VISIBLE THINKING 2022

Undergraduate Research Symposium

VISIBLE THINKING

Visible Thinking is hosted by the Undergraduate Research Support Office of Trinity College of Arts & Sciences at Duke University.

The URS Office promotes undergraduate research at Duke through workshops, the annual Visible Thinking Symposium, funding independent research, assistantships, and conference attendance, and by providing support for summer research programs.

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Table of Contents

What is URS? 4
Community Engaged Research 5
Visible Thinking Undergraduates 6
Behavioral Sciences and Psychology 7
Biological Sciences 23
Creative Arts 71
Health / Clinical Research 73
Humanities 87
Physical Sciences 98
Quantitative Sciences 107
Social Sciences 111
Meeting of the Minds 120
Index 121

WHAT IS URS?

MISSION

The Undergraduate Research Support Office (URS) promotes undergraduate research at Duke through workshops, the annual Visible Thinking Symposium, funding independent research, assistantships and conferences and by providing support for summer research programs.

LEADERSHIP

Director of URS Office: Dr. Sarah Russell
Program Coordinator: Sharon Coglianese

VISIBLE THINKING

Visible Thinking is a campus-wide symposium that celebrates undergraduate research in all disciplines. The event is held every spring.

EVENTS

URS offers outreach events that educate students about research opportunities at Duke and the research process.

RESEARCH SUPPORT

URS provides support for several undergraduate research endeavors, including the Dean's Summer Research Fellowship, the Biological Summer Undergraduate Research Fellowship, and several other term and year-long projects.

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Community Engaged Research

Throughout this book, you will see abstracts with the symbol below. This identifies projects as Community-engaged Research.

Community-engaged Research (CER) projects have met some combination of the following 4 criteria:

- There were conversations with the community on the purposes of the CER.
- There was collaboration in the design of the project with a community partner.
- There was collaboration in the implementation of the project with a community partner.
- There will be public dissemination of the results of the CER.

Visible Thinking Undergraduates

Behavioral Sciences and Psychology

Economic Situations and Social Distance: Taxation and Donation

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Behavioral Sciences / Psychology

It has been well documented in social psychology that reduced social distance, or the degree of closeness between two people in a social situation, leads to greater prosocial behavior, or behavior that is performed for the benefit of others. However, the effect of prosocial behavior on social distance has not yet been studied. This study addressed this issue by examining the effects of two different prosocial economic situations – taxation and donation – on the social distance between participants in these situations. This study employed a novel online survey framework in which subjects (n = 957) earned money, gave that money to other real people in the economic situations, and rated their social distance – in terms of similarity and commonality – with the recipient of their money. The results revealed little difference in social distance between the two economic situations, except that the amount of money transferred by taxation was found to negatively predict similarity. The novel survey framework allowed for an effective comparison of situations of taxation and donation and the isolation of the effect of the economic situation on social distance. Because the framework established by this study is the first of its kind and allows for the exploration of novel areas of investigation, this framework is this study's major contribution to the field. Overall, this study was successful in being the first to explore a previously unexplored research question and was able to establish an original survey method that can be used to explore similar research questions.

Keywords: Economic Situations, Donation, Prosocial Behavior, Social Distance, Taxation

Psychology- Graduation with Distinction candidate

Young Children's Understanding of Inclusive "We"

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Behavioral Sciences / Psychology

For children, the task of comprehending and producing complex pronouns presents a unique challenge. While significant research has been conducted on the development of comprehension and production for singular pronouns, there is a lack of analysis focusing on plural pronouns, particularly the pronoun "we". Shared intentionality theory postulates that children undergo a shift in group understanding at the age of 3, wherein perception of group interaction develops from dyadic to larger group comprehension. The current study seeks to analyze whether this shift in group understanding affects the natural interpretation of the pronoun "we". We hypothesize that children younger than 3 will interpret an ambiguous "we" as referring to themselves and the speaker while children older than 3 will interpret an ambiguous "we" as referring to themselves, the speaker, and others present in the group. We hypothesize that linguistic cues like "we both" and "we all" will affect participants' group inclusion selections compared to an ambiguous "we". An online study was conducted with 2.5-year-olds and 4.5-year-olds that had participants select whom to include in an ambiguous "we" group and linguistically primed "we both" and "we all" groups to test these hypotheses. Generalized linear mixed models were conducted on the data, revealing no significant effect of age or condition on children's responses, except for a statistically significant effect of "we all" among 2.5-year-olds. At both ages and across conditions, children tended to interpret "we" as a dyadic group. This study reveals interesting clues as to how young children naturally interpret ambiguous plural pronouns and provides further insight into the development of inclusive language comprehension.

