadvertent variations in distance and turn angles between the crossed and uncrossed trajectories. To address this issue I have designed novel curved paths that control for these variables, allowing for a more accurate comparison between the two conditions. Using the Unity Game Engine, I created these paths, for testing in an immersive virtual reality environment. To ensure the participants only rely on self-motion cues, they are guided through the paths by vanishing poles in otherwise darkness, which disappear as they come into contact with them. At the end of each path, participants are instructed to point to where they believe their starting position was using handheld controllers. I will analyze the distance and angle errors in their pointing responses. Although I am still in the early stages of data collection, which will continue into the Spring semester, I anticipate that crossing one's trajectory will result in lower accuracy in determining the starting position. These results will enhance our understanding of how we perceive space and the factors that influence it. Additionally, the findings could contribute to improving computational models of navigation.

INFLUENCE OF MATERNAL EDUCATIONAL ATTAINMENT ON TREATMENT SUCCESS IN PRESCHOOL-AGED CHILDREN WITH DEVELOPMENTAL LANGUAGE DISORDER

***ARENA HAUGHT*, ELENA PLANTE**

Untreated Developmental Language Disorder (DLD) can manifest itself in a higher risk for school failure, poor employment outcomes, and a host of behavioral problems. One successful intervention for children with DLD is conversational recast, which is used for the correction of morphological and grammatical errors specific to the English language. Literature has found that mother's education level impacts young children's language skills. We are interested in seeing if mother's education has an impact on the treatment progress of preschool aged children (4-5 years) with DLD who received conversational recast treatment. This study used a sample of 57 children with DLD, ages 48 months to 74 months (mean=60.666 months). Mother's educational levels varied between 11 years to 17 years (mean=14.438 years). This was used as a proxy for socio-economic status as mother's education level has been found to significantly impact children's language development. We correlated

mother's education with an effect size that represented individual treatment gains to determine the role of this component of socio-economic status on treatment outcomes. The implications of these results are discussed.

DOES INHIBITING ABHD6 IN MALE MICE MITIGATE PAIN AND DEPRESSIVE BEHAVIORS?

***RILEY HAVEMAN*, IRENE RUIZ, TALLY LARGENT-MILNES, TODD VANDERAH**

According to recent studies, up to 17% of the general population struggles with neuropathic pain. Looking to the endocannabinoid system as a source for treatment, this study examines the effect of the compound KT-182, an irreversible ABHD6 inhibitor. ABHD6 is an enzyme that breaks down the endocannabinoid 2-arachidonoylglycerol (2-AG), thus through inhibition of its activity, 2-AG levels increase and are able to continue binding to cannabinoid receptors in the cell. KT-182 has successfully mitigated pain in a medication overuse headache model, but has not been examined in a neuropathic pain model, such as partial sciatic nerve ligation (pSNL). This study seeks to better understand whether KT-182 is able to diminish chronic neuropathic pain and resulting depressive behaviors. In order to model neuropathic pain, male mice undergo the pSNL surgery on their right sciatic nerve. Using behavioral assays, such as Von Frey and Forced Swim to assess allodynia and depressive behaviors respectively, data is then collected on the development of each behavior. Throughout the study, mice are separated into a KT-182 and a control group in order to determine the success of the compound. The findings of this pilot study suggest that KT-182 is able to attenuate pain responses in the mice, but has decreased effectiveness when being dosed chronically. Moreover, the data shows that the compound has no significant effect in preventing or reversing depressive behaviors. In the future, more studies with larger sample sizes using different injection strategies will be conducted to continue exploring the analgesic effects of KT-182 on pain behaviors and possible effects on decreasing depressive behaviors.

ELECTROPORATION OF STAPHYLOCOCCUS HAEMOLYTICUS: EXPLORING THE OBSTACLES TO SUCCESSFUL TRANSFORMATION

***ARIEL HEINRICH*, FREYA ALLEN, WILLEM VAN SCHAIK, DAVID BALTRUS**

Staphylococcus haemolyticus is a Gram-positive bacterium most often associated with the skin microbiota of healthy individuals. However, it has also been known to cause a variety of opportunistic infections, including implant-associated biofilm infections and infantile late-onset sepsis. Furthermore, S. haemolyticus has been shown to serve as a reservoir of antibiotic resistance genes, and is able to transfer these genes to related pathogens such as Staphylococcus aureus, a highly virulent human pathogen, making subsequent infections by these gene-transfer recipients more difficult to treat. Although S. haemolyticus has been responsible for an increasing number of hospital outbreaks, rigorous genetic studies on the bacterium continue to be sparse, and universal protocols on producing mutations in the genome of S. haemolyticus do not exist. Here, we report our attempts to introduce mutagenesis plasmids into strains of S. haemolyticus via electroporation, which is the first step in the process of generating targeted mutant strains of bacteria. Five different protocols for electroporation were tested for efficacy in S. haemolyticus. Additionally, four different strains of plasmid-containing Escherichia coli were tested for plasmid-methylation compatibility with S. haemolyticus strains. We conclude that there exist major obstacles to the electroporation of S. haemolyticus, as protocols that successfully transform Staphylococcus aureus, and other Gram-positives, have been unsuccessful in S. haemolyticus. Further research is needed to understand how electroporation protocols can be optimized to allow genetic studies in this pathogen.

LIPIDOMIC COMPOSITIONAL ANALYSIS OF CAROTID ATHEROSCLEROTIC PLAQUE IN TYPE-2 DIABETES PATIENTS.

***DYLAN HERMOSILLO*, BONNIE HURWITZ**

Cardiovascular disease is the leading cause of death among individuals with diabetes, accounting for two-thirds of the reported fatalities. Atherosclerosis, characterized by the buildup of fatty plaque on the artery walls, obstructs blood flow and can rupture, leading to the formation of blood clots. Specific lipid species may influence the severity of arterial plaque accumulation. Our study aims to identify, characterize, and compare lipid compositions among symptomatic and asymptomatic diabetic, non-diabetic, and prediabetic patients to determine if significant lipid species emerge as potential biomarkers for future prognostic analysis. We analyzed lipids using liquid chromatography-mass spectrometry (LC-MS) from 71 surgically removed endarterectomy specimens from the following groups: diabetic asymptomatic (n=11), diabetic symptomatic (n=9), non-diabetic asymptomatic (n=12), non-diabetic symptomatic (n=20), prediabetic asymptomatic (n=9), and prediabetic symptomatic (n=11) patients. The lipid data was cleaned, log2 normalized, and analyzed using P-Mart and MetaboAnalyst statistical tools to identify significant differences in lipid composition and species among the various patient groups. Initial results suggest that glycerophospholipids are significantly more abundant in symptomatic diabetics compared to asymptomatic diabetics and are even more prevalent in symptomatic prediabetics than in either diabetic group. We also find that glycerol lipids are significantly more abundant in symptomatic diabetics compared to asymptomatic diabetics. These results suggest that lipid species could act as important biomarkers in understanding atherosclerosis disease progression in individuals with diabetes.

EFFICIENCY ASSESSMENT OF BRD4/PLK1 DUAL DEGRADERS: A PROMISING APPROACH TO CANCER TREATMENT

***ANDREA HERNÁNDEZ*, MENGYANG CHANG, WEI WANG**

Small-molecule inhibitors block the activity of enzymes involved in cancer. However, achieving high affinity requires high drug concentrations, increasing the risk of toxicity, drug resistance, and undesired outcomes. Molecular glues are novel molecules that address these problems. They alter the surface of either an E3 ligase or a protein of interest to stabilize their interaction and promote proteasome degradation. Most molecular glues against cancer focus on degrading a single protein, ignoring the possible benefits of eliminating a pair of tumor-related proteins. We attempted to evolve the mechanism of molecular glues by simultaneously degrading two overexpressed proteins in cancer: BRD4 and PLK1. The degradation ability, apoptosis/DNA levels, and cell viability of dual degrader candidates were tested by Western Blot assay, flow cytometry, and CCK8 assay. The BPD-05 compound displayed great degradation and similar cell activity to the commercial BRD4/PLK1 inhibitor in Molm-13 leukemia cell line.

MICROBIAL FERTILIZER: AN EXPLORATION OF ARIZONA NITROGEN FIXERS

***EMILY HERRERA*, ALONSO FAVELA**

Nitrogen is an essential nutrient for all organisms, yet the most abundant nitrogen source is not derived from the soil but rather exists as atmospheric nitrogen gas (N2). To meet the nitrogen demands of crops, the agricultural industry has long relied on inorganic nitrogen fertilizers to supplement soil nitrogen levels. However, certain bacteria living in the rhizosphere can naturally access atmospheric N2 and convert it into ammonia (NH3) through a process called nitrogen fixation. Our current agronomic practices are circumventing these biological means of nitrogen nutrition, likely because of a lack of understanding of how these N-fixing microbes work in the agroecosystem context. Here, we aimed to better understand how diverse bacteria with the capacity to fix nitrogen influence key plant productivity metrics, including biomass, growth rates, and soil moisture content. To achieve this we created a diazotroph isolate collection with a selective N-free media. On individual isolates, we carried out a N-fixation assay (via NH3 accumulation) and determined 4 relevant phenotypic groups. Three distinct microbes with high, medium, and low N-fixing rates were cultured from a Durham wheat soil sample and inoculated onto alfalfa sprouts. Surprisingly, we found that all 3 nitrogen-fixing microbes demonstrated similar productivity and nitrogen-fixing rates when introduced to alfalfa soil. This suggests that isolates may not significantly alter a plant's N-fixing capacity through symbiotic relationships or other mechanisms. In our recovery trial, we could not culture the original morphologies; instead, new microbes colonized our samples. This finding underscores the challenges of establishing nitrogen-fixing microbes in new environments. This may explain why microbes are not widely utilized as substitutes for inorganic fertilizers in agricultural systems. To address this limitation, we are conducting follow-up studies to investigate whether cultivating novel N-fixing microbial communities under conditions selective for N-fixers can produce more notable impacts on plant productivity.

ANALYZING THE EFFECTS OF HORIZONTALLY TRANSFERRED EF-TU

***CIARA HIMES*, DAVID BALTRUS**

A megaplasmid is a very large plasmid—a (usually) circular strand of extrachromosomal DNA that can be found in the cytoplasm of bacteria. Plasmids may contain genes that can be horizontally transferred, which can enable changes in the host phenotype. These changes can be beneficial, for example many plasmids contain genes that code for antibiotic resistance in their hosts. However, unless the host is in an environment that selects for this beneficial change, there is usually thought to be a fitness cost with maintaining a plasmid. Moreover, there are certain genes—known as housekeeping genes—that are thought to not be typically horizontally transferred.

A megaplasmid in L58, a strain of Pseudomonas, contains an identical copy of a critical gene, tufA, seen on the chromosome. TufA encodes for elongation factor Tu (EF-Tu), which is a protein responsible for bringing tRNA to the ribosome. Bringing tRNA to the ribosome allows for elongation of the polypeptide chain that is being formed, and ultimately protein production. Divergent copies of tufA might encode proteins that don't cleanly interact with proteins already in the cell, disrupting the protein production process taking place at the ribosome and potentially causing a fitness defect in the host. However, the tufA gene in the megaplasmid is horizontally transferred, which is unusual and rare. It is the only known case where a plasmid encodes tufA and, seeing as there is already a copy of this gene on the chromosome, the purpose of the megaplasmid tufA gene is unclear. This project aims to determine whether or not the megaplasmid tufA gene is functional and if the protein it encodes has any impact on its host's fitness.

ENHANCING SEGMENTATION: *EVEN-SKIPPED* ENHANCERS IN *TRIBOLIUM*

***SHEA HOLLIS, CAROLINE VASQUEZ*, VIOLET ROWLAND, MARYALEE ROAZEN, NINA KOYILLA, LISA NAGY**

The evolution of segmentation remains a mystery. Segmentation is the process of dividing an organism's body into repetitive units. Arthropods, annelids, and chordates are all segmented. However, their closest sister groups are not. Did they have a common ancestor, or did segmentation evolve three separate times? Segmentation mechanisms differ between organisms even within a phylum, such as the two arthropods *Drosophila melanogaster* (the fruit fly) and *Tribolium castaneum* (the red flour beetle). Drosophila segments simultaneously, whereas *Tribolium*, and most other segmented animals, form segments sequentially from the posterior. Despite these differences, the first genes expressed are pair-rule genes, which are expressed with two-segment periodicity, including the gene *even-skipped (eve)*. In *Drosophila*, the simultaneous production of eve stripes is regulated by stripe-specific enhancers; nothing is known about *Tribolium's eve* enhancers. Based on background knowledge, we hypothesize that *Tribolium* will have enhancers that undergo cycling in the posterior in addition to stripe-specific enhancers. Through bioinformatic tools and published ATAC-Seq data, we identified three potential eve enhancers: an upstream 2.0kb region, an intron unique to *Tribolium eve*, and a downstream 3.6kb region. Transgenic beetle lines were created to analyze enhancer-driven expression by immunofluorescence and hybridization chain reaction (HCR). The 3.6 region expression follows the early stripe 2 and 3 formation of endogenous eve. All three enhancer regions are expressed early in the posterior growth zone (PGZ) and late in the terminal regions, indicating they are likely shadow enhancers. The intronic enhancer is also expressed in the presumptive mesoderm. HCR experiments on later staged embryos will determine if the intron harbors a muscle-heart enhancer. Further exploration through CRISPR knockouts will reveal the direct effects of each potential enhancer on *Tribolium* eve expression. Understanding how transcription of the *Tribolium eve* gene is controlled gives insight into how arthropod segmentation has evolved.

COLONIC BUTYRATE ACTIVATES VAGAL AFFERENT NEURONS TO IMPROVE GLUCOSE TOLERANCE

***REBEKAH HUBBLE*, HALLIE WACHSMUTH, FRANK DUCA**

Signals from the gastrointestinal tract are known to influence glucose homeostasis. Vagal afferent neurons (VANs) directly link the gut to the brain and have been previously demonstrated to be involved in regulating hepatic glucose production via small intestinal nutrient-sensing mechanisms. However, consumption of a western-style diet (WD), high in fat and sugar and low in dietary fiber blunts VAN signals, resulting in dysregulated glycemia. Data from our lab shows WD impairs glucose homeostasis and is associated with an altered gut microbiome and reduced butyrate production, a short chain fatty acid produced by bacterial fermentation of dietary fiber in the large intestine. Exogeneous butyrate administration improves glucose homeostasis in WD-fed rodents, but the mechanisms are not fully understood. Previous studies have shown that butyrate induces glucagon-like peptide-1 (GLP-1) release from colonic enteroendocrine cells, and VANs that express the GLP-1 receptor innervate the colon and portal vein. Therefore, we hypothesized that colonic butyrate acts via GLP-1 to increase VAN signaling and regulate glucose homeostasis. To test this, we infused butyrate or saline into the colon of rats during a glucose tolerance test. Rats receiving butyrate infusion had improved glucose tolerance and increased VAN activation compared to saline-infused controls. Next, TRAP2 mice were used to determine if butyrate-induced VAN activation is sufficient to improve glucose homeostasis in WD-fed mice. TRAP2 mice express a tamoxifen inducible cre under the early activation gene (Fos promoter) which is rapidly expressed upon neuronal activation. Bilateral nodose injections of a cre-dependent excitatory DREADD were injected into TRAP2 mice, and two weeks later implanted with a colon catheter. Butyrate-responding neurons were then 'trapped' during a 15 minute colonic infusion of butyrate. Activation of the butyrate 'trapped' VANs improved glucose homeostasis in mice compared with the control. These studies establish that colonic butyrate improves glucose tolerance via VAN signaling.