The Effects of the Timing of Trust Violations based on Political Ideology and Group Membership

Megan Gerges
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Behavioral Sciences / Psychology

Human interactions are essential to the functioning and flourishing of society. One important element of successful interactions is trust. Not much research, however, has been done on the effects of trust violations on subsequent cooperation levels. The literature, however, does suggest that early trust violations tend to have a greater negative impact on Americans compared to trust violations that occur later in an interaction. This study aims to better understand the effects of early versus late trust violations within the American population, based on the political affiliation of participants (conservative or liberal) as well as based on whether participants are partnered with an ingroup member (a fellow conservative or fellow liberal respectively) or an outgroup member (a liberal or conservative respectively). Based on past literature, we predict that all participants will be impacted by an ingroup/outgroup bias in which early trust violations are more harmful when paired with outgroup partners and late trust violations are more harmful when partnered with ingroup members. However, we predict that conservative participants will be impacted by this bias to a greater extent than liberal participants. Furthermore, we expect conservatives will be more impacted, overall, by early trust violations whereas liberals will be more impacted, overall, by late trust violations. However, we also expect to replicate the general findings of previous literature that American participants are more negatively impacted by the earlier trust violations.

Mental Health in Korean American (MIKA) Communities Project

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Behavioral Sciences / Psychology

According to the US Census, Asians are the fastest growing immigrant population, and Koreans constitute the third largest Asian group within this category. The U.S. experienced a 55% increase in the total Korean population between 2000 and 2019 yet are consistently under sampled in health surveys. National health surveys that collect information on Asian-Americans often omit this population in research reports; when health data are reported for Asian-Americans, it is often reported for the aggregated group. This masks the health differences between Asian-American subgroups and have led to incorrect extrapolation of health outcomes in one subgroup to all Asian ethnicities. Currently, there are no comprehensive statistics of Korean Americans suffering from mental health disorders (such as depression, anxiety, and stress) given that national and local health datasets do not disaggregate Asians by ethnicity, nor purposefully collect data among Korean Americans. Due to the lack of information on the current makeup of the Korean American mental health in the US, we lack comprehensive understanding of their mental health wellbeing. This study was designed to examine the mental health experiences of Korean immigrants and Korean Americans aged 18 and older living in the Durham/ Triangle area of the Southeastern United States. Using a descriptive study design, we collected data via an anonymous 60-question survey from local participants. The frequency data and continuous variables data were analyzed using Chi square tests and two-sample t-tests, respectively. Our findings indicate a wide variation of mental health status and experience and access to mental health care among the participants. Continued collaboration among community leaders and healthcare professionals is needed to discuss the mental health concerns of Koreans and address the unmet needs of these populations. Social determinants of Health (SDoH), cultural competency, and awareness are themes that emerged from the data collected to help address barriers to utilization of healthcare services. The survey results evaluated to assess the mental wellbeing of local Korean populations serves to begin needed discussions and partnerships between healthcare professionals and the community to develop sustainable programs that will ensure awareness of mental health symptoms and meaningful access to care.

Childhood Teachers' Responses to Their Students' Emotions: An Early Look at a Novel Measure

Sonia He
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Behavioral Sciences / Psychology

Childrens' ability to influence their emotions (i.e., emotion regulation) is crucial to their academic and social success. There are many ways that an adult might influence a child's development of emotion regulation, including how they respond to the child's emotional displays. Studies have shown that the way that parents respond to their child's emotional displays seems to significantly impact how well the child is able to manage their own emotions. However, not much is known about the impact of teachers' responses, possibly because there is a lack of adequate measurement tools for studying teachers' responses to their students' emotions. Thus, to overcome this barrier, the overarching goal of this research is to develop a self-report scale to measure the extent to which teachers of elementary school-aged and younger children assist their students with using the five emotion regulation strategies proposed by the Process Model of Emotion Regulation (e.g., situation selection, situation modification, attentional deployment, cognitive reappraisal, response modulation) when responding to their emotional expressions at school. In Study 1, we determined which student emotions to include in item stems by examining daycare, preschool, and elementary school teachers' (N = 23) perceptions of which emotions their students most frequently express at school. In Study 2, we explored the viability of initial candidate items of the novel scale by administering a 15-minute online survey via Qualtrics containing the initial version of the scale to a sample of preschool and elementary school teachers (N = 208). The importance of this scale lies in its potential future uses: by having a validated scale to measure teacher's use of emotion regulation strategies to influence their students' emotions, future researchers can design studies to empirically test the link between teachers' use of different external emotion regulation strategies and various student outcomes (e.g., students' emotion regulation, academic achievement) to determine which ways of managing students' emotions are best. Long term, these results can be used to inform school policies on how teachers should properly respond to their students' emotions and designs for school mental health interventions.

Psychology- Graduation with Distinction candidate

Examining the relationship between social identity motivation and anti-vaccine beliefs in varyi

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Behavioral Sciences / Psychology

In recent years, there has been an increase in health misinformation and conspiracies theories spread online via blogs, social media, and prominent public figures. Whilst there is a wide range of health misinformation being spread, anti-vaccine information has posed a unique threat to public health efforts both in terms of its content and virality.