IMAGE ANALYSIS OF MECHANICALLY CONDITIONED BREAST CANCER TISSUE IN MOUSE MODELS

***SOMTO IKE*, MARCO PADILLA RODRIGUEZ, FAITH RICE, JENNIFER BARTON, GHASSAN MOUNIEMMNE**

The study of cancer metastasis is critical for understanding the progression and treatment of cancer. This research focuses on the image analysis of tissue cells obtained from mouse models to evaluate the extent of metastasis. Using advanced imaging techniques, including NIS-Elements and other imaging tools, we analyzed matrix conditioned cells (cell pulling on its matrix) , obtained with multiphoton spectroscopy, to determine the degree of collagen interaction and overlap, a hallmark of aggressive cancer growth.

Our approach involved quantifying the crossing frequency of the collagen fibers and recording the number of collagen branches and endings. We hypothesized that increased collagen matrix overlaps correlate with heightened cancerous activity due to the rapid and unregulated proliferation of cancer cells, leading to random and excessive collagen crossing. The results of this study provide valuable insights into the mechanical and structural characteristics of cancerous tissues, potentially informing better diagnostic and therapeutic strategies.

This work aligns with the Bioimaging & biomarkers in physiology category, as it leverages cutting-edge imaging techniques to reveal critical biomarkers of cancer metastasis. The findings could have broad implications for understanding cancer progression and developing novel treatment approaches.

CHARACTERIZING THE COPPER STRESS RESPONSE USING MULTI-OMICS DATA

***ISABELLA IRBY*, BRADFORD HULL, GEORGE SUTPHIN**

In Arizona, 1 in 4 adults is 65 years old or older. To promote healthy aging and reduce the risk of age-related illnesses, our research aims to increase the human healthspan. We study how exposure to copper, an environmental stressor commonly found in Arizona, can protect humans from the toxic effects of other stressors using the model organism Caenorhabditis elegans. We discovered that copper acts through a non-canonical mechanism and are now integrating multi-omics data with PANDA to complete transcription factor analysis and follow-up RNAi knockdowns to explore the genes involved in this non-canonical copper stress response pathway. By better understanding the mechanism of copper stress, we can better equip our community to treat age-associated diseases correlated with copper stress.

We hypothesized that transcription factors predicted by PANDA to control upregulated genes under copper stress would reveal key regulators of this pathway. PANDA analysis identified notable transcription factors, including CEH-36, NHR-42, and CEH-23, linked to metabolic adaptation, sensory response, and developmental regulation. Zinc-finger transcription factors (ZTFL-2, ZTFL-3) and helix-loop-helix transcription factors (HLH-10, HLH-27) were implicated, suggesting a complex regulatory network mediating neuronal, developmental, and detoxification processes under stress. To validate these findings, we are currently running RNAi knockdowns of candidate transcription factors, analyzing their effects on lifespan under copper and control conditions. This approach directly connects transcription factor activity to stress resilience.

This research enhances our understanding of the copper stress response and its implications for age-associated diseases, particularly in populations vulnerable to environmental toxins. By identifying key transcription factors, we elucidate regulatory mechanisms underlying copper toxicity and pave the way for targeted interventions. These findings inform public health strategies and therapeutic approaches to mitigate the risks of age-related diseases linked to environmental factors related to copper, ultimately improving health outcomes for aging populations in regions like Southern Arizona.

DETECTING MOVEMENT OF SIRNA FROM SEED COAT TO ENDOSPERM

VIDYA IYER

ANTENATAL DEPRESSIVE SYMPTOMS PREDICT CESAREAN BIRTH

***RUESHUNDA JIM*, ERIN GEORGE, ELISE ERICKSON**

In 2023, the Cesarean Delivery (CD) rate in the United States rose to 32.4%, with a notable increase in nulliparous patients. Concurrently, maternal depression remains a significant concern, with 1 in 5 women not being screened for depression during prenatal visits. Previous studies have not established a clear association between antenatal depression and CD, although women of color experience higher rates of complications, including CD. This study aims to examine the relationship between antenatal depressive symptoms and the mode of delivery, utilizing data from the Nulliparous Pregnancy Outcomes Study: Monitoring Mothers-to-Be (NuMoM2b), which enrolled first-time mothers and assessed depression using the Edinburgh Postnatal Depression Scale (EPDS) given during the patients' third trimester. Statistical analyses, including chi-square tests and logistic regression, were performed to explore the impact of depressive symptoms, maternal characteristics, and comorbidities on CD. The study included 2,273 participants, after inclusion criteria were applied with significant findings across racial groups. Results indicated that a 1-point increase in EPDS score was associated with a 2.6% increase in the odds of CD ($p=0.024$). Furthermore, increased BMI and age were also linked to higher CD rates. The treatment of depression was associated with a 27% reduction in CD odds. This study highlights the potential influence of untreated depressive symptoms on delivery outcomes, suggesting that maternal mental health interventions may reduce CD rates. Further research is needed to understand the direct and indirect impacts of depressive symptoms on pregnancy outcomes.

USING ENTEROIDS TO EXAMINE SMALL INTESTINAL NUTRIENT SENSING MECHANISMS

***NIKHIL JOHNSON*, SAVANNA WENINGER, JULIA MORRIS, CURTIS THORNE, FRANK DUCA**

Ingested lipids signal from the gut to the brain through a complex pathway that has yet to be fully elucidated, making it unclear how changes in the small intestinal microbiota improve lipid sensing. High fat (HF)-feeding impairs gut-brain signaling in response to small intestinal nutrients, which is restored following treatment with the prebiotic oligofructose (OFS). However, it is unclear how OFS impacts small intestinal nutrient sensing mechanisms. This study aimed to investigate these mechanisms using a small intestinal organoid model. Organoids from HF and HF-OFS mice were treated with oleic acid to assess nutrient-induced GLP-1 secretion. HF-OFS organoids exhibited significantly increased GLP-1 secretion, Cd36 mRNA expression, and increased number of enteroendocrine cells compared to HF organoids, all suggesting enhanced nutrient sensing in organoids from HF-OFS mice. However, it is unclear how OFS supplementation improves small intestinal nutrient sensing machinery, although we hypothesize a possible mechanism involving the gut peptide GLP-2. To explore the role of GLP-2 in gut peptide secretion and Cd36 expression, organoids were treated with GLP-2. However, no changes in nutrient receptor expression were detected, as expected given intestinal epithelial cells do not express GLP-2 receptor. These results indicate that GLP-2 likely acts on paracrine tissues rather than directly on the epithelium, possibly via insulin-like growth factor 1 (IGF-1) signaling from subepithelial myofibroblasts. Therefore, organoids derived from HF-fed mice were treated with IGF-1. IGF-1 treatment increased GLP-1 secretion in response to oleic acid and increased expression of nutrient sensors in organoids generated from HF-fed mice. These findings demonstrate that this organoid model offers a valuable high-throughput system to study nutrient-induced GLP-1 release and changes in nutrient receptor expression in response to diet or treatments. Additionally, the model highlights the potential for intestinal organoids to replicate animal phenotypes in short-term experiments, offering insights into intestinal nutrient sensing mechanisms.

GERMLINE MUTATION SPECTRUM, DEMOGRAPHICS, AND OUTCOMES IN PATIENTS AT THE UNIVERSITY OF ARIZONA CANCER CENTER: A RETROSPECTIVE ANALYSIS

***KAYLA JONES*, LAUREN MAYNARD, SETSUKO CHAMBERS, SIMA EHSANI**

Cancers can be categorized as ‘hereditary’, caused by inherited genetic mutations, and ‘sporadic’, caused by spontaneous mutations accumulated over a person’s life due to various risk factors. Most cancers are sporadic, while 5-10% are hereditary due to germline mutations. Genetic counseling and testing provide insights into germline mutations and its correlation with cancer risks. The purpose of this study was to compare 1) the characterized cohort of patients who have undergone genetic counseling and testing at the University of Arizona Cancer Center (UACC) and tested positive for germline mutations related to cancer risks (Progeny Cohort) to 2) the overall UACC cancer patient database (UACC Cohort). This retrospective analysis utilized the medical and ancestry information from UACC’s clinical genetic counseling patient database ‘Progeny’ (N=904) and the overall UACC cancer registry from years 2019-2023 (n=11878) to evaluate potential health disparities by analyzing cancer diagnoses, germline mutations, race/ethnicity, gender, age, and zip code. The most common mutations seen in the Progeny database were *BRCA2* (N=211, 23.34%) and *BRCA1* (N=121, 13.38%), with breast cancer being the most common cancer diagnosis for those with germline mutations. Skin and breast cancer were the most commonly diagnosed cancers within the UACC cohort. The age at cancer diagnosis, although similar between the two groups, had a curve shift that was younger for those with germline mutations. Females received genetic counseling and testing at a higher rate than males ($p<0.001$). Patients in the Progeny Cohort were more likely to be from Arizona when compared to the UACC Cohort who 1) received diagnosis and care at UACC or 2) transferred care to UACC ($p<0.001$). Because patients’ racial and ethnic demographics were comparable between Progeny and UACC, there is indication that the UACC genetics clinic aids a representative cohort.

FLUORESCENT NANODISCS REPORTERS FOR LIPIDOMIC LIPID EXCHANGE-MASS SPECTROMETRY

ISAAC KAILAT

TRACKING SOIL PROPERTIES, WATER-HOLDING CAPACITY, AND INFILTRATION IN RESPONSE TO DROUGHT STRESS

***YVONNE KAN*, ALONSO FAVELA**

Beneficial microbiome interactions with plants have been shown to boost plant growth by altering soil characteristics in response to environmental factors. Stressors such as drought could elicit higher production of extracellular polymeric substances (EPS) or allow microbes to change the soil density, thereby changing the amount of water the soil could hold. However, there is still much unknown about the conditions in which microbes readily alter soil and how these properties benefit the plant. To understand more about these plant-microbe interactions, we set up mesocosms to control the type of microbes present (no microbes, an isolated microbe, and a community of microbes) and the amount of water available in the system. To capture the microhydrology of the mesocosms, we measured water holding (WH), infiltration, evaporation, and soil properties we believe microbes are altering (EPS, pore space, etc.) We hypothesized that the no diversity (i.e. uninoculated) systems would hold the least amount of water because there are no microbes altering soil properties. Unexpectedly, no diversity systems had higher water holding than the low and high diversity systems that we tested. We believe that this was caused by a difference in infiltration driven by microbial diversity. High diversity systems appear to accumulate biomass, EPS, and pore space that allows water to move into the soil profile, without changing the water holding capacity. Interestingly, if we cyclically re-wet systems, we observe that high-diversity systems are modifying their hydrology through time. In conclusion, it appears that soil microbial communities play an active and understudied role in the movement of water in soils. In arid regions, deepening our microbes-water relationship will be essential for advancing sustainable, low-water agricultural practices and enhancing resilience to water scarcity.

TUMOR INFILTRATING MONOCLONAL ANTIBODY PRODUCTION

DANI KHATIB, COLIN FIELDS, STEPHANIE WORRELL, DEEPTA BHATTACHARYA

The current state of cancer treatment is largely dependent on the use of chemotherapy and radiotherapy—two treatments that cause inadvertent damage to many healthy cells within patients. Immunotherapy, on the other hand, is a method of treating cancer using the patient's own immune system. One of the mechanisms through which the human immune system promotes tumor elimination is the production of antibodies that bind to cancerous cells. Therefore, characterizing both the antibodies and their respective cancer-dependent epitopes is vital for the development of monoclonal antibodies (mAbs). As a part of immunotherapy, mAbs can be administered to cancer patients, where they bind to cancerous cells and allow for the patient's immune system to specifically target cancerous cells while minimizing harm to healthy cells. The aim of this research project is to produce tumor-infiltrating monoclonal antibodies for immunotherapeutic treatment of lung cancer. Such research is essential for the advancement of the field of immunotherapy in order to implement a more direct treatment for lung cancer

that is significantly less harmful to healthy human cells. Additionally, identifying the distinguishing cell surface markers between healthy and cancerous cells can uncover the fundamental process to break the immune system's self-tolerance.

SHORT TERM VS LONG TERM RESPONSE OF PRIMARY FIBROBLASTS TO MERKEL CELL POLYOMAVIRUS LARGE T-ANTIGEN (LT) EXPRESSION

***DAE-KYUNG KIM*, MIREYA HERRERA-HERRERA, MEGHA PADI**

Merkel Cell Carcinoma (MCC) is a rare, aggressive type of neuroendocrine skin cancer that arises when there are mutations or infection of skin cells such as fibroblasts, pro B cells, and neural progenitors. MCC can be caused by UV radiation or by integration of the Merkel cell polyomavirus (MCPyV) genome into normal skin cells. The MCPyV large T (LT) antigen inhibits the retinoblastoma (Rb) protein, a vital tumor suppressor, promoting uncontrolled cell division while avoiding cytotoxic effects. Our goal is to characterize how skin cells respond to LT expression in the short-term, including the immediate downstream effects of Rb inhibition, and in the long-term, encompassing potential epigenetic and developmental changes in cell state. We used neonatal human dermal fibroblast (nHDF) and MRC5 embryonic lung fibroblasts expressing green fluorescent protein (GFP) as a control, or MCPyV LT, under the control of a doxycycline-inducible promoter. By observing the cells' behavior and analyzing which genes are upregulated and downregulated, we associated gene expression with functional outcomes like increased cell proliferation, differentiation, and loss of adhesion leading to a floating cell phenotype resembling neurospheres. Our results suggest that MCPyV LT could induce skin cells to develop neural progenitor characteristics that allow them to circulate freely in bodily fluids, opening up future research directions in metastatic mechanisms of Merkel cell carcinoma.

FETAL GROWTH RESTRICTION CAUSES DYSREGULATION OF GENES IN THE INSULIN SIGNALING PATHWAY CONTROLLED BY TGF*β* IN SATELLITE CELLS FROM LAMBS

***KAYLEE KIMBRELL*, ROSA LUNA, SEAN LIMESAND**

Fetal growth restriction (FGR) caused by placental insufficiency (PI) reduces skeletal muscle mass in fetuses. Skeletal muscle is essential for metabolic functions and deficits increase future risk of metabolic diseases. Insulin growth factor (IGF-1) promotes muscle growth through the insulin signaling pathway (ISP), whereas transforming growth factor beta (TGFβ) inhibits muscle satellite cell (SC) proliferation and differentiation. Crosstalk between IGF-1 and TGFβ produces opposing effects on muscle growth. Altered expression of genes related to IGF-1 or TGFβ affect muscle mass. We have shown differential methylated status on gene promoters in the ISP between FGR and control (CON) SC. Three promoters for INSR, PI3K-CD, WNT1 were hypomethylated in FGR SC and the DAXX promoter was hypermethylated. We hypothesize that TGFβ responsiveness in FGR SC was lower due to hypomethylation of INSR, PI3K-CD, and WNT1 promoters.

SC were collected from 30-day old FGR and CON lambs. SC were differentiated in low-serum media and treated with either TGFβ or TGFβ inhibitor (A83-01) for 6 hours. Chromatin immunoprecipitation (ChIP) was then performed to verify access of RNA Pol II to gene promoters. ChIP was achieved through fixation and collection of treated cells, sonication of chromatin, treatment with anti-RNA Pol II antibody, and immuneabsorption with magnetic beads. Captured DNA was amplified with qPCR using primers to INSR, PI3KCD, DAXX, WNT1 promoters.

INSR, PI3KCD, and WNT1 promoter concentrations were greater in FGR SC treated with TGFβ compared to FGR SC treated with A83-01, while CON SC were unresponsive. Conversely, TGFβ regulation of apoptosis gene (DAXX) was reduced in FGR SC. These data confirm enhanced transcription of genes regulating differentiation in the ISP despite TGFβ stimulation. Furthermore, our findings support the DNA methylation status results in FGR SC and suggest that FGR SC are less responsive to TGFβ due to dysregulation of key promoters in the ISP.

ROLE OF RAP1 VERSUS PIP3 IN THE REGULATION OF MTORC2

***CALEB KONECEK*, ISAIAH TOTH, GENESIS CAHIGAS, SHANNON COLLINS, PASCALE CHAREST**

Chemotaxis is a directed cell migration in response to external chemical stimuli that is critical for biological processes like development and immune response. Chemotaxis dysregulation has been linked to disease spread like cancer metastasis, but its disruption isn't fully understood. Research has shown that the mechanistic Target of Rapamycin Complex 2 (mTORC2) is crucial for chemotaxis regulation and cytoskeleton structural protein rearrangement. Despite mTORC2's implications in chemotaxis, its precise role and regulation are unclear. Recently, we have identified the small GTPase Rap1 as a binding partner of the SIN1 component of mTORC2, and experiments have linked Rap1 overexpression to increased mTORC2 activity in mammalian cells. Additionally, evidence suggests that the membrane phospholipid PI(3,4,5) (PIP3) similarly regulates mTORC2 activity by binding it to the plasma membrane with experiments demonstrating decreased activity following inhibition of PIP3 production. Although Rap1 and PIP3 positively regulate mTORC2, there is a critical gap in understanding their relationship in its regulation and localization to the plasma membrane. Our research attempts to clarify their relationship as binding partners of SIN1 to provide insight into chemotaxis and disease processes. We hypothesize that Rap1 and PIP3 independently regulate mTORC2 activity in HEK293 cells by playing similar roles in its localization. To test this, we over expressed Rap1 and used a PIP3-production inhibitor, examining their effects on mTORC2 activity in HEK293 cells in response to stimulation by insulin, a strong activator of mTORC2. So far, our research shows no significant difference between the effects of Rap1 and PIP3 on mTORC2 activity.

MICROSCOPY STRATEGIES FOR 3D RECONSTRUCTION OF VISCOELASTIC PROPERTIES OF TISSUE

KAYMA KONECNY

EXPRESSING RECOMBINANT HUMAN PAPILLOMAVIRUS GENOMES WITH SELECTABLE TRAITS IN TONSILLAR KERATINOCYTES

***DAVID KOSANKE*, ISABELLE TOBEY, KELLY KING, KOENRAAD VAN DOORSLAER, SAMUEL CAMPOS**

Human papillomavirus (HPV) is one of the most common sexually transmitted infections and is responsible for approximately 5% of cancers worldwide, with high-risk types such as HPV16 and HPV18 being particularly prevalent. HPV16 is notably the most common type associated with HPV-positive head and neck cancers, though the molecular mechanisms behind this prevalence are not yet understood. This is closely followed by HPV18, although this type does not generally cause cancer in this niche, making it a useful control. In this study, we constructed a recombinant HPV16 genome that expresses a selectable

neomycin-resistance trait, termed HPV16 Neo.We transfected three primary human tonsil keratinocyte cell lines with HPV16 or HPV18 to investigate the effects of these two HPV types on head and neck cancer development. This approach successfully generated six cell lines—one HPV16 Neo and one HPV18 Neo line per keratinocyte lineage. qPCR and western blot analysis confirmed the presence of viral genomes, transcripts, and proteins in all six lines. Furthermore, the recombinant HPV genomes enabled tonsil keratinocytes to form robust colonies under neomycin selection, confirming successful expression of HPV-related traits in these cell models. Future experiments with these cell lines will focus on investigating the role of HPV oncoproteins in suppressing innate immune pathways and exploring why HPV16 genomes integrate into host chromosomes at a significantly higher rate than other HPV types.

DEMOGRAPHIC HISTORY AND FITNESS EFFECTS IN ALLOPATRIC MIDAS CICHLID SPECIES

LILITH KOTLER

INVESTIGATING THE POTENTIAL OF VAGUS NERVE STIMULATION AS A PRIMER FOR NEURAL PLASTICITY

***KAITLYN LAI, GAVIN ARNOLD, CATHERINE JEZERC*, MARK SUNDMAN, YING-HUI CHOU**

Neural plasticity can be defined as the brain's ability to reorganize itself, a feature of paramount importance for healthy cognitive functioning and rehabilitation of injured or impaired brains. Notably, this feature exists along a continuum, fluctuating due to interpersonal factors such as age and lifestyle, as well as intrapersonal 'day-to-day' variations such as in mood or sleep. Increased plasticity corresponds to an enhanced ability to form memories, learn new information, recover from injuries, etc. Therefore, a burgeoning frontier of cognitive neuroscience is to develop experimental techniques that increase the brain's neuroplastic potential, hence 'priming the brain' to maximize human cognition and brain rehabilitation. Transcutaneous auricular vagus nerve stimulation (taVNS) is an emerging method that displays the potential to enhance neural plasticity. Its widespread projections allow taVNS to non-invasively stimulate the nerve through the ear, and prior research has shown positive associations between taVNS and enhanced learning and memory in healthy adults.

This project aims to integrate this novel technique alongside an established secondary non-invasive stimulation tool, transcranial magnetic stimulation (TMS). Decades of literature support the use of TMS over the motor cortex to non-invasively measure plasticity of the brain by collecting motor evoked potentials (MEPs) from the APB and FDI muscles of the hand. Additionally, short-latency afferent inhibition (SAI)—a paired pulse protocol that uses TMS—will be utilized to measure cholinergic activity of specific regions of the brain. As taVNS modulates neural activity through noradrenergic and cholinergic mechanisms, SAI serves as another measure of its effects. It is hypothesized that the average MEP amplitude will increase post-taVNS and there will be an enhancement of SAI due to taVNS' modulation through cholinergic pathways. Therefore, a single session of taVNS will be conducted among healthy young adults to investigate its capabilities in 'priming the brain' by increasing neuroplasticity as measured with TMS.

EFFECT OF FETAL GROWTH RESTRICTION ON SKELETAL MUSCLE GROWTH IN SHEEP FETUSES

***SANVI LAMBA*, MARIANGEL VARELA, WEICHENG ZHAO, SEAN LIMESAND**

Fetal growth restriction (FGR) is a significant obstetric complication associated with increased perinatal morbidity and mortality. FGR is closely tied to deficient skeletal muscle growth as skeletal muscle is the largest soft tissue by mass in the fetus. This study aimed to investigate the effects of FGR on fetal musculoskeletal development. FGR fetal sheep (n = 6) was induced from exposing pregnant ewes to hyperthermia between d40 and d95 of gestation (term gestation = d149). Control fetuses (n = 8) were from pregnant ewes reared in thermoneutral conditions. Fetal arterial blood were collected from surgically indwelling catheters at late gestation for blood gas and biochemistry analysis. Growth parameters, including fetal and placental weights, crown-rump and hindlimb lengths, and hindlimb muscle masses, were also evaluated at necropsy. At late gestation (d133), FGR fetuses had lower plasma glucose (11.7 ± 1.0 vs. 15.5 ± 0.9 mg/dL, P = 0.039) and reduced blood total oxygen content (2.8 ± 0.3 vs. 3.6 ± 0.2 mmol/L, P = 0.01) compared to control fetuses. Both fetal weight (2246 ± 213 vs. 3485 ± 197 g) and placental weight (230 ± 40 VS. 412 ± 37 g) were reduced in the FGR group (both P < 0.01). FGR fetuses exhibited a higher brain-to-liver weight ratio (0.65 ± 0.07 vs. 0.43 ± 0.06, P = 0.001), indicating the asymmetrical growth pattern and the brain-sparing effect. FGR fetuses had shorter crown-rump length (40.2 ± 1 vs. 47 ± 1 cm, P = 0.001) and hindlimb length (33 ± 1 vs. 38 ± 1 cm, P = 0.005). Hindlimb muscle mass was also reduced in FGR fetuses, including the biceps femoris (13.4 ± 2.5 vs. 18.9 ± 2.3 g, P = 0.049), semitendinosus (4.2 ± 0.7 vs. 6.0 ± 0.7 g, P = 0.034), gastrocnemius (8.4 ± 1.0 vs. 12.9 ± 1.0 g, P = 0.003), and tibialis anterior muscles (3.7 ± 0.4 vs. 5.7 ± 0.4 g, P < 0.001). In summary, FGR fetuses exhibited hypoglycemia, hypoxia, and reduced placental function, likely contributing to impaired hindlimb muscle development. These findings suggest that nutrient deficiency and hypoxic stress are key factors driving the observed growth impairments. Further research is warranted to elucidate the molecular mechanisms underlying the reduced muscle growth in FGR fetuses.

RYANODINE RECEPTOR LEAK AND OXIDATIVE STRESS CONTRIBUTE TO ARRHYTHMOGENESIS IN THE CALM2-D132E(+/-) RAT MODEL OF CALMODULINOPATHY

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Calmodulinopathy is a life-threatening arrhythmia syndrome associated with mutations in calmodulin. The phenotype is variable, but patients typically present with catecholaminergic ventricular tachycardia (CPVT) or long QT syndrome, arrhythmias associated with intracellular Ca2+ mishandling. A subset of calmodulinopathy patients also demonstrate significant cardiac remodeling, but underlying molecular mechanisms remain unclear. Given that the endoplasmic reticulum (ER) stress system has been implicated in other Ca2+-dependent arrhythmias, and is also critical for the hypertrophic response, we hypothesized that maladaptive ER stress contributes to cardiac remodeling and the arrhythmogenic phenotype in this calmodulinopathy subset. To test this, we have generated the first rat model of the disease, induced by CALM2-D132E(+/-) mutation. Similar to the patients, CALM2-D132E(+/-) rats present with bidirectional VT accompanied by torsade-de-pointes, characteristic of mixed CPVT/long QT syndrome. Echocardiography revealed significant structural remodeling and cardiac dysfunction. Western blot demonstrated increased markers of ER stress in diseased ventricles. Assessment of intracellular Ca2+ transients in CALM2-D132E(+/-) ventricular myocytes (VMs) under b-adrenergic stimulation revealed reduced Ca2+ transient amplitude, spontaneous Ca2+ wave latency and caffeine-sensitive SR Ca2+ content, all indicative of significant Ca2+ mishandling. Of note, diseased VMs demonstrated increased SR Ca2+ leak measured with biosensor G-CEPIA1er, indicative of RyR2 hyperactivity. As RyR2 is sensitive to redox modifications, we sought to test whether ER stress disturbed SR redox status. Indeed, experiments utilizing targeted biosensor ERroGFP_iE revealed increased oxidative stress in the SR. Importantly, genetic inhibition of ER stress components normalized SR redox status and attenuated RyR2 activity in diseased VMs, reducing pro-arrhythmic spontaneous Ca2+ release. Collectively, our data implicate ER stress crosstalk with SR Ca2+ release in the arrhythmogenic phenotype of CALM2- D132E(+/-) rats. In future studies, we will test whether benefits of reducing ER stress in calmodulinopathy extend beyond normalizing intracellular Ca2+ handling to attenuating pathological cardiac remodeling.

EFFICACY OF A NOVEL PROTEASE-ACTIVATED RECEPTOR-2 (PAR2) BIASED BETA-ARRESTIN ANTAGONIST (C957) IN RESPONSE TO ACUTE ALLERGEN EXPOSURE OF MICE EXPRESSING HUMAN PAR2

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Asthma is a heterogeneous disease that affects millions of people worldwide. Current treatments for asthma are limited and mostly focus on reducing symptoms rather than targeting the cause of the problem. Despite recent advances in biological therapies, there remains a need for new therapeutic options that target upstream regulators. Protease-activated-receptor 2 (PAR2) is a G protein-coupled receptor that has been shown to play a role in allergic asthma and is a promising drug target for new treatments. In this study, I describe the in vitro and in vivo action of C957, a new PAR2-biased antagonist. A human airway epithelial cell line that naturally expresses human PAR2 was used to evaluate the ability of C957 to alter ligand- or protease-activated PAR2 signaling in vitro. C957 reduced both ligand- and trypsin-induced PAR2 in vitro physiological signaling. Further examination of PAR2-dependent intracellular signaling showed that C957 acts as a biased-ligand antagonist, effectively limiting β-arrestin/MAPK signaling while having no effect on Gq/Ca2+ signaling pathways. I next evaluated C957's effects on allergic asthma using an acute allergen (Alternaria alternata) challenge of a mouse that expresses both human and mouse PAR2 (hmPAR2). C957 effectively limited A. alternata-induced airway hyperresponsiveness, had a sex-specific effect on limiting, and had no effect on mucus overproduction. My work shows that PAR2 β-arrestin/MAPK-biased antagonist C957 is an effective treatment for allergen-induced airway hyperresponsiveness and inflammation in mice. Further studies on pharmacokinetics and pharmacodynamics will establish C957 as a novel drug hit for the treatment of allergic asthma.

EVALUATION OF EXOSOMES CONTAINING EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR): ROLE AS A BIOMARKER

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It has been hypothesized that exosomes containing Epidermal Growth Factor Receptor (EGFR) could be used as biomarkers for a variety of clinical applications. This extends to monitoring disease progression and evaluating the efficacy of a sorting nexin peptide inhibiting drug, cSNX1.3, a therapeutic agent used to induce regression in lines of triple negative metastatic breast cancer. In this study, we developed a methodology for extraction, concentration, and purification of exosomes, followed by the assessment of protein markers. Through the analysis of our samples, we confirmed the presence of EGFR, as well as multiple exosome markers, including TSG-101, and two tetraspanins: CD81 and CD9. Treatment with cSNX1.3 led to the cessation of exosome release, marked by suppressed levels of detectable EGFR & exosome markers. Proteomic analysis revealed a significant down-regulation of EGFR as well as exosome markers such as CD44 and CD9 in cSNX1.3 treated cells. Further analysis using a retrograde trafficking inhibitor, Retro-2, suggested that the role of retrograde trafficking may not have a direct relationship to exosome formation and release. Retro-2 treatment inhibited exosome release in MDA-MB-468 cells but had no effect in HCC70 cells. Moreover, treatment with cSNX1.3 appeared to alter exosome contents in HCC70 cells rather than suppress exosome formation or release. Future studies will continue exploring the characterization of EGFR as a biomarker of response to cSNX1.3 treatment, and experiments will be expanded to include other cell lines of triple negative metastatic breast cancer.

BACTERIAL IMMUNE PRIMING IN DROSOPHILA MELANOGASTER IS LONG-LASTING, BUT NOT PATHOGEN-SPECIFIC.