There have been many theoretical and conceptual attempts to understand the allure of conspiracy and anti-vaccine rhetoric. Social identity refers to social group memberships that become part of the self, which are a powerful psychological phenomenon that generates community and creates a shared reality that people can accept, further entrenching them in their beliefs about the world. Very recent research published in May 2021 by Motta et al. developed a novel anti-vax social identification (AVSID) measure and established with predictive validation analyses that AVSID is associated with higher opposition to vaccine requirements. In other words, the more one identifies socially with the label of an “anti-vaxxer”, the higher likelihood that one will oppose vaccine requirements. However, this research has two notable limitations. First, since the study was cross-sectional rather than longitudinal, the causal direction of AVSID and vaccine-sceptical views is unclear. Second, there is a lack of exploration of this phenomenon across physical geography and policy environments. Essentially, Motta et al. established a link between social identification and vaccine opposition, but the details of this relationship are still ambiguous. In this thesis project, I designed a survey to address this second limitation.

This research project pairs vaccine belief and behaviour measures developed by Heidi Larson and pair them with Motta's AVSID measure to evaluate the relationship between anti-vax self-identification and vaccine opposition in varying policy environments across the USA. Data will also be collected on medical folk wisdom, anti-expert attitudes, subjective health assessments and parental status. Participants will be recruited using Lucid and will be restricted to those who are: over the age of majority; located in the USA in the policy environments of choice. Participants will be compensated for their time through the Lucid platform; general demographic data such as sex and age will be collected.

An examination of adults' use of mutual exclusivity in response to mispronunciations

Jingxuan Liu
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Behavioral Sciences / Psychology

People frequently encounter phonologically ambiguous labels (e.g. "dat") that could be interpreted as either mispronunciation of familiar words (e.g. "bat") or novel words. Understanding the ways people make sense of these labels can contribute to our knowledge regarding how people weigh environmental context (e.g. the type of objects present) and phonetic cues in their processing of ambiguous speech. In the current research, we examined how adults interpret mispronunciations of familiar labels (e.g. "crig") when both familiar (e.g. crib) and novel (e.g. spiky toy) objects are present. Specifically, we are interested in adults' use of mutual exclusivity (ME, the bias to attribute a novel label to a novel object over a familiar object) and potential drivers of their ME usage in response to mispronunciations. Through two looking-while-listening eye-tracking studies, we presented participants with mispronunciations that varied by level (e.g. number of phonetic features), with each in the presence of a pair of novel and familiar objects. Participants were asked to select their object of choice, and their looking behavior was tracked during the experiment. Study 1 followed the paradigm of Moore & Bergelson (2021) and verified their finding that adults seemingly adopt two different approaches (ME using vs ME eschewing) that guide their use of ME. Study 2, which further manipulated the level of mispronunciation, found that while participants were fairly consistent in using their approach, their ME usage was also impacted by the level of mispronunciation. The further the mispronunciation was from the correct pronunciation, the more likely participants were to apply ME. Taken together, our findings suggest that adults' processing of mispronunciations in ambiguous situations is driven by both top-down personal approaches and perceptual signals like phonetic cues.

Psychology- Graduation with Distinction candidate

Using visualization to reduce test anxiety in collegiate student athletes

Zoe Lusk
Faculty Mentor(s): Nancy Zucker
Behavioral Sciences / Psychology

Visualization is a method that many athletes have in their toolbox to cope with anxiety in competition and improve performance. Visualization, or 'mental imagery', requires athletes to imagine every detail of their competition in their mind from start to finish. Since athletes already have visualization skills that they take advantage of in an athletic context, this study proposes to redirect these skills towards another aspect of the student-athlete experience, performance in school. Test anxiety is a feeling of stress during evaluative circumstances that is significant enough to impair academic performance and overall well-being. Analogous to anxiety in sports competitions, this study aims to see if visualization can also reduce test anxiety for students in academics. Current established treatments for test anxiety are not ideal for the typical college undergraduate, as they are time-consuming and not easily accessible to everyone. Conversely, this intervention is a five minute audio-guided visualization that can be done anywhere at any time of the day. This study tested the efficacy of a visualization intervention for test anxiety (Aim 1), examined if factors such as prior visualization experience are moderators of the effectiveness of the visualization intervention (Aim 2) and synthesized a literature review to reveal the neural underpinnings of the visualization intervention. The study is a single arm trial where student-athletes are exposed to an audio guided visualization intervention and their test anxiety scores from before and after the intervention were compared. After the intervention levels of test anxiety were significantly lowered, specifically the 'Emotionality' component of test anxiety. Greater reductions in test anxiety scores were correlated with higher levels of initial test anxiety, suggesting that the intervention works better for those with high levels of test anxiety. Prior levels of visualization experience, competitiveness and interoceptive scores did not correlate with the effectiveness of the intervention. Additionally, levels of interoception did not change from before and after the intervention, although some aspects of interoception were correlated with levels of test anxiety. This preliminary look into visualization as an intervention for test anxiety suggests that it is an effective method to decrease levels of test anxiety in collegiate student-athletes.