***MAYA LEONARD*, EMILY BURKE, TODD SCHLENKE**

Invertebrates lack an adaptive immune system, which produces antibodies critical to fighting repeated infections in vertebrates. Despite relying solely on their innate immune system, invertebrates can acquire immunity against pathogens similar to how vertebrates respond to vaccination. This phenomenon, called "immune priming," remains mechanistically elusive despite extensive investigation. This is partially due to an inconsistency of model organisms, pathogens, infection methods, and results across various studies, with many not observing immune priming at all. Our lab successfully demonstrated consistent priming in D. melanogaster, a prominent genetic model organism used to study innate immune responses and host-pathogen interactions, placing our methodology as a viable framework for future immune priming studies. Additionally, our lab discovered D. melanogaster exposed to low-dose, live injections of bacteria (E. faecalis or S. marcescens) displayed improved survival against a subsequent lethal infection compared to mock-infected controls. We revealed that immune priming is long-lasting, but not pathogen-specific. This work contributes to scientific understanding concerning the mechanism of immune priming, but also how to take advantage of its capabilities. In the future, we may leverage these mechanisms to boost the immune responses of invertebrates beneficial to human society such as pollinators and livestock.

CHRONIC KIDNEY DISEASE OF UNKNOWN ETIOLOGY (CKDU) IN WORKERS: CUMULATIVE RISKS AND KNOWLEDGE GAPS

***IKA LIN*, AISHWARYA DABHOLKAR, MARY FOX**

Emerging research highlights Chronic Kidney Disease of unknown etiology (CKDu) as a growing global health concern. Characterized by declining renal function in the absence of traditional risk factors (e.g., diabetes, hypertension), CKDu primarily affects agricultural workers involved in physically demanding occupations co-exposed to heat stress.

One population of concern is Nepali migrant workers, as labor migration has been integral to Nepal's socioeconomic landscape. Driven by high unemployment rates, food insecurity, and rising living costs, international remittances comprise over a quarter of Nepal's Gross Domestic Product (GDP).

Once abroad, migrant workers are often employed in low-skilled occupations (e.g., construction, manufacturing) in working conditions that pose significant health concerns. Gulf Cooperation Council countries and Malaysia – the destinations of 88% of Nepalese migrant workers – are subject to extreme heat events and high temperatures. Compounded by long working hours and limited access to drinking water, heat-related illnesses – including acute kidney injury – have been increasingly reported.

Research by the Nepal Development Study (NeDS) in 2022 found that 31% of patients receiving treatment at two large dialysis centers in Kathmandu were returnee migrants. While CKDu has been rising in prevalence, definitive risk factors and underlying etiologies are yet to be identified. As such, this umbrella review aims to summarize the existing body of CKDu literature with a focus on identifying associated exposures, synthesizing knowledge deficits, reporting research, policy, and practice recommendations, and assessing the inclusion of migrant populations.

The umbrella review findings will inform the Enhanced Network for Safety and Occupational Health Utilisation and Risk Evaluation (ENSURE) project. Spearheaded by La Isla Network, ENSURE-Nepal is a four-year project to assess the socioeconomic and health exposures that prompt migration and develop scalable interventions. Ultimately, this project will aid in designing and implementing multidisciplinary efforts to protect migrant workers' health.

EVALUATING MACHINE LEARNING MODELS TO PREDICT ALZHEIMER'S DISEASE SEVERITY FROM QMRI METRICS IN HIPPOCAMPAL SUBFIELDS

CASSIDY LITTLE

NEUROFILAMENT H CHANGES IN THE RETINA AFTER SIX WEEKS OF DIABETES

***VIJETHA LOGAPRABHU*, ANDREA WELLINGTON, ERIKA EGGERS**

Diabetic retinopathy is a complication of the chronic disease diabetes, which affects the eyes leading to vision loss or blindness caused by damage to the blood vessels in the retina. In this research project, we are examining a specific type of cell within the retina known as the alpha ganglion cells to study if there is any neuronal damage and its extent in these cells in people with diabetes as the cells play a critical role in the integration and transmission of visual information form the retina to the brain. For example, the somas of the retinal ganglion cells are responsible for integrating visual information from bipolar cells and other retinal neurons while the axons are responsible for transmitting the integrated visual information from the retina to the brain.

In this study we used the SMI32 antibody to label for Neurofilament H, which is a structural protein that is key to the composition of the structural components of the axons and somas of alpha ganglion cells in the retina, in 6 week diabetic and non-diabetic mice. Then using the Image J/ Fiji computer program and GraphPad PRISM applications we performed analysis on the whole-mount retina from diabetic and non-diabetic mice to examine if there is a difference in the number of somas and or axons of the retinal ganglion cells in the two different groups of mice.

Our analysis illustrated that there is a significant difference (p - value of 0.006) in the number of alpha ganglion retinal cell somas in diabetic and non-diabetic mice and no significant difference (p - value of 0.5) in the number of alpha ganglion retinal cell axon bundles in diabetic versus non-diabetic mice, suggesting that in the population of ganglion cells there is a change in the number of somas in diabetic animals.

MITOCHONDRIA ON THE MOVE: EXPLORING HOW GLIA AID NEURONS IN INJURY MODELS OF D. MELANOGASTER

***GAMALIEL LUNA AHUMADA*, ADITI GHOSH, SUSAN SHARPE, KIARA BACHTLE, MARTHA BHATTACHARYA**

Mitochondria are crucial for energy regulation and cell survival, especially for neurons which have an energy-intensive nature, making them particularly susceptible to stress and injury. Maintaining proper mitochondrial function is thus essential for neuroprotection. Additionally, mitochondria have been shown to transfer between glial cells and neurons after nerve injury in a process called mitochondrial transcellular transfer (mitoTCT). This exchange of mitochondria presents a novel method of cell-to-cell communication and may be involved in whether neurons undergo protective or destructive consequences following damage and glial cell intervention. While neuron-glia mitoTCT has been observed in cultured conditions and mouse stroke models, the major pathways regulating mitoTCT remain unclear.

Our project employs confocal microscopy and imaging flow cytometry to visualize and quantify neuron-glia mitoTCT frequency post-injury in D. melanogaster. We hypothesize that neuronal injury will trigger an increase in mitoTCT frequency between neurons and glia as a rescue mechanism. Here, we present a detailed methodology of the two imaging techniques used, as well as our preliminary findings. Understanding the triggers and regulation of mitoTCT will provide insight into intercellular communication, especially in cases of neurodegeneration, where mitochondrial dysfunction is considered a major contributor.

THE EVOLUTION OF METAL USAGE IN ANCIENT PROTEIN DOMAINS

***NANDINI MANEPALLI*, SAWSAN WEHBI, JOANNA MASEL**

Almost half of all proteins bind metals, and are referred to as metalloproteins. For example, binding transition metals is often a key component in catalyzing biochemical pathways across all three domains of life. Many metalloproteins are ancient, dating back to the last universal common ancestor's (LUCA) and the even older pre-LUCA communities. Here, we examine which metals and metal clusters were used more often by ancient proteins compared to proteins that emerged more recently. We specifically analyze the metal usage across metal-binding protein domains—the basic evolutionary units of proteins, which usually fold independently. We annotate 72, 488, 1004, and 483 metal-binding protein domains previously classified as being present in pre-LUCA, LUCA, ancient but post-LUCA, and modern (present in prokaryotic supergroups) respectively. We use the BioLIP Database to identify which metal ions and/or clusters bind to protein domains. We therefore infer which metal types were essential in early life metabolism versus which metals gained popularity more recently, subsequently deducing the different geochemical environments of ancient microbial communities. This study has implications for understanding the environmental conditions and metal availability conducive to the origins of life on Earth and even elsewhere in the universe.

INVESTIGATING DEMOGRAPHIC INFERENCE UNDER A REALISTICALLY HIGH DELETERIOUS MUTATION RATE WITH REGARDS TO A BRIEF GENETIC BOTTLENECK

***MICAILA MARCELLE*, JOSEPH MATHESON, ULISES HERNÁNDEZ, JOANNA MASEL**

Linked background selection (BGS) is well known to reduce genetic diversity in a population via selection against deleterious alleles at linked loci. Relative to this phenomenon, unlinked BGS is widely considered to be negligible. It is thus frequently omitted from population genetics models, especially considering that the inclusion of unlinked BGS means tracking the whole genome rather than more efficiently examining only a smaller genomic window. Recent research by Matheson and Masel (2024) has shown, however, that under a realistically high deleterious mutation rate, unlinked BGS is not only significant, but it has a greater impact on neutral diversity than linked BGS. Hence, failing to include unlinked BGS may limit the accuracy of genetic inference. This is particularly true when it comes to inferring the demographic history of a population in the wake of a

brief genetic bottleneck using its site frequency spectrum (SFS), which is a statistical summary of the distribution of allele frequencies across the genome. Bottlenecks tend to increase the prevalence of recessive deleterious homozygotes, causing prolonged, intensified BGS. As a result, the SFS may mimic that associated with an extended population size reduction, even in the case of rapid regrowth, thanks to an enrichment for common variants rather than rare ones. My research aims to utilize the Masel lab's whole-genome forwards time simulation to investigate the demographic inference of a brief bottleneck under a realistically high deleterious mutation rate, considering how the inclusion of unlinked BGS may impact the SFS.

OPTOGENETIC ACTIVATION OF BEHAVIORAL SEQUENCE ACROSS TIME

***ELLA MARSHALL*, GOWRI SOMASEKHAR, SEAN CADIGAN, MELVILLE WOHLGEMUTH**

When performing naturalistic orienting behaviors, animals utilize a multimodal plan to reorient their bodies toward the stimulus of interest. In humans, this typically involves eye movements, head movements, and even body movements. While multifaceted orienting behaviors are ubiquitous, most laboratory research focuses on single behavioral events (e.g., eye movements) rather than how the brain sequences behaviors for naturalistic orienting. To characterize these sequences, we study the natural orienting behaviors of the echolocating bat. The echolocating bat performs a series of behaviors for their sonar imaging system, including ear movements, head movements, and sonar vocalizations. Our goal was to explore the brain's orienting motor space for initiating sequenced behaviors by focusing on the superior colliculus, a midbrain structure essential for generating species-specific spatial attention behaviors. To drive naturalistic behaviors, we developed a wireless method to activate brain activity in behaving animals. This system involves driving the expression of optogenetic proteins in the brain using the AAV system, then wirelessly shining light on the transfected neurons using a miniature device developed with the Gutruf Laboratory in Biomedical Engineering to control their actvity. We targeted different regions of the superior colliculus to initiate different orienting plans and used various light stimulation protocols to activate neurons in different rhythms. Exploring activation sites and patterns within the superior colliculus network, we have initiated diverse spatial orienting sequences. We find that hemispheric implantation sites lead to motor movement on the opposite side, in addition to symmetrical movements like ear vergence. We also notice behavioral sequences such as ear movements followed by asymmetrical head movements and vocal output. Future work will continue to target different regions of the superior colliculus, and employ these methodologies in flying bats hunting prey.

USING NANODISCS TO STUDY LIPID EXCHANGE

***TYLER MARTINEZ*, ANNIKA SILVERBERG, MICHAEL MARTY**

Lipids are known to modulate membrane protein structures and functions. To study lipid binding affinity to different membrane proteins, nanodiscs, a membrane mimetic, can undergo lipid exchange (LX). Nanodiscs used to study lipid exchange are assembled with lipids, membrane scaffold protein (MSP), membrane proteins, and detergent. After growing MSP from BL21 E. coli, immobilized metal affinity chromatography (IMAC) is used to purify Histidine (His) tagged MSP. The additional step of adding Tobacco Etch Virus protease cleaves the His-tags, and the His-cleaved MSP is purified again through IMAC. This allows the presence or absence of His tags from nanodiscs, enabling post-LX purification through IMAC. To separate different nanodisc populations pre-LX size exclusion chromatography is used. We established an LX control by observing the LX between nanodiscs without membrane proteins. We have successfully exchanged 100% phosphatidylcholine (POPC) nanodiscs with 90% POPC and 10% cholesterol nanodiscs to see lipids reach an equilibrium of 95% POPC and 5% cholesterol in both populations of nanodiscs. Once an exchange is performed with a membrane protein nanodisc, this equilibrium of lipids will shift, indicating a binding affinity for certain lipids to that protein. LX is analyzed using liquid chromatography coupled with mass spectrometry (LC-MS). Here, we are interested in how cholesterol impacts the structure and function of serotonin receptors. Recent studies show that patients who are taking cholesterol lowering medications experience increases in negative mental health effects, but it is unclear what this interaction looks like in natural systems. Future directions include using natural brain polar lipid extract within

this process, and inserting the M2 influenza membrane protein, which has known interactions with cholesterol, into a nanodisc to observe its exchange behavior. Furthermore, we will observe the kinetics of cholesterol exchange between empty nanodiscs to gain insight into the rate at which cholesterol exchanges between nanodiscs.

INVESTIGATING THE EFFECTS OF GLYPHOSATE EXPOSURE ON FOLLICLE COUNTS IN THE OVARY

TAYLOR MASSEY

INVESTIGATING STATE POLICIES THAT IMPACT CANCER CARE ACCESS FOR INDIVIDUALS WITH INTELLECTUAL AND DEVELOPMENTAL DISABILITIES

ESHA MATHUR

LYSINE DEACETYLASE-CONTAINING COMPLEXES AID IN FACILITATING TRANSCRIPTION OF GLUCOCORTICOID RECEPTOR-TARGETED GENES

***GWENDOLYN MCKAY*, MICHAEL AROWOSEGBE, SARAH OLSON, MEGAN CARVER, CATHARINE SMITH, CATHARINE SMITH**

It has been widely accepted that lysine deacetylases (KDACs) facilitate the repression of gene transcription through their post-translational modification of histone proteins. However, recent research has revealed that KDAC1, a Class I lysine deacetylase, is able to activate glucocorticoid receptor (GR) target gene transcription. Many factors are unknown about the function of KDAC1 as an activator of GR transcription, therefore we are currently focusing on determining how the KDAC1-containing protein complexes are important for GR transcription, and how they can be located within the genome. There are various kinds of protein complexes which contain KDAC1, but the Corepressor of Repressor Element-1 Silencing Transcription factor (RCOR/CoREST) complex has been shown to be the most significant promoter for transcription of GR-regulated genes. In the RCOR/CoREST complex, there exists a scaffold protein of which one paralog–RCOR3–has been observed to play a crucial role in KDAC1 function. Through a process of depleting RCOR3 protein from the RCOR/CoREST complex, we have observed impairments in basal and induced transcription of four GR targeted genes, indicating the scaffold's necessity to KDAC1 function. Moving forward with the knowledge of RCOR3's importance, we are developing a plasmid with a biotin-ligase insert in order to observe–through proximity profiling–other proteins that may interact with the complex. By identifying which transcription-necessary proteins are biotinylated, we can then identify those which are acetylated, and examine their function as possible substrates for KDACs. Researching Class I KDACs and their substrates can provide a deeper understanding of their inhibitors, which can aid in our comprehension of how these drugs affect endocrine pathways.

NON-CROSSLINKED STIFFNESS GRADIENT GRANULAR MICROGELS FOR 3D CANCER MODELING

KAITLYN MCKNIGHT

UNDERSTANDING THE EFFECTS OF OLFACTION ON MEMORY: INSIGHTS FROM NOVEL ODOR RECOGNITION IN FERRETS

***JESUS MENDOZA*, LAUREL DIECKHAUS, ALVARO CRUZ, LEILI FALBINAN, ELIZABETH HUTCHINSON**

Ferrets have an excellent sense of smell which is used for hunting, scavenging, and communicating in the wild. Many neurodegenerative diseases like Alzheimer's Disease (AD) often have symptoms such as loss of smell which develop early on, making olfactory dysfunction a potential marker for these conditions. Previous experiments from Schwerin (2022) using the novel object recognition (NOR) task in ferrets indicated high variability across animals which we have observed as well in our female ferret cohort. This may be attributed to the fact that NOR may be less relevant for the ferret's natural behavior repertoire. To examine this hypothesis, we focused on a behavior that ferrets specialize in, novel odor recognition. We developed a novel odor recognition (NOdorR) paradigm to assess whether ferrets of different ages (age ranges=1-4years) could identify new smells following a variable delay (30-60 minutes). To quantify NodorR task performance, we used the machine learning pose estimation software DeepLabCut (Mathis, 2018) to label the nose of each ferret and quantified how many frames the nose was in proximity of odor 1 and odor 2. Our hypothesis is that younger ferrets (1-2 years of age) will be able to better discriminate between a new and familiar odor. Additionally, we anticipate that there will be less variability in time spent with novel odors compared to habituated odors over multiple sessions. To correlate brain structure with behavior we also investigated the relationship between NoDoR performance and cortical thickness in the piriform and entorhinal cortex, two regions involved in olfactory processing.

CHARACTERIZATION OF COMMENSAL NEISSERIA POLYSACCHARIDE CAPSULE

***AKSHAY MENGHANI*, EVY NGUYEN, KATHERINE RHODES**

The genus Neisseria consists of a diverse set of gram-negative bacteria which exhibit both pathogenic and commensal behavior in their hosts. Human commensal Neisseria are common members of their host mucosal microbiota, colonizing the oral cavity and nasopharyngeal regions. These species have high genetic similarity with the pathogens N. gonorrhoeae and N. meningitidis, and encode several shared host-interaction factors. By studying these host-interaction factors within commensal Neisseria, we aim to characterize their adaptation to host environments and better understand the relationship between the microbiota and the host. This study focuses on the polysaccharide capsule—a layer of complex sugar molecules surrounding the bacterium's outer membrane involved in host adhesion and persistence. We utilized molecular cloning and gene expression techniques to examine the capsule's role in survival for N. subflava, a commensal of the human upper respiratory tract, and N. musculi, a commensal of the mouse oral cavity and gut. We deleted biosynthesis genes in the capsule locus of N. musculi and N. subflava and tested the mutants for capsule production using SDS-PAGE and alcian blue staining. Future directions include measuring the expression of these genes under various environmental conditions, such as nutrient and temperature stress, to determine how they are regulated. Survival phenotypes for each mutant will be tested by phagocytosis, complement evasion, and desiccation tolerance assays to evaluate the capsule's protective role in different environments. Finally, we will use N. musculi as a surrogate for human commensal Neisseria species, to examine polysaccharide capsule function in vivo. Our findings will contribute to the understanding of basic mechanisms of Neisseria adaptation and host interaction.

TESTING THE MITOCHONDRIAL EFFECTS AND THERAPEUTIC EFFICACY IN AGING PARKINSONIAN MICE

JASMINE MEREDITH

THE EFFECTS OF A PHTHALATE MIXTURE EXPOSURE ON FATTY ACID SYNTHESIS AND METABOLISM

VIVIANNA METZLER

INVESTIGATING THE ROLE OF HSC70 ON TDP-43 SOLUBILITY

AMELIA MITCHELL**, LUCAS MARMORALE, ROSS BUCHAN**

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by the death of motor neurons leading to muscle weakness, respiratory failure, and death. In over 95% of ALS cases, the nuclear RNA binding protein TDP-43 is mislocalized to the cytoplasm and forms aggregates, which may be responsible for neuronal cell death. Reducing cytoplasmic TDP-43 has been found to improve cell viability, thus a main focus of our lab is understanding cytoplasmic TDP-43 clearance. Our lab discovered a macroautophagy-independent endolysosomal clearance pathway wherein TDP-43 is ubiquitinated and trafficked to the multivesicular body (MVB). The pathway discovered by our lab shares some key qualities with a previously characterized pathway called endosomal microautophagy (eMI). eMI requires the chaperone protein Hsc70 for trafficking TDP-43 to the MVB membrane. By assessing the role of Hsc70 in our pathway we can begin to understand whether TDP-43 is a substrate for eMI. If TDP-43 is not cleared effectively, it becomes more insoluble and tends to aggregate. Therefore, we used solubility assays to determine if TDP-43 is being cleared effectively. We treated HEK293 WT and TDP-43 overexpression cells with a variety of drugs and found that in WT cells, MG132 (proteasome inhibitor) caused TDP-43 to shift to a more insoluble state, and in TDP-43 overexpression cells, DBeQ (VCP inhibitor) caused TDP-43 to shift to a more insoluble state, whereas Dyngo (dynamin inhibitor), and YM201636 (PIKfyve inhibitor) caused TDP-43 to shift to a more soluble state. Interestingly, we found that overexpression of Hsc70 seems to shift the TDP-43 into a more insoluble state, however, this requires further investigation. Future work will focus on achieving efficient genetic inhibition of HSC70, potentially using CRISPRi, and using immunoprecipitation experiments to observe the interaction between TDP-43 and Hsc70 in response to various knockdowns of proteins within our pathway.

BRIDGING SOIL AND GUT: IRON SPECIATION CHANGES IN WARMING SOIL AND THEIR RELEVANCE TO HUMAN MICROBIOME RESEARCH

CASSANDRA MONTANO ARELLANO

EVALUATING THE IMPACT OF AGE AND SEX HORMONES ON THE PROLIFERATION OF ADULT NEURAL STEM PROGENITOR CELLS

***TYLER MONTGOMERY*, MADDY SKODA, SREE VANI PILLUTLA, MANDI CORENBLUM, LALITHA MADHAVAN**

Understanding the interplay between neurogenesis and aging is fundamental to deciphering neurodegenerative disease progression and identifying novel therapeutic targets. Our prior studies have highlighted a critical period during aging in male rodents—between 13 and 15 months—where neurogenesis declines significantly. Since corresponding research in female rodents remains limited, our current work investigates the effects of aging and female sex hormones, 17β-Estradiol (E2) and progesterone (P4), on adult neurogenesis. Female F344 rats of four age-groups – 2, 6, 9 and 14 months – were either ovariectomized (OVX) or kept intact (Sham), to simulate acute hormone loss. The animals were treated with the thymidine analog bromodeoxyuridine (BrdU), a marker of dividing neural stem progenitor cells (NSPCs) and processed for histological analysis. Brain sections were immunohistochemically probed with a BrdU targeted antibody. Unbiased stereological estimation of BrdU labeled cells was conducted in biologically relevant niches of the brain: the subventricular zone (SVZ) and dentate gyrus (DG) of the hippocampus. Results from the SVZ reveal a general decline in NSPC proliferation with age, with a significant decline noted at 9 months in the OVX group. Analysis of the DG is currently ongoing. In terms of estrogen receptor expression, western blot data showed that ERα and ERβ expression increased in the 9-month OVX animals compared to shams. In the DG, no significant differences were noted at the 9-month aging stage, although a significantly decreased ERα expression was noted in 6-month OVX animals compared to controls. These findings align with prior cognitive behavioral assay data, which also show significant functional declines in neurogenesis-related behaviors at 9 months of age, emphasizing a connection between neurogenesis, cognitive function, and female sex hormones at this age. These results suggest a critical vulnerability to E2/P4 loss at 9 months of age in the female brain, marked by notable changes in neurogenesis.

UNDERSTANDING ANTAGONISTIC INTERACTIONS IN COMBINED CELLULAR STRESS RESPONSES

***MICHAEL MROZ*, BRADFORD HULL, GEORGE SUTPHIN**

The accumulation of cellular stress is intricately linked to the onset and progression of age-related diseases. Persistent challenges, such as oxidative damage, protein misfolding, and DNA instability, compromise cellular integrity over time. While stress response pathways initially work to mitigate harm, aging exacerbates the cumulative burden of stress, leading to diminished pathway effectiveness and greater cellular vulnerability. Exploring how combined stressors influence these dynamics not only builds on prior studies of individual stressors but also reveals new complexities that are essential for understanding lifespan and resilience.

Past work has shown that individual stressors like copper sulfate, sodium chloride (NaCl), golgicide A, and dithiothreitol (DTT) affect cellular function and lifespan. Building on this foundation, we determined dose-response effects for these stressors and explored how their combinations influenced lifespan. Notably, copper sulfate paired with NaCl, golgicide A, or DTT showed

antagonistic non-additive interactions, creating lower mortality compared to what would be expected from additive effects. These findings suggest the chemicals may interact to counterbalance each other's harmful effects, highlighting complex cross-talk between stress response pathways.

To investigate these interactions further, we conducted preliminary RNA sequencing (RNA-seq) analyses to screen gene expression changes under the combined stress condition of copper sulfate and NaCl. These analyses are being utilized to identify genes of interest and stress response pathways involved in this potential combined stress interaction.

By providing novel insights into the effects of combined stressors, this work enhances understanding of cellular stress responses and offers potential avenues for therapeutic interventions targeting age-related diseases. Exploring multi-stressor environments is essential for developing comprehensive approaches to mitigating cellular decline and identifying therapeutic targets.

This work is supported by NIH R35GM133588 and T32GM136536-04.

INTEROCEPTIVE MANIPULATION AFFECTS NEURAL RESPONSE TO SOCIAL TOUCH

***GABRIEL NEAL*, RYAN LE, ALEXIS MORRISON, MICHAEL CARDENAS, KATALIN GOTHARD**

Theories suggest that interoceptive signals interact with the processing of exteroceptive information. Exteroceptive sensory neurons transmit information to the brain about external stimuli, such as touch. Interoception, the process of sensing the body's physiological state, occurs within a closed-loop circuit, where autonomic efferents from the brain adjust the activity of internal organs in response to behavioral demands and interoceptive sensory neurons relay information back to the brain about the physiological state of the body. In non-human primates, social and affective touch, like grooming, is a form of emotional signaling associated with specific emotional and physiological states. To evaluate the effects of social touch on the body's physiological state a rhesus macaque was either groomed by a trusted human or received gentle airflow stimulus delivered to the same areas of the skin. Compared to airflow, grooming decreased heart rate by an average of 10 beats per minute (p<0.01) and changed the baseline firing rates of ~25% of neurons in the amygdala (p<0.001), an area of social and emotional processing in the brain. Next, we examined whether experimentally induced physiological states (indexed by increasing heart rate) can disrupt the correlation between the heart rate and baseline firing rates in the amygdala. A sympathetic-dominated physiological states was induced by the administration of glycopyrrolate which does not cross the blood-brain barrier. Glycopyrrolate led to a significant increase in heart rate relative to saline injections of ~20 beats per minute (Wilcoxon rank-sum test p<0.01). We found that, in parallel with an increase in heart rate, baseline firing rates of ~50% of neurons in the amygdala and somatosensory cortex were changed (p<0.05). These changes were likely induced by interoception. We are in the process of evaluating the role of glycopyrrolate-induced heart rate on the neural processing of social (grooming) and non-social (airflow) exteroceptive stimuli.

SELECTIVE HEAT SHOCK PROTEIN 90-β INHIBITION AS AN ALZHEIMER'S DISEASE TREATMENT STRATEGY USING AN AMYLOID-β INTRACEREBROVENTRICULAR INJECTION MOUSE MODEL

***CLAIRE NIELSON*, BRITTANY GRATREAK, JOHN STREICHER**

Alzheimer's Disease (AD) impacts over 6 million people in the United States and has limited treatment options. Early intervention strategies are critical for preserving cognitive function and improving quality of life. Previous research has established non-selective heat shock protein 90 (Hsp90) inhibitors as a viable therapeutic for AD via anti-inflammatory immune modulation of microglia. While pan-Hsp90 inhibitors have robust effects in treating AD pathology by reducing memory loss and neuroinflammation, their use is limited by toxicity due to inhibition of the Hsp90-alpha isoform. By targeting the Hsp90-β isoform, we aim to achieve similar therapeutic benefits without toxic side effects. Additionally, we address key challenges in AD research by supplementing long-term experiments and transgenic models with a shorter-term intracerebroventricular (ICV) model. ICV injections of amyloid-beta (Aβ) in wildtype mice induces AD-like cognitive impairments in a shorter time frame. Previous research has established the overexpression and accumulation of Aβ in mouse models as a pathological hallmark of AD. We hypothesize that the Aβ-injected mouse model will exhibit behavioral symptoms consistent with AD-driven memory loss, and that selective inhibition of Hsp90-β will mitigate AD pathology in C57BL/6 mice by reducing inflammatory microglial activation.

Cognitive assessments included novel object recognition (NOR), Morris Water Maze (MWM), and nest-building assays to evaluate the therapeutic potential of this approach. We performed Aβ peptide (1-40) or vehicle ICV injections, followed by daily injections of the Hsp90-β selective inhibitor NDNB-01 (1 mg/kg, SC) or vehicle. Aβ/NDNB-01-treated mice performed better in nest building than Aβ/vehicle-treated mice, and subtle memory enhancement trends were seen in the number of investigations of novel objects in Aβ/NDNB-01 mice compared to Aβ/vehicle mice in NOR. These studies lack the statistical power to analyze sex differences and nuanced memory changes, but ongoing studies with additional cohorts are pending.

PREVALENCE OF FUNGAL DISEASE IN THE UNITED STATES BASED ON THE ALL OF US RESEACH PROGRAM

EMERAL NORZAGARAY

PIKFYVE: A NOVEL TARGET FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA

SOFIA ORRANTIA

OVARIAN FOLLICLE SURVIVAL MEASURED THROUGH COMMERCIALLY AVAILABLE CELL-BASED LUMINESCENCE ASSAYS

TALIA OWEN

ENDOCRINE DEVELOPMENT AND CORTICAL THINNING AS DEVELOPMENTAL MARKERS IN MACAQUE ADOLESCENCE

***AVA PAL*, ARCHER BOWRIE, LAUREL DIECKHAUS, ALEXIS MORRISON, BETH HUTCHINSON, KATALIN GOTHARD**

During adolescence, the thickness of the cerebral cortex is reduced via synaptic pruning. However, cortical thinning is unequal across brain areas. In primates, the maturation of the prefrontal cortex coincides with the onset of adult-like behaviors, yet the timing relative to the onset and completion of adolescence remains unknown. Macaque adolescence is typically defined as 2.5-5 years of age. We collected yearly MRI scans to longitudinally monitor changes in regional cortical thickness for six adolescent male rhesus macaques, resulting in 14 scans spanning 2.5-5.5 years of age. We analyzed the MRIs using two different MRI processing pipelines, ANTs and CIVET-Macaque, and found that the prefrontal cortex thins between the ages of 40 and 50 months, plateauing afterward. We then correlated the changes in cortical thinning with endocrine measures of adolescent maturation and found that cortical thickness is anticorrelated with estradiol levels. Estradiol is a more reliable predictor for cortical thinning in the prefrontal cortex than age (estradiol: Spearman $p < 0.05$, rho = -0.7416; age: Spearman $p > 0.05$, rho = -0.6242). This may indicate that endocrine development plays a larger role in the time course of brain development during adolescence than age alone.