Effects of Explanation and Framing on Belief and Sharing Behavior

Emily Mawyer
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Behavioral Sciences / Psychology

Concerns are growing that online misinformation can have real effects on people's beliefs and behavior. These impacts demonstrate the importance of creating strategies that can reduce belief in false information. The current study tested whether asking participants to explain why something is true or false would reduce belief in false information. Although participants who wrote explanations gave lower truth ratings for false items, this difference was not significant. The experimental condition did show a reduced belief in novel truths compared to repeated truths that was not evident in the control condition. Participants who wrote explanations were more accurate on known items. Explanation also resulted in the removal of a preference for headlines that imply a correlational relationship over ones that imply a causal relationship. Age was found to be the biggest predictor of sharing behavior for both causal and correlational headlines with younger adults sharing more headlines. These results suggest that younger adults may be more appropriate targets for strategies to reduce misinformation. Although this intervention was not effective at reducing belief in false information, it may encourage participants to rely on their own knowledge and evaluate underlying causal mechanisms.

Psychology- Graduation with Distinction candidate

Exploring the Relationship Between Discrimination and Maternal Fetal Attachment

Emily Raich
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Behavioral Sciences / Psychology

The caregiver-child attachment relationship begins early in life. Existing attachment literature has focused on the period from birth to one year old. However, a mother's relationship with her child begins before birth, while the fetus is in utero. Maternal fetal attachment (MFA) describes a pregnant woman's emotional bond with her unborn child. MFA manifests through maternal attitudes and behaviors and is associated with infant health and developmental outcomes. MFA predicts future attachment during childhood, which influences cognitive and socio-emotional development. A mother's environment during pregnancy impacts MFA, and several studies have examined the relationship between MFA and factors such as anxiety, depression, age, social support, prenatal testing, etc. However, no study to date has examined the relationship between discrimination and MFA despite known disparities in maternal and infant health. Moreover, discrimination during pregnancy adversely impacts maternal physical and mental health as well as infant development. The current study aimed to fill this gap in MFA research by including a diverse sample and examining the relationship with discrimination. The study collected self-report data from pregnant mothers in their third trimester (N = 61) and tested a moderated-mediation model between discrimination and MFA. The hypothesized model included depression as a mediator and social support as a moderator of the relationship between discrimination and MFA, based on previous findings. The moderated-mediation analysis was not significant and there was no direct correlation between discrimination and MFA. There were significant positive correlations between discrimination and depression and MFA and social support, and negative correlations between depression and MFA and depression and social support. These findings were in the expected directions and built upon previous studies. Future research should examine the proposed model in a larger and more diverse sample and include longitudinal data across trimesters through infancy.

Psychology- Graduation with Distinction candidate

Knowledge Structures: Top-Down vs. Bottom-Up Strategies

Paige Sevchik
Faculty Mentor(s): Ruth Day
Behavioral Sciences / Psychology

Students may do well on course exams, demonstrating good knowledge of the subject matter. But what about the structure of that knowledge – do they see relationships among course concepts or see them as relatively unconnected? To examine knowledge structures, participants completed an online sorting task with key concepts from an introductory psychology course. They sorted the concepts into piles based on "similarity" – they could define similarity in any way they wished. To examine the effect of different amounts of knowledge and exposure to psychology concepts on how the sorting task was performed, three experiments were conducted using different participant groups – beginning students (currently completing the course), advanced students (teaching fellows in the course), and the general public. Results from the sorting task revealed strong knowledge structures for the advanced students, modest to weak structure for the beginning students, and virtually no structure for the general public. The project reported here focused on the strategies that participants used to sort the concepts. For example, did they use a Bottom-Up strategy, focusing on individual concepts one at a time? Or did they use a Top-Down strategy, inspecting the concepts first, creating mental categories for them, and then sorting them into piles reflecting these categories? The distinction between Top-Down vs. Bottom-Up processes has been observed in other types of cognitive tasks. To determine whether they were used here, after performing the sorting task participants reported how they performed the task by listing the steps they used to sort the concepts. A coding scheme was developed to identify types of steps and content analyses were performed to identify the strategy used by each participant. In addition to the Top-Down and Bottom-Up strategies, a Hybrid strategy combining both approaches was also identified. The frequency of usage for each type of strategy was calculated and the results reveal how strategy usage differs across participants with different knowledge levels and experience with the subject matter. This research holds implications for both cognitive psychology and course instruction.