CHARACTERIZING MICROBIOTA USING PAPER MICROFLUIDIC CHIPS AND MACHINE LEARNING

***ARYAN PANDEY*, JOCELYN REYNOLDS, JEONG-YEOL YOON**

Currently, most microbiota profiling techniques are expensive and time-consuming (like PCR, RNAseq). This project seeks to develop a cheap, simple, and rapid alternative to traditional microbiota profiling techniques. The project's goal is to analyze the interactions between non-specific biomolecules and microbiota on paper microfluidic chips. In order to accomplish this, paper microfluidic chips are wetted with samples containing the biomolecules, carboxylated particles, and microbiota. Each channel of a paper microfluidic chip is pre-loaded with a small protein, peptide, or amino acid. Then, a smartphone records the flow velocity of the wetting front as it passes up the chip. Thus, the flow velocities which are collected from these multi-channels represent the molecular interactions between these biomolecules and bacterial species. A learning database is collected to build a machine learning model to determine the dominant and pathogenic species of microbiota. This methodology could be applied to profile the skin microbiome, detect pathogenic species within water samples, etc.

TESTING EFFECTIVE PLOIDY IN RNA-DIRECTED DNA-METHYLATION IN THE GENUS *CAPSELLA*

KATHRYN PANFEROV

EXPLORING DISPARTITIES IN PEDESTRIAN FATALITIES: UNDERSTANDING DEMOGRAPHIC FACTORS THROUGH A PUBLIC HEALTH LENS

***JULIANA PANHORST*, ALYSSA RYAN**

Motor vehicle traffic crashes remain the second leading cause of death among unintentional injury causes, while concurrently, rates of pedestrian travel continue to increase. With this rise, it is more critical than ever to explore why this public health crisis occurs, and understand methods of mitigation. Thus, this analysis provides a comprehensive view of pedestrian fatality statistics across demographics, insights into the risk factors for examined populations, and recommendations for future traffic safety implementations from a CDC dataset. We analyzed how sex, age group, ethnicity, race, and urbanization level, sourced from the CDC's National Vital Statistics System (NVSS), impacted pedestrian fatality rates from 2011 to 2019. Specific population-level analyses were conducted, allowing for comparison among subgroups. Significant findings highlighted the highest annualized death rates present among American Indian or Alaska Native populations at 0.62 per 100,000 population and among the 85+ age group at 0.67 per 100,000 population. Outcomes in subgroups such as gender exhibited large disparities, where males have an annualized death rate 2.5 times that of females, which points towards a variety of unique risk factors associated with pedestrian fatality prevalence. Examining these results, a further review of past literature was performed, gleaming information regarding potential explanations for these outlined correlations. For instance, higher annualized pedestrian fatality rates among American Indian or Alaska Native populations may be attributed to a variety of factors, including limited infrastructure on reservations, or rates of alcohol-related motor vehicle collisions. Understanding these statistics within the context of these associations allows for the formulation of fatality prevention strategies. This study highlights the importance of interpreting fatality statistics in the context of their impacts on the distinct and diverse populations affected.

UNCOVERING HPV HELPERS: USING A SPLIT TURBOID SYSTEM FOR PROXIMITY BIOTINYLATION OF PMLNBS DURING HPV L2 INFECTION

***AVRIL PEREZ*, ZACHARY WILLIAMSON, ASHLIN SCHAEFBAUER, SAMUEL CAMPOS**

Human papillomavirus (HPV) is an oncogenic dsDNA virus that typically infects the anogenital region, causing STIs. HPV is made up of two capsid proteins, L1 and L2, which interact with host cell proteins during host entry. L2, the minor capsid protein, aids in entering host keratinocytes and reverse trafficking into the nucleus, where the virus is then dependent on mitosis to replicate its viral genome. Recent studies show that the virus interacts with proteins in promyelocytic leukemia nuclear bodies (PMLNBs) in the nucleus in order to facilitate replication and transcription of the viral genome. In order to find how the minor capsid protein L2 interacts with PMLNB proteins, we engineered the promiscuous biotin ligase UltraID as a C-terminal fusion to the L2 capsid protein. UltraID converts biotin into biotin-AMP, a highly reactive intermediate that tags nearby proteins through biotinylation. Unfortunately this approach failed due to excess self-biotinylation that deactivated the system during production of viruses. Here we are attempting to engineer a split model (two fragments of TurboID, N and C) which is a more controlled system that we will use to determine which proteins are in close proximity with L2. We hope to use the split TurboID stystem to gain a better understanding of the mechanisms and interactions of L2 during early stages of infection.

SYNTHESIS AND CHARACTERIZATION OF MOXIFLOXACIN IONIC LIQUIDS TO IMPROVE OCULAR DRUG DELIVERY

***TOM PHAM*, JOSEPH ADAMS, ABHIJIT DATE**

Moxifloxacin, a fourth-generation 8-methoxy fluoroquinolone, is effective against a broad spectrum of gram-positive and gram-negative bacteria commonly implicated in ocular infections. Approved by the FDA in 1999, moxifloxacin hydrochloride is marketed as Vigamox, a 0.5% ophthalmic solution for treating bacterial conjunctivitis. It inhibits bacterial replication by targeting DNA gyrase and topoisomerase IV, enzymes critical for DNA synthesis. Despite its potency and broad-spectrum antimicrobial activity, moxifloxacin's high aqueous solubility poses challenges for achieving optimal bioavailability when applied topically to the eye. This is due to high tear turnover and the lipophilic nature of the corneal epithelium, which limits drug retention and penetration. Current guidelines recommend administering moxifloxacin hydrochloride eye drops three times daily for seven days, but this regimen risks low patient compliance and the development of antibacterial resistance.

To address these challenges, ionic liquids (ILs) present a promising solution. Ionic liquids are salts that remain liquid below 100°C due to their disrupted crystalline lattice structure, resulting in lower melting points. My research demonstrates that combining moxifloxacin hydrochloride with fatty acid counterions converts the crystalline drug into an ionic liquid, significantly enhancing its physicochemical properties. This transformation increases the drug's lipophilicity, thereby improving corneal permeability—an obstacle for many aqueous ophthalmic formulations. The use of an antibacterial ionic liquid formulation offers the potential for greater bioavailability, superior therapeutic efficacy, and improved patient compliance in clinical practice. By optimizing the physicochemical profile of moxifloxacin, this approach could set a new standard for treating bacterial eye infections.

DOES BACE1 INHIBITION IMPAIR RECOVERY FROM STROKE IN MOUSE MODELS OF ALZHEIMER'S DISEASE?

***ESTHER QIU*, BOAZ MAIYO, SANNA LOPPI, JENNIFER FRYE, KRISTIAN DOYLE**

BACE1 is an enzyme responsible for cleaving amyloid precursor protein (APP), a process that leads to the formation of amyloid-beta (Aβ), a hallmark of Alzheimer's disease (AD) pathology. BACE1 inhibitors were initially proposed as a therapeutic strategy to slow the progression of AD by reducing Aβ accumulation. However, clinical trials have largely failed, potentially due to the role BACE1 plays in the neuregulin 1 (NRG1) pathway crucial for myelination. We hypothesized that inhibiting BACE1 in post-stroke AD mice might produce mixed effects, reducing Aβ aggregates while impairing re-myelination, an essential component of recovery. To test this hypothesis we performed a model of middle cerebral artery occlusion (MCAO) stroke in 5xFAD transgenic AD mice, followed by treatment with the BACE1 inhibitor MK-8931 for seven weeks post-stroke. We then assessed cognitive and motor function, amyloid accumulation, and myelination. Our results showed that MK-8931 impaired motor function, but had no significant effect on cognitive function, Aβ accumulation, or myelination as assessed by CNPase levels, though some trends supported our hypothesis. We conclude that while BACE1 inhibitors may hinder motor recovery after stroke, their impact on repair mechanisms requires further investigation.

A ROLE FOR PROTEASE ACTIVATED RECEPTOR 2 IN ASTHMA

***ZAINAB RAMADAN*, TRUC LE, AYLEEN MENDOZA, SCOTT BOITANO**

Protease-activated receptor-2 (PAR2) is one of a group of four G-protein coupled receptors that are activated by proteases. Protease cleavage of the NH2 terminus results in a tethered peptide sequence that acts as the natural ligand. Activation of PAR2 results in multiple signaling pathways, most prominently a G-protein/Ca2+ signaling pathway and a β-arrestin/mitogen activated protein kinase (MAPK) signaling pathway. Activation of PAR2 has been associated with a variety of inflammatory

diseases, including asthma. In asthma, PAR2-dependent β-arrestin/MAPK signaling promotes inflammation and mucus production, while the G-protein/Ca2+ signaling pathway results in bronchorelaxation. Over the summer, we evaluated C957, a β-arrestin/MAPK signaling PAR2 antagonist, in a unique mouse model that expresses human PAR2 for its ability to limit allergen-induced asthma. We specifically monitored the ability for C957 to limit lung inflammation, mucus production, and airway hyperresponsiveness using FlexiVent ventilator experiments and cell slide scoring. We found that C957 was effective against allergen-induced asthma when administered oropharyngeally two hours prior to the allergen delivery. Since oropharyngeal delivery mimics inhaler use, we propose that C957 is a viable drug lead for the treatment of allergic asthma.

CHARACTERIZING THE DYNAMICS OF A NOVEL REGULATOR OF NUTRIENT SIGNALING AND TRANSPORT IN THE TORC1/SEAC INTERACTOME

***NATALIE RAWLINGS*, JEAHO LIM, ANDREW CAPALDI**

The Target of Rapamycin Complex 1 (TORC1) is a key regulator of eukaryotic cell growth and metabolism. Although this 2-billion-year-old, highly conserved protein complex is a central control hub for growth, proliferation, autophagy, and stress response, many mechanisms of its regulation remain unclear. Mutations in proteins involved in the TORC1 pathway are correlated with numerous human diseases, including cancer, diabetes, and epilepsy, and as such, building a comprehensive, quantifiable model of the nuanced regulation of this pathway will lay the foundation for developing novel TORC1-based therapeutics. Previous work in Saccharomyces cerevisiae has highlighted the role of the vacuolar GPCR-like protein Ait1 in TORC1 regulation via interactions with the small GTPases Gtr1/2. In characterizing the behavior of Ait1, the protein Vsb1 was identified as a potentially novel regulator of the TORC1/SEAC interactome of similar significance to Ait1. Building upon this foundation, this research focuses on Vsb1, a vacuolar arginine transporter with secondary activity in histidine and lysine transport, and its impact on TORC1 dynamics. Preliminary findings suggest a functional interaction between Vsb1 and Ait1 in amino acid signaling, with significant overlapping response patterns during histidine starvation. Using western blot analyses, we extend methodologies from earlier studies on Ait1, examining TORC1 activity in various mutant strains under numerous starvation conditions. This work aims to elucidate whether Vsb1 exerts its regulatory effects on TORC1 through either a direct or indirect interaction with Ait1 and other intermediate pathways, and to determine how Vsb1 itself is regulated, ultimately to create a more comprehensive model of TORC1 regulation.

BACTERIAL MORPHOTYPES DO NOT CONVEY RELATEDNESS

***TYLER REESE*, MAE BERLOW, KATRINA DLUGOSCH**

There is substantial bacterial diversity found within and on the surface of plant roots. These bacteria can be categorized based on the morphological characteristics of their colonies, such as color, shape, margin, and texture. DNA sequencing can also be used to phylogenetically classify bacteria based on the nearest matches. DNA sequencing involves amplifying specific regions of a bacterial genome and then comparing these regions to known bacteria in a database. The goal of this research was to find whether a relationship exists between bacterial morphotype and phylogeny. To test this, we investigated two relationships. First, we compared the morphotypes of different bacteria that belonged to the same genera. Then, we took a cluster of bacteria with the same morphotype and compared their sequence-based taxonomic relationships. We found a variety of morphotypes in the same bacterial genera and a diverse range of bacteria sharing a morphotype, indicating that morphotype and phylogeny are not closely linked. This study demonstrates the ability of closely related bacteria to rapidly evolve different morphotypes, which may be advantageous in novel environments. It also highlights the importance of relying on genetic or chemical identification methods rather than just visual inspection when identifying bacteria.

DI-N-BUTYL PHTHALATE CAUSES FOLLICULAR GROWTH INHIBITION BY TARGETING THE INTRAFOLLICULAR GRANULOSA AND THECA CELL COMPARTMENTS

VIVIANA ROMERO

OPTICAL COHERENCE TOMOGRAPHY AND ELASTOGRAPHY FOR TISSUE IMAGING AND STIFFNESS QUANTIFICATION IN BREAST TUMORS

***CAITLIN RUHLAND*, ALANA GONZALES, PHOTINI RICE, GHASSAN MOUNEIMNE, JENNIFER BARTON**

This project focuses on validating a benchtop method using optical coherence tomography (OCT) and optical coherence elastography (OCE) for ex-vivo tissue imaging and stiffness quantification. OCT is a non-destructive imaging technique that measures reflected near-infrared light to create high-resolution (<10 µm), cross-sectional images. This can be used to visualize tissue layers up to 2 mm in depth and detect early morphological changes characteristic of cancer. OCE uses the images generated by OCT to accurately determine tissue stiffness, a key biomarker for cancer. Tumor-induced changes in the extracellular matrix often increase tissue stiffness, and higher stiffness is correlated with a greater likelihood of metastasis. Imaging was conducted using a benchtop OCT system (Thorlabs Telesto TEL221). A phantom with constant mechanical properties was placed on the tissue, and an axial force was applied to compress both by a known distance, measured using a micrometer. The applied force was measured using a capacitive force sensor (SingleTact 4.5N). MATLAB was used to calculate the resulting strain of the phantom, providing relative measurements of tissue stiffness. Future work aims to quantify the mechanical properties of the phantoms, including their Young's modulus, to enable accurate calculation of the Young's modulus for the imaged tissue rather than relative strain measurements. Additionally, future efforts will involve implementing endoscopic OCT and OCE for early-stage in-vivo cancer detection.

CNP-MIR146A ACCELERATES DIABETIC WOUND HEALING BY PROMOTING A PRO-REGENERATIVE IMMUNE RESPONSE

***ALYSSA SAN AGUSTIN*, KATHARINA FISCHER, BAILEY LYTTLE, ANISHA APTE, EFUA BOLOUVI, AILA HAUGER, STACY SKOPP, JIMENA CANCHIS, KELLEN CHEN, GEOFFREY GURTNER, KENNETH LIECHTY**

Background: The wound healing impairment in diabetic patients is multifactorial, with chronic inflammation and an oxidant/antioxidant imbalance leading to increased oxidative stress as central features. We developed a novel strategy targeting both the dysregulated inflammatory response and elevated ROS in diabetic wounds. By selecting cerium nanoparticles (CNP) for their antioxidant properties and conjugating a miR-146a mimetic to CNP (CNP-miR146a), to also target proinflammatory signaling. However, the exact mechanisms by which oxidative stress and inflammatory signaling affect wound

healing at the cellular level remain unclear. We hypothesize that CNP-miR146a improves healing in diabetic wounds by modulating the various cell types within the wound microenvironment.