Behind the Curtain of Competency: Assessing Racial Colorblindness of Premedical Students

Clarke Shead
Faculty Mentor(s): Sarah Gaither and Keisha Bentley-Edwards
Behavioral Sciences / Psychology

“Racism is a public health crisis,” has become the slogan for a new revolution in healthcare. Previous literature describes poor treatment health outcomes as a result of bias in physician and medical students. However, there is a lack of research and attention on the bias of pre-health students prior to medical school matriculation. To improve these outcomes, early bias interventions and cultural competency trainings may better prepare students to provide culturally competent care. This study uses Racial Colorblindness and Multi-Cultural Competence scales to explore the current biases held by the pre-medical student population. Key findings express a similar amount of colorblindness amongst all racial groups in the pre-medical student population with little influence from racial exposure. Furthermore, racial exposure had an influence on multicultural competency, a variable found to have a strong relationship with colorblindness. Although the social influence of bias may be impossible to change, educational strategies may be impactful in the way biases are addressed to ultimately make strides towards equitable healthcare.

Psychology- Graduation with Distinction candidate

Young children judge defection less negatively when given a good excuse

Aren Tucker
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Behavioral Sciences / Psychology

The present study was designed to assess the degree to which providing excuses of varying qualities for promise-breaking behavior impacts a child's moral judgment of the behavior and of the transgressor responsible for said behavior. Moral judgment was assessed through measurement of participants' explicit judgments ("right" or "okay"), justifications for said judgments, interpersonal liking, tattling behavior, and partner selection. The sample included two age groups: 3.5-year-olds (n = 32, M = 42 months) and 5.5-year-olds (n = 32, M = 66 months). All participants were asked to judge each wrongdoer's actions as right or wrong, and justify their judgments. Judgment was assessed through open-ended questioning regarding each transgression. Justifications were recorded and categorized based on references to promises, and use of normative or descriptive statements. The study was conducted over Zoom, using pre-recorded videos that simulated puppets engaging in hypothetical promise-breaking scenarios. Results indicated first, that children judged moral transgressors less harshly when they were presented with a good quality excuse, as opposed to a bad excuse, or no excuse. Second, in regards to justifications, 5.5-year-olds used more normative statements when reasoning about puppets with good excuses than when reasoning about either "bad excuse" puppets or "no excuse" puppets. Third, 5.5-year-olds used more descriptive statements when reasoning about either "bad excuse" puppets or "no excuse" puppets than when reasoning about "good excuse" puppets. Overall, results suggest a significant transition in moral development occurs between the ages of 3.5 years and 5.5 years, during which children begin to adopt and refine their moral reasoning skills, increasingly using normative and descriptive statements to justify moral judgments.

Jury Cognition: Understanding the Insanity Defense

Jonathan Victor
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Behavioral Sciences / Psychology

Jurors must reach verdicts in criminal cases. How do they do this? They must consider everything presented in the trial, including physical evidence, witness testimony, and legal arguments. However, they often have trouble understanding the jury instructions given by the judge before they retire to the jury room to begin their deliberations. Jury instructions are based on criminal law and can change depending on what type of defense a defendant uses. This is especially problematic for the "Insanity defense," where defendants claim they are not guilty because they have a mental disease or defect. It is not clear whether a priori attitudes about insanity, murder, or the jury instructions themselves are responsible for jury problems in such cases. To examine this problem, an experiment on jury cognition was conducted, based on a paradigm developed in the Day Cognition Lab at Duke. That research used jury instructions for the Insanity defense in murder cases and found that overall accuracy was poor – people made correct decisions about guilt only 66% of the time. The current experiment expanded this paradigm to study multiple types of defenses and crimes. Participants were adults in the general population with a wide range of demographics (age, gender, ethnicity, education, and geographic location in the US), like those who serve on juries. They read jury instructions for a specific type of defense and crime and then used it to decide court case scenarios. Across different participant groups, there were three types of defenses (insanity, medication side-effect, self-defense) and two types of crimes (murder, theft), yielding a total of six experiment conditions. Overall accuracy was again poor – only 59% correct. Furthermore, it was unaffected by the type of defense or crime. These results suggest that preexisting attitudes about insanity or murder are not responsible for jurors' inability to make accurate decisions. Instead, the structure of jury instructions appears to be responsible. Therefore, the results have considerable generality across multiple types of legal cases. This research may also suggest how to improve jury instructions to increase juror decision accuracy.

Psychology- Graduation with Distinction candidate

Harnessing Stress in Sports & School: Testing A Stress-Mindset Intervention for Student-Athlete

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Behavioral Sciences / Psychology

Student athletes represent a diverse subset of the college student population in America, and the unique demands in their lives balancing athletic and academic responsibilities introduces an increased burden in their lives. Statistically, these student-athletes are often sleep deprived, leading to increased difficulties paying attention in class, and are at higher risk for diminished academic performance and likelihood of graduation when compared to the regular student population. Additionally, student-athletes are at increased risk for developing mental health conditions or symptoms. Stress plays a major role in shaping how student-athletes respond to and manage their responsibilities, and understanding how student-athletes can change their perception of stressors can help to understand how to improve the experiences of these students. Collegiate athletics is governed by the National Collegiate Athletic Association (NCAA), and research findings from the current study have the potential to lead to nationwide impact for college student-athletes. Prior research on the role of mindsets and their impact on the stress response has been conducted on school age children and working adults, but limited knowledge in the field exists for student-athlete populations. Furthermore, no current research exists for stress-mindset interventions targeting student-athletes. The goal of this study was: (1), to understand how student-athletes perceive and think about stress differently in two domains, academics and athletics, (2) how these mindsets may be changed through the use of a novel video intervention, and (3) how student-athletes may be uniquely prepared to deal with stress. The study utilized an experimental design delivered electronically via Qualtrics to assess the stress mindsets of student-athletes in both domains and deliver the intervention material, and a follow up study allowed the researchers to assess the impact of the intervention in changing student-athletes stress mindsets. The study successfully replicated some of the current findings on the relationship between stress mindsets and psychological outcomes, but no significant differences were noted in stress mindset between the intervention and control group following completion of the intervention.

Psychology- Graduation with Distinction candidate

Biological Sciences

A new in vitro model of arrhythmogenic cardiomyopathy and calcium leak

Alexander Behura
Faculty Mentor(s): Andrew Landstrom and Robin Perelli
Biological Sciences

Arrhythmogenic cardiomyopathy, where heart muscle is replaced by scar and fat, is a major cause of sudden cardiac death in children. The replacement of muscle tissue with scar and fat tissue can ultimately lead to life-threatening arrhythmias. Arrhythmogenic cardiomyopathy has been related to genetic variants in genes coding for proteins of the cardiac desmosome, a specialized cell structure for cell-to-cell adhesion. However, these variants only account for 60% of victims of arrhythmogenic cardiomyopathy; for the remaining 40% of patients, the cause of disease is unknown, and for all cases there exists no treatment to prevent or cure the disease. We have identified a family with arrhythmogenic cardiomyopathy that has variants in a gene that has not been studied in the heart, a non desmosomal gene, and this could give us insight into understanding genotype negative arrhythmogenic cardiomyopathy. We have generated induced pluripotent stem cells from this family and differentiated them into cardiac myocytes to study arrhythmogenesis. We hypothesized that the loss of this gene results in a calcium leak which leads to delayed after depolarizations, which can cause arrhythmias.

Identifying Inhibitors of Hsp70 in Plasmodium using Thermal Shift Assay

Elizabeth Boger
Faculty Mentor(s): Emily Derbyshire and Aaron Keeler
Biological Sciences

Malaria, a mosquito born disease, is responsible for over 200 million infections each year. Malaria parasites are members of the Apicomplexan family. Plasmodium is a type of Apicomplexan that is specifically responsible for the spread and infection of malaria in humans. Certain proteins are essential to each step of the life cycle of plasmodium, one of which is Hsp70. Hsp70 is a heat shock protein produced in plasmodium in response to exposure to stressful conditions, like extreme heat. This protein is hypothesized to play an integral role in the life cycle of plasmodium, acting as a chaperone and stabilizing the digestive vacuole membrane. Without the assistance of Hsp70, research has shown that parasite death ensues. This past semester, we have been studying a library of roughly 3400 drug compounds, selecting for those that bind to Hsp70, and observing the impact that binding to this drug has on the viability of Hsp70 and the resulting parasite condition.

We used a thermal shift assay to filter through this library of drugs and select compounds that bind to Hsp70. Thermal shift assays measure the change in thermal denaturation temperature and, in turn, the stability of a protein under various conditions. When a drug binds, the protein is stabilized. This assay allows us to examine the extent of the resulting binding affinity. Of the 3400 compounds, roughly 20 compound hits were confirmed. Our current project endeavors include running additional assessments to test the specificity of the binding to malaria parasites and exploring their phenotypes. This research leads to a better understanding of what environments influence the viability of Hsp70 and ultimately how to induce malaria parasite death.

Effects of ground angle on human vertical jump performance

Julia Choi
Faculty Mentor(s): Daniel Schmitt
Biological Sciences

The emphasis on improvement in elite sports is like an arms race of actual human limbs; technology, data analytics, fear of competition, and scientific understanding all feed into a steady drive to improve peak physical performance in humans. Some specialized training practices designed for this goal involve the use of plyometric exercises with rapid and explosive movements in order to improve power production in the lower extremities by hopping on inclines. To assess the value of plyometric approaches to athletic training and to better understand the relationship between tendon length and power production in humans, this study aimed to investigate the relationship between tendon stretch and power production through the following question: How does flexion of the ankle, determined by angle of the ground surface, affect human vertical jump performance by changing the length of the Achilles tendon? Fifteen amateur female athletes aged 18 to 22 years old were recorded performing one ballistic calf raise and three maximum vertical jumps at ground angles of -20, -10, 0, 10, and 20 degrees, and a proxy for length of their achilles tendon was measured by ultrasound at each condition. The force traces were integrated to compare vertical jump height, maximum force, maximum power, and overall work performed across the five conditions. It was found that when normalized to baseline values for each individual, jump height and force, but not work nor power, increased as the starting position of the ankle was shifted from plantarflexion to dorsiflexion.

Characterization of GAT1 interactors in NCR in Cryptococcus neoformans

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Additional Mentor(s): Julia Palmucci
Biological Sciences

Cryptococcus neoformans, a ubiquitous infectious yeast, proliferates in cerebrospinal fluid (CSF) causing fungal meningitis in immunocompromised individuals. CSF is deficient in nitrogen, a necessary growth nutrient. To overcome this hostile growth environment and maximize fitness, many fungi employ nitrogen catabolite repression (NCR) in which genes required to break down unfavorable nitrogen sources are repressed when more favorable nitrogen sources are present. Other fungal genera like Saccharomyces and Aspergillus have well-studied NCR pathways, but NCR has not been thoroughly investigated in C. neoformans. Current research suggests that Tar1 and Ure3 in C. neoformans may be orthologous to NCR gene repressors in Aspergillus and Saccharomyces (NmrA and Ure2, respectively), and may regulate the GATA transcription factor Gat1—the master NCR regulator. A Gat1::GFP strain was constructed, grown in replete and deplete nitrogen sources, and observed to show some differential fluorescence between the two types of nitrogen, and possible differential fluorescence between different hours of growth. Tar1 and Ure3 proteins were fluorescently tagged and transformed into wild-type (H99). These strains will also be transformed into a Gat1::GFP background to determine the localization of these proteins during nitrogen replete and deplete conditions and co-localization with Gat1. Co-localization of Ure3 or Tar1 with Gat1 would indicate a C. neoformans NCR pathway similar to Saccharomyces or Aspergillus, respectively. Negative results may indicate a unique regulatory system in C. neoformans. Further experimentation would examine additional NCR interactors and investigate their effect(s) on C. neoformans survival in CSF.

Investigating Aquaglyceroporins and Host-Parasite Interactions in Liver Stage Malaria

McKenna Crawford
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Biological Sciences

Malaria is caused by the apicomplexan parasite from the genus Plasmodium, which undergoes an asymptomatic developmental stage in the liver of its host before entering the symptomatic blood stage. Host aquaglyceroporins, such as host aquaporin-3 (AQP3), have been previously shown to be involved in liver stage malaria, as well as in infection caused by the apicomplexan parasite Toxoplasma gondii that is used as an in vitro model for malaria. Host AQP3 is known to be upregulated and localized to the host-pathogen interface at the parasitophorous vacuole membrane (PVM) during liver stage infection, and thus we propose that host aquaglyceroporins are hijacked by the parasite for nutrient acquisition. However, many details regarding the nature of this dynamic host-parasite interaction remain to be elucidated. In this work, the tubovesicular network (TVN) that protrudes from the PVM at the host-pathogen interface of liver stage Plasmodium parasites was characterized for the first time in the human-infective Plasmodium vivax species. In P. vivax, host AQP3 was found to co-localize to some TVN features by high-resolution confocal microscopy analysis. Host aquaglyceroporins were characterized in P. berghei and T. gondii by genetic and chemical approaches. Additionally, RT-qPCR analysis during T. gondii infection demonstrated that the T. gondii aquaglyceroporin (TgAQP) was downregulated at the same time that host AQP3 has been previously shown to be upregulated. This finding suggests that the parasites may exploit host aquaglyceroporins instead of using their own. Future work needed includes an analysis of parasite aquaglyceroporin regulation and localization upon host aquaglyceroporin inhibition, along with an analysis of any functional redundancy of different host aquaglyceroporins. While the vast majority of antimalarial drugs currently target the symptomatic blood stage, this work aims to characterize potentially druggable host proteins that are exploited during the asymptomatic liver stage of malaria pathogenesis.