Methods: We employed single-cell RNA sequencing to interrogate cell-specific gene expression across more than 5,000 individual genes. The study included three murine treatment groups: non-diabetic wounds (D3 -DB, and D7 -DB), untreated diabetic wounds (D3 +DB -T, and D7 +DB -T), and diabetic wounds treated with CNP-miR146a post-wounding (D3 +DB +T, and D7 +DB +T). Subsets of these wounds were harvested on days 3 and 7 post-wounding. We used specific cell markers to identify the distribution of various cell populations in each group.

Results: On day 3, non-diabetic wounds exhibit a dominant presence of pro-inflammatory macrophages with pro inflammatory neutrophils present in lower quantities (Pro I Mps 60%; Pro I Neu 38%), and minimal populations of monocyte, dendritic cells (DCs), and other macrophage types (<1%). In contrast, untreated diabetic wounds show a reduction in pro-inflammatorymacrophages (Pro I Mps 26%) and an increase in pro-inflammatory neutrophils (Pro I Neu 72%). Diabetic wounds treated with CNP-miR146a demonstrate an increased presence of pro-inflammatory neutrophils (Pro I Neu 80%) compared, along with a reduction in pro-inflammatory macrophages (Pro I Mps 19%). By day 7, the non-diabetic wounds show a high population of pro-regenerative macrophages (44%), monocyte populations were at 40%. Untreated diabetic wounds display a upregulation in DCs (14%) and pro-inflammatory neutrophils (9%), along with a slight presence of pro-regenerative macrophages (23%), neuronal signaling cells (28%), and lower expression of monocytes compared to non-diabetic wounds (26%). Treatment with CNP-miR146a in diabetic wounds on day 7 maintains an elevated level of pro-regenerative macrophages (42%) similar to those of the non-diabetic wounds. The monocyte population within this group showed a similar distribution to the untreated diabetic wounds (28%).

Conclusion: Overall, our data highlights the adverse impact of diabetes on wound healing and suggests that targeting inflammation and oxidative stress with CNP-miR146a treatment can modulate and restore immune cell populations to enhance healing in diabetic wounds.

PRECISION REGULATION OF INTEGRIN MECHANOSENSING RECEPTORS IN CANCER INVASION

***ISHNOOR SANDHU*, JAIME GARD, ANNE CRESS**

Integrins are cell surface adhesion receptors that respond to tissue microenvironments to promote cell-cell and cell-extracellular matrix (ECM) interactions necessary for early development and adult tissue remodeling. In cancer, integrins help cells invade and migrate to distant sites by initiating adhesion to the ECM and directing the formation of reversible focal adhesion complexes. Integrins are type I membrane heterodimers with alpha and beta subunits; the alpha subunit dictates the ECM molecules used for adhesion, and the beta subunit stimulates focal adhesion formation using a cytoplasmic adapter protein called kindlin2 (K2). It is currently unknown how the alpha subunit influences K2 binding and focal adhesion formation. Our previous work revealed that a6b1 integrin and a tumor-specific form (a6pb1) are required for tumor invasion into muscle. A6pb1 integrin is formed by an ectodomain-specific post-translational modification (PTM). While both a6b1 and a6pb1 integrin forms bind K2, neither result in focal adhesions compared to the a5b1 integrin. This study aimed to test whether the PTM acts as a switch to release K2 from the a6b1:K2 complex and increases the ability of a5b1:K2 to form focal adhesions. We predict that the a6b1:K2 exists with K2 as an inactive multimer, explaining the constitutive lack of a6b1:K2 within focal adhesions. To test our hypothesis, we used cells with an inducible form of the a6pb1 integrin to control the PTM, paxillin to indicate focal adhesions, and K2 to localize it to either a6 or a5 integrins. Using immunofluorescence microscopy revealed the appearance, distribution, and composition of focal adhesions. Exploring how K2 interacts with the a6b1 and a5b1 integrins will allow us to

understand how prostate cancer cells use integrins to metastasize, which can be targeted to prevent systemic disease and improve cancer outcomes.

ANALYSIS OF ALTERNATIVE METHODS TO TRACTOGRAPHY

***VICTOR SANDRIN*, LAUREL DIECKHAUS, ELIZABETH HUTCHINSON**

Diffusion Tensor Imaging (DTI) and Diffusion Tractography offer the capability to image the structure and orientation of white matter fibers throughout the brain non-invasively, via the Brownian motion of water. We can use this diffusion data to represent "tracts" of white matter, or the projections of a group of neuron's axons with diffusion tractography. Current research indicates that DTI and the derived data from diffusion tractography might be able to provide early identification of neurodegenerative diseases such as Alzheimer's Disease (AD) in specific brain regions by observing these comparative abnormalities. Current models of diffusion tractography are limited, for example, certain tracts take tortuous and highly angled paths, sometimes feeding into and splitting from other tracts that connect to entirely different regions and crossing over each other constantly. There is an immediate need for improved models of tractography for proper discernment of white matter tracts and the complicated geometries they must assess. This project compares the industry standard software MRtrix3 (constrained spherical deconvolution of a fiber orientation diffusion function) with QUEST GO-ESP (biased random walk entropy spectrum pathways probability model) and their tract generation methods from data retrieved from Magnetic Resonance Imaging (MRI). MRI data of bonnet macaque brains (n=8) was analyzed by both software programs to create tractography pathways across the whole brain and in various pathways implicated in Alzheimer's disease such as the locus coeruleus to thalamus and corticospinal tract. Currently, it is apparent that MRtrix was able to successfully recapitulate all of the locus coeruleus tracts while GO-ESP was unable to find any connectivity in at least 3 of the specimens specifically between the entorhinal cortex, indicating the parameters for tract generation of GO-ESP must be further investigated. Testing these various parameters will help us better capture the potential of GO-ESP and allow direct comparison with MRTRIX.

OPTIMIZATION OF ANTISENSE OLIGONUCLEOTIDE POSITIONING FOR IMPROVED SPECIFICITY IN RAPID SARS-COV-2 DETECTION: BRIDGING THE GAP BETWEEN LABORATORY DIAGNOSTICS AND POINT-OF-CARE TESTING

ORLI SANYAL

OREXIN 2 RECEPTOR AS A TARGET IN CHRONIC PAIN MECHANISMS

JOSUE SARMIENTO

ANALYZING THE VOCABULARIES OF AUSTRALIAN-ENGLISH-SPEAKING TODDLERS

***ELISSA SCHIFF*, SARAH MASSO, ELISE BAKER, NATALIE MUNRO, MARY ALT**

Words can be classified by their phonological complexity, which takes into account words' speech sounds, syllable structures, and stress patterns. This measure can be used to assess the accuracy of young children's productions of words (e.g., how many sounds in the word "donkey" do they produce correctly?). Words' phonological complexities may determine when a child starts to say them. For example, the word "mom" is not phonologically complex, as its speech sounds are typically acquired early and it consists of one syllable. To understand the phonological complexity patterns of toddlers' early words, the vocabularies of 910 Australian-English-speaking toddlers were analyzed. Each child's vocabulary was evaluated out of a set of 290 words that they either produced or did not produce. The findings speak to the larger question of what drives children's early lexicons and may inform target word selection for speech and language treatments for children with communication disorders.

PROMOTING EMBRYONIC STEM CELL DERIVED CARDIOMYOCYTE MATURITY BY GENE EDITING SARCOMERIC PROTEINS

ERIN SCHUETTE

ELEVATING REFUGEE PERSPECTIVES ABOUT ACCESS TO DISABILITY SERVICES IN ARIZONA

***SALEHAH SHABAZZ*, JACY FARKAS, DIBA FALLAH, JULIE ARMIN**

Arizona is one of the top 10 states to resettle refugees in America with nearly a thousand refugees resettled each year. Refugees with disabilities face obstacles when it comes to receiving the adequate health care and services that they deserve. The project team received funding from the Arizona Developmental Disabilities Planning Council in October of 2022 to begin the 15-month community-based, qualitative research on ways in which Arizona disability services can improve a diverse group of refugees' access to disability services that they require and deserve. The project team worked with refugee resettlement organizations and disability systems to collect information from refugees that volunteered from the Tucson and Phoenix areas. Through these interviews, the team was able to gain insight into the disability service systems adequacy from the refugee's perspective. The team published a report, Elevating Refugee Perspectives about Access to Disability Services in Arizona (2024), which highlights the barriers that these refugees experience when navigating through the Arizona disability service systems and ways in which the disability services in Arizona can work to provide them with equitable care. The team created codes of the in-depth interviews of the refugees' experience and are working on creating subthemes to help provide evidence for each code. We will submit our findings as a manuscript to a peer review journal for interpretation and eventual dissemination to an international audience of researchers working to solve similar issues.

KNOCK, KNOCK - KNOCKING OUT EVERY OTHER BEETLE SEGMENT

***JEREMIAH SHANKAR*, CONNOR CARNEY, VIOLET ROWLAND, SUSAN HESTER, LISA NAGY**

Segmentation is essential to the development of vertebrates, annelids, and arthropods, and disruptions to it result in deformed or unviable phenotypes in many species. Insects—which also segment—are a cornerstone of global ecological systems and work for and against the productivity of the agricultural sector. Two modes of segmentation have been discovered—simultaneous segmentation in Drosophila melanogaster (the fruit fly) and sequential segmentation, which occurs in most segmented animals, including vertebrates and our model organism Tribolium castaneum (the red flour beetle). Tribolium segmentation relies on a molecular oscillator composed of three genes—even-skipped (eve), odd-skipped, and runt, driven by a posterior gradient of the transcription factor caudal. Knockdown of eve breaks the oscillator, resulting in an asegmental phenotype. eve is initially expressed in stripes with a double periodicity that split into primary and secondary stripes. How the eve locus produces this pattern is unknown but a computational model predicts it emerges as a consequence of falling levels of caudal. A 4.0 KB region downstream of the eve open reading frame coupled with an mCherry reporter expresses exclusively in the secondary eve stripes. The Tribolium 4.0 KB expression pattern is identical to that of a Drosophila eve enhancer. While the expression pattern of these enhancers is known, their function remains unexplored in both species. We hypothesize that the 4.0 region is necessary for stripe splitting. We expect that the loss of the 4.0 region, or some transcription factor binding sites within it, will result in the failure to form the secondary stripes and an embryo with half as many segments. To test this hypothesis, we perform CRISPR-Cas9 targeted knockdowns within the 4.0 region. We will visualize the resulting phenotypes through live imaging and immunohistochemistry and determine sequence-specific changes created through the CRISPR-Cas9 injections.

ALBUMIN CONJUGATION WITH IRON PROCHELATORS: PREDICTIONS AND SCREENING FOR CANCER THERAPEUTICS

JAKE SHAW

PURIFICATION OF RECOMBINANT SPYCATCHER-TAGGED CARDIAC MYOSIN BINDING PROTEIN-C (cMYBP-C) WITH HCM-ASSOCIATED MUTATION N755K

***KRITIKA SINGH*, ANGELA GREENMAN, RACHEL SADLER, SAMANTHA HARRIS**

Cardiac myosin binding protein-C (cMyBP-C) is a thick filament-bound sarcomeric protein that regulates interactions between thick and thin filaments in cardiac muscle. Mutations in MYBPC3, the gene encoding cMyBP-C, are linked to hypertrophic cardiomyopathy (HCM), a major risk factor for sudden cardiac death in young people and athletes. N755K is one such mutation associated with HCM, yet its impact on cMyBP-C function remains poorly understood. This study aimed to investigate the N755K mutation through recombinant protein purification using *E. coli* as the expression system. Site-directed mutagenesis introduced the N755K mutation into the MuC0C7Sc plasmid, which contained murine cDNA encoding cMyBP-C domains C0-C7 with a SpyCatcher tag for future in situ functional testing. Sanger sequencing verified the mutation, and bacterial transformation followed by nickel-affinity chromatography was used to grow and purify the mutant protein. Initial recombinant purification of MuC0C7Sc N755K suggested potential haploinsufficiency, indicating that the N755K mutation compromises cMyBP-C stability, reducing functional cMyBP-C levels below the threshold required for normal cardiac muscle contraction, potentially contributing to diseases like HCM. However, improved protocols, such as maintaining lower temperatures to prevent overgrowth, yielded purer samples without aggregation, highlighting the importance of experimental conditions in addressing protein stability. These findings suggest that while the N755K mutation may predispose cMyBP-C to haploinsufficiency, other mechanisms might also influence its function. To further investigate this, future studies will involve selectively removing endogenous cMyBP-C from cardiomyocytes or myofibrils engineered with a SpyTag peptide and replacing it with the mutant, SpyCatcher-tagged construct. This construct will bind to SpyTag on the thick filament, reconstituting a full-length N755K mutant

version of cMyBP-C. This approach will enable us to gather functional data on the impact of the N755K mutation on cMyBP-C function.

ANOXIC INCUBATION EXPERIMENTS: HOW FE3+ REDUCTIVE DISSOLUTION FRACTIONATES SOIL ORGANIC MATTER (SOM) ACROSS VARYING CLIMATES AND DEPTHS

JORDAN SINGLETON, **ALEXANDER EDERER**

DEVELOPMENT OF AN INDUCIBLE CONDITIONAL MARCKS KNOCKOUT MOUSE MODEL FOR RESEARCH ON LUNG VIRAL DISEASES

MELISA SMITH

GENERATING MHCI AND MHCII KNOCKOUT M12 CELL LINES USING CRISPR-CAS9

HARITI SONI

STRATEGY DIFFERENCES BETWEEN YOUNG AND OLD RATS PERFORMING A SPATIAL MEMORY TASK

MIA SPONSELLER**, SAHANA SRIVATHSA, CAROL BARNES**

In an increasingly aging population, over 20% of adults above the age of 60 suffer from neurodegenerative disorders. Thus, it is imperative that we understand what memory deficits are caused due to normative aging before we can understand the pathological memory deficits. Episodic memory, including spatial memory, is one of the most common cognitive impairments that occurs with aging. It has been shown that spatial memory and navigation are more impaired in older animals when compared to younger animals. The Morris Water Maze task is used to study these differences. In this task, animals utilize external contextual clues in the environment to generate a spatial map and navigate to a hidden platform. When young (9 months) and old (22 months) Fischer 344 rats are trained across four days on this task, the old rats tend to take longer paths to the target platform that are less optimal than young rats. However, this does not capture potential strategy differences that are being employed by the age groups. In order to study these differences, we utilize a package in R called Rtrack. This package allows us to compare rats from multiple cohorts in order to identify the strategies used by each rat. Nine different strategies, categorized as non-goal-oriented, procedural, or allocentric search, are determined based off of the path that the rat takes from the drop point to the platform or endpoint. Our analysis allows us to identify how these strategies are differently

employed by young and old rats in learning this task. These differences may suggest that spatial memory is impaired in aged rodents in specific ways.