Identifying the Site of Action of Spastin

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Biological Sciences

The spastin gene plays a role in microtubule severing, and when mutated in humans leads to Autosomal Dominant Hereditary Spastic Parapelgia (AD-HSP), a disease that impairs motor function in the legs. When spastin is mutated in flies, they also suffer from impaired motor function alongside abnormal neuromuscular junction morphology. These spastin null larval neuromuscular junctions are characterized by a high terminal bouton count with a "bunched" phenotype. Although the spastin null flies rarely survive to adulthood, the ones that do survive exhibit symptoms that are AD-HSP-like, such as limited flying abilities and impaired motor function in the legs. However, the site of action of Spastin remains unknown, as past studies of spastin have been based on ubiquitous knockouts. Our aim is to study spastin mutant phenotypes in a tissue-specific manner to identify the site of action of the protein. Additionally, other studies have indicated increased CRISPR knockout efficiency with the use of two guide RNA sequences. Overall, using CRISPR/Cas9 technology, a two gRNA strategy, and tissue specific drivers in Drosophila, our goal is to compare various tissue-specific spastin knockout lines and observe the phenotype seen in larval neuromuscular junctions. With a successful spastin knockout, we should see the spastin mutant phenotype in the ubiquitous knockout larvae. If the site of Spastin action is in glia, then knocking out spastin in glia specifically would be sufficient to induce the bunched phenotype. If the site of Spastin action is in neurons, then knocking out spastin in neurons specifically would be sufficient to induce the bunched phenotype. Understanding the tissue in which Spastin acts would better our comprehension of how Spastin regulates NMJ bouton morphology, AD-HSP, and shed light on potential treatment options for AD-HSP.

Location Bias Contributes to Functionally Selective Responses of Biased CXCR3 Agonists to Regul

Julia Gardner
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Additional Mentor(s): Dylan Eiger
Biological Sciences

G Protein-Coupled Receptors (GPCRs) represent the largest and most diverse family of cell receptors in the human genome and are targeted by ~35% of all FDA-approved drugs. GPCR signaling is mediated by various effectors, including G proteins, β-arrestins, and GPCR kinases (GRKs). Recent studies have uncovered that different ligands can bind to the same GPCR and differentially activate specific signaling pathways, a phenomenon known as biased agonism. Most research has focused on characterizing biased agonism at the plasma membrane, but GPCRs are also known to traffic to and signal from a variety of subcellular compartments. To determine if GPCR subcellular localization contributes to biased signaling, we studied how the chemokine family GPCR CXCR3 signals from endosomes following stimulation by the three endogenous ligands CXCL9, CXCL10, and CXCL11.

We determined that the biased ligands of CXCR3 promote different amounts of receptor mediated endocytosis and also show markedly different G protein and β-arrestin signaling profiles at both the plasma membrane and the endosome. The biased activation of G proteins and β-arrestins promotes differential activation of cytoplasmic and nuclear ERK1/2, as well as cellular transcription in both HEK293 cells and primary CD8+ T-cells. Inhibition of receptor internalization greatly diminishes the biased signaling observed between the endogenous chemokines. Finally, we demonstrate in a murine model of contact hypersensitivity that receptor internalization is a key component of the ability of a synthetic β-arrestin biased CXCR3 agonist to potentiate the inflammatory response. The present study shows the CXCR3 biased ligands induce unique signaling profiles at the plasma membrane and the endosome, and that signaling from endosomes greatly contributes to the overall biased responses seen at CXCR3. These results demonstrate that signaling from subcellar compartments is a critical component of GPCR signaling. Our findings have implications in the development of pharmaceutical drugs targeting chemokine receptors and other GPCRs.

LowCostomy bag for low-income ostomy patients

Amy Guan
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Biological Sciences

There is an immense need for low-cost colostomy appliances among ostomy patients living in low-income countries in Sub-Saharan Africa. There are an estimated 1.2 million new cases globally of colorectal cancer annually (1). Additionally, instances of colorectal cancer and the need for colostomies is increasing in Sub-Saharan Africa at an annual rate of up to 6.8% in certain countries (2).

Without access to colostomy products, low-income patients often resort to handmade solutions, such as taping a plastic bag to one's stoma. However, these makeshift solutions are not safe, odor-proof, or leak-proof, which lead to a decreased quality of life for these individuals. To combat this problem, we have developed LowCostomy: a highly affordable (<5 cent) colostomy appliance that capitalizes on utilizing low-cost, recycled materials in addition to a novel, all-natural formula of beeswax and pine resin that replaces the expensive adhesive material found in traditional adhesives for colostomy appliances. Existing skin-care products that separately contain beeswax and pine resin have been proven to be safe, non-toxic, and non-corrosive (7, 8,9, 10); however, there are still potential risks for rash, irritation, infection and other topical reactions upon the combination of these two substances.

The purpose of this study is to investigate the safety of a beeswax-pine resin mixture as it acts as a buffer between the patient's peristomal skin and the colostomy appliance. Rather than testing the entire LowCostomy system, this study will focus solely on the safety of the beeswax-pine resin buffer. The effect of having the beeswax-pine resin buffer adhered to the skin on the abdomen over a one-week period will be analyzed independently by two separate licensed physicians, with an emphasize on assessing changes in color, odor, texture, and overall skin health. Subject surveys will also be utilized to record subject experiences and will include quantitative sections like the 5-D itch scale. Compiled