USING TOXOPLASMA STRAIN-SPECIFIC DIFFERENCES IN ENCYSTMENT TO IDENTIFY GENES ESSENTIAL FOR THE BRADYZOITE STAGE

***ARUNA SREENIVASAN*, CHANDRASEKARAN SAMBAMURTHY, JOSHUA KOCHANOWSKY, ANITA KOSHY**

Toxoplasma gondii is an intracellular parasite that chronically infects up to one-third of the world. Toxoplasma's chronic infection is mediated by switching from a fast-growing tachyzoite to a slow-growing bradyzoite that encysts in multiple cell types, including neurons. While two master regulators of stage conversion are known, the functions of the ~2000 downstream bradyzoite-specific genes are not. To address this gap, we analyzed RNA-seq data from primary murine neurons infected with a type II Toxoplasma strain (fast, efficient encystment) or a type III Toxoplasma strain (slow, inefficient encystment). We focused on genes upregulated in type II versus type III parasites and cross referenced these genes with "bradyzoite" genes identified through a publicly available dataset. This analysis identified 173 genes that we hypothesize are core genes linked to stage conversion and/or bradyzoite persistence. To test this hypothesis, we selected four genes that we confirmed were upregulated in an independent study of primary murine neurons infected with type II vs type III parasites. Three of the four are hypothetical proteins while the fourth was previously identified as being involved in brain colonization, though limited mechanistic work has been done on it. To define the role of these genes, we are currently generating individual knockouts and complemented strains in a type II strain. Once the knockout and complemented strains are generated, we will use in vitro assays and in vivo experiments to determine how each of these genes influences stage conversion and chronic infection.

DETERMINING THE ROLE OF HRAS ALTERATIONS ON THE PI3K/MTORC2/AKT SIGNALING AXIS IN MCF10A BREAST EPITHELIAL CELLS

***DOUGLAS SWANGO*, MOLLIE WIEGAND, PASCALE CHAREST**

Ras is a small GTPase mutated in approximately 30% of cancers, leading to hyperactivation, increased proliferation, and migration of tumors. Migration is of particular concern due to increased metastatic potential, which increases the risk of death from cancer. Ras has been associated with PI3K, AKT, and mTORC2, proteins involved in signaling pathways related to migration. However, its exact role in the PI3K/mTORC2/AKT signaling axis requires further study. This project examined the role of Ras transformations and PI3K inhibition on PI3K and mTORC2-dependent AKT phosphorylation sites. This was done by genetically altering HRas in MCF10A breast epithelial cells via overexpression or constitutively active mutation and by pharmacological PI3K inhibition via wortmannin. We found that both Ras-overexpressing and constitutively active cells saw increases in PI3K and mTORC2-dependent AKT phosphorylation compared to control cells, with more statistically significant increases seen in Ras-overexpressing cells. PI3K inhibition decreased PI3K and mTORC2-dependent AKT phosphorylation in all cell lines, suggesting PI3K is not functioning independently from Ras-mediated mTORC2 activity in these cells.

IMPROVING SKIN CANCER AWARENESS AND EDUCATION AMONG AMERICAN INDIAN COMMUNITIES

***HALIE TEWA*, ROBIN HARRIS, DYLAN MILLER, JULIE ARMIN**

Skin cancer is the most common form of cancer in the United States (US). Melanoma, the most severe form of skin cancer, has been increasing over the years in Arizona. Excessive exposure to ultraviolet radiation (UVR) is the biggest risk factor for all skin cancers. UVR exposure leads to damage of DNA in skin cells, and is a cause of all skin cancers, including melanoma. Arizona's climate is becoming increasingly hot and the UV index for Phoenix ranged from "high" to "extreme" for 6 months in 2024. The key to lowering your risk for melanoma and other skin cancers is becoming aware of how to avoid excess UVR and to know the signs of skin cancer to catch it early so it can be treated. Although people with lighter skin are more at risk of getting skin cancer, it is a huge misconception that American Indians can't get skin cancer. However, there is a lack of skin cancer awareness and education in American Indian communities. Project SASS (Students are Sun Safe) is a program at the University of Arizona that provides sun safety messages to all communities and all ages. My project was to become trained in skin cancer prevention, and to use that information to create a Hopi-tailored powerpoint presentation to highlight the A.C.E. message (Avoid excess sun exposure, Cover-up as necessary, and Examine your skin regularly). These materials were reviewed by 20 persons in the Hopi Health Department. The next step is to provide several community presentations that include survey evaluations to assure the presentation is well-received. All materials will be made available to Hopi Cancer Support Services.

CHARACTERIZING THE ROLE OF CTR2 IN LATENT TOXOPLASMA GONDII INFECTION

JADEN TODD-NELSON, CHANDRASEKARAN SAMBAMURTHY, ANITA KOSHY

Toxoplasma gondii (*T. gondii*) is an intracellular parasite that persistently infects the central nervous system (CNS) of up to a third of the world's population. This CNS persistence can have significant consequences for the immunocompromised. To establish a persistent infection, *T. gondii* differentiates from its acute, lytic, fast-growing, tachyzoite stage to a latent, slow-growing, bradyzoite stage which persists within cysts. This project focuses on a putative copper transporter, Ctr2, that is predicted to be dispensable in tachyzoites and shows higher expression in bradyzoite-inducing conditions. To determine the role of Ctr2 in stage conversion and persistence, we generated a *T. gondii* strain that lacks Ctr2 (IIΔCtr2) as well as an ectopically expressed, HA-tagged complement strain (IIΔCtr2::Ctr2HA). As expected, IIΔCtr2 parasites show no lytic cycle defects.; However, under high pH, low CO2 conditions, IIΔCtr2 cysts show high levels of abnormal morphology (~60%), relative to wild-type and complemented strains (<20%). Consistent with a lack of copper homeostasis driving this phenotype, copper supplementation rescues the IIΔCtr2 cysts. Preliminary in vivo data suggest that IIΔCtr2 parasites show a decreased level of dissemination to the CNS at three weeks post infection. Current work is focused on defining the viability of bradyzoites within the abnormal cysts; quantifying CNS cysts in IIΔCtr2, wild-type, and IIΔCtr2::Ctr2HA infected mice; verifying that Ctr2 is copper transporter using yeast functional complementation studies; and determining changes in Ctr2 localization under stress and copper-supplemented conditions.

MODULATION OF BOVINE LIVER GLUTAMATE DEHYDROGENASE ACTIVITY THROUGH FILAMENT FORMATION

ETHAN TONTHAT

THE MADAGASCAR HISSING COCKROACH AS A LOW-COST ALTERNATIVE TO MAMMALIAN MODELS FOR STUDYING ACETAMINOPHEN HEPATOTOXICITY

***VICTOR VIGBEDORH*, ULISES RICOY, KONRAD ZINSMAER**

Drug-induced acute liver failure [ALF] can occur from overdosing on the widely used analgesic acetaminophen [APAP]. According to the NIH, APAP hepatotoxicity [APH] accounts for over 50% of overdose-related ALF in the United States alone. The worldwide use of APAP and the likelihood of overdoses make research on APH valuable to prevent and treat ALF.

When consumed, APAP is metabolized in the liver mainly through glucuronidation, followed by sulfation, where products are excreted via blood and bile. However, 5–8% of APAP is converted by cytochrome P450 and 3A4 into N-acetyl-p-benzo-quinone imine [NAPQI] which is usually excreted through urine. NAPQI is a highly reactive and electrophilic toxic metabolite that interferes with the formation of covalent bonds in proteins. During an overdose, NAPQI is produced in high quantities where it is not as effectively detoxified and excreted out of the body, resulting in cell or organ failure.

Extensive research has been and is being conducted on the processes governing APH development, and how to counteract its effects. However, these studies tend to be conducted on mammalian models. Although sufficient, the cost of care and utilization of these models can make relevant studies inefficient and difficult to conduct. The complexity of these models can also make understanding the more basic and underlying mechanisms difficult.

In this study, Madagascar hissing cockroaches were utilized as possible surrogates for modeling APH. The cockroaches were put under lab conditions like those of mice models where histological and immunohistochemical assays were conducted to identify the presence of necrotic tissue and indirect measures of molecular activity in response to APH (e.g., concentrations of CYP40, various reactive oxygen species, glutathione, and alanine aminotransferase).

UNCOVERING SECRETS OF THE MYSTERIOUS ANAEROBIC ARCHAEA

TU VO

CHARACTERIZING LIPOGENIC GENE EXPRESSION OF NON-LIPOGENIC ABCA1 INDUCER COMPOUNDS FOR ALZHEIMER'S DISEASE THERAPY

KATRINA VOLLMER

THE RELATIONSHIP BETWEEN MATERNAL PRE- AND POSTPARTUM OXYTOCIN LEVELS AND SIGNIFICANT CHILDHOOD ADVERSITY IN HUMANS

PAIGE WAGSTAFF

AUTOMATIC FLUORESCENCE IMAGING ROBOT

***LAINEY WAIT,* SAMUEL FREITAS, GEORGE SUTPHIN**

Many labs study aging and genetics using the roundworm *Caenorhabditis elegans*. One advantage of *C. elegans* as a model system is their transparent body structure, which allows fluorescently labeled proteins and other biomarkers to be visualized in live, free-crawling animals. Imaging tools that automate the measurement of lifespan, health, and molecular biomarkers in *C. elegans* are important as counting *C. elegans* is one of the most time consuming processes. We are working on creating a compact robotic imaging platform that is designed to automate the collection and analysis of fluorescence data. In this system, worms are cultured in an environment that isolates individual animals and allows us to track them over time. These environments currently require hundreds of individual wells to be filled manually, which is time consuming. The imaging system on this robot uses a camera, a filter cube, and a few simple lenses to create a high quality image of 2 wells of the tray of *C. elegans*. The robot is enclosed in a light-tight enclosure that will keep any stray light from hitting the camera sensor. It can be controlled via arrow keys using a simple Python script or set to automatically image each well on the plate one at a time, saving the images to a user-designated folder. This system is customizable and can be used on any tray if given the coordinates of each well. Future work will improve the signal to noise ratio of the images while maintaining the cost effective nature of the robot.

LASMIDITAN IMPROVES COGNITIVE FUNCTION IN AGING PARKINSONIAN MICE

***PAIGE WENE*, ATSUSHI ISHII, JASMINE MEREDITH, MANDI CORENBLUM, RICK SCHNELLMANN, LALITHA MADHAVAN**

Parkinson's disease (PD) is an age-related, progressive neurodegenerative disorder characterized by motor deficits and cognitive decline. However, there are no treatments that can slow or prevent PD. Mitochondrial dysfunction is known to be a key contributor to cell death in PD. Given this, the presented study investigated the therapeutic effects of Lasmiditan, an FDA-approved 5-HT1F receptor agonist, capable of inducing mitochondrial biogenesis or the production of new mitochondria. Specifically, the study examined Lasmiditan's effects in PD mouse model, namely Thy1-ASyn mice. Two age-groups of mice were assessed: young (4-5.5 months of age) and old (10-11.5 months of age), representing earlier and more advanced stages of PD, respectively. The mice received Lasmiditan (1 mg/kg, i.p.) or vehicle every other day for 1.5 months and wild-type mice served as controls. Following this treatment, the animals were subjected to several cognitive and motor behavioral assessments, followed by cellular and molecular analyses. Focusing on the behavioral analysis, we present data from underwent Y-maze, novel object recognition, nest building, and open field activity tests in the young mice, and novel object recognition (NOR), open field and inverted screen tests in the old mice. Results indicate that in the younger mice Lasmiditan improved object recognition memory and working memory tested by the NOR and Y-maze task. However, no significant improvements in the nestlet and open field tests, that assessed motor function, were observed. In the old mice, similar to the young animals, Lasmiditan improved performance in the NOR task but did not mitigate motor deficits in the open field or inverted screen.

Overall, these findings indicate that Lasmiditan may have potential in improving cognitive function across different stages of PD progression, but its effects on motor symptoms appear limited.

PANK2 DEFICIENCY LEADS TO IMPAIRMENTS IN AUTOPHAGY IN A CELL MODEL OF LIPOTOXICITY

BRYCE WILSON

EFFICACY OF NON-LIPOGENIC ABCA1 INDUCER CL3-3 FOR TYPE 2 DIABETES AND ALZHEIMER'S DISEASE

***ABBY WOLF*, MAHA IBRAHIM SULAIMAN, ANANDHAN ANNADURAI, GREGORY THATCHER**

Alzheimer's disease (AD) is a progressive neurodegenerative disease that constitutes a growing health crisis in the United States. Simultaneously, chronic metabolic diseases such as type 2 diabetes (T2D) are on the rise due to the prevalence of obesity and other risk factors. Previously, mouse models have been explored to test effective therapeutics for AD and comorbidities such as T2D. Apolipoprotein (APOE) therapeutics remain a priority as this is the dominant genetic risk factor for AD. Meanwhile, high-fat diets (HFD) have been observed to accelerate AD neurodegeneration. As determined in previous studies, the CL3-3 compound, an ABCA1 inducer enhancing cholesterol efflux, was found to be orally bioavailable and a promising lead for further development in AD and T2D treatments. To address the effectiveness of CL3-3, we used APOE3 and APOE4 mice as an Alzheimer's disease model to study the physiological relationship between HFD-induced metabolic defects and AD pathology. Study results indicate that CL3-3 improves cognitive deficit in all APOE4 mice and improved glucose tolerance in HFD APOE4 mice, whereas APOE3 mice displayed no significant results.

PHYSIO-CHEMICAL AND BIOLOGICAL PROCESSES AFFECTING BIOSORPTION OF METALS ON BIOTIC AND ABIOTIC POLYMERS CHITIN AND CELLULOSE

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Harmful metals like arsenic (As) and uranium (U) are present in the environment due to mining, smelting, ore processing, and industrial activities causing potential exposure to human health and the environment. The physical and biogeochemical characteristics of As and U affect their fate and transport in the environment. Several considerations for addressing environmental contamination in ecosystems due to these metals include ion exchange, adsorption, bioremediation, and advanced oxidation. Previous work has shown the potential for As and U adsorption using biopolymers, but there is a need to understand removal from environmental systems with mixed metals (U and As) and with environmentally relevant nutrients like phosphate. This project integrates laboratory batch experiments, geochemical modeling, spectroscopy, microscopy and microbiology tools to determine metal speciation and rate of adsorption onto biotic and abiotic biopolymers at pH 4 and 6 under surface oxidizing conditions. Biogeochemical insights gained from this project will help us differentiate between abiotic

and biotic processes involved in the removal of mixed metals in environmental systems near rural and Native communities impacted by contamination.

DOES ULTRASOUND THERAPY REDUCE ASTROGLIOSIS?

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Traumatic Brain Injury (TBI) causes a series of changes in the brain that can sometimes require invasive treatments, especially in severe cases. One key response to TBI is astrogliosis, where a type of brain cell called astrocytes reacts to the injury. This response can vary, from temporary changes in cell behavior to lasting structural changes in brain tissue. Astrogliosis is an important indicator of how much damage the brain has sustained.

The study investigates whether Low-Intensity Pulsed Ultrasound (LIPUS) can help reduce this astrocyte response after a TBI, promote brain repair, and prevent further damage. LIPUS is a non-invasive treatment that uses sound waves to stimulate tissue. We believe it could be a promising way to aid recovery from brain injuries. To test this, we used mice and divided them into four groups. The first group was uninjured and served as the control. The second group had TBI induced by a controlled impact. The third group received LIPUS treatment during the first week after the injury, and the fourth group received LIPUS treatment during the second and third weeks post-injury. We studied the brains of these mice using a marker called GFAP (Glial Fibrillary Acidic Protein), which highlights areas of astrogliosis. The study combined this with MRI scans to map changes in the brain near the injury site. Preliminary results showed that TBI caused significant astrogliosis near the injury and changes visible on MRI scans. Our ongoing work focuses on comparing how LIPUS treatment affects these responses across the different groups. By studying this, we aim to understand whether LIPUS can limit damage and promote healing after TBI, potentially leading to better, less invasive treatments for brain injuries in the future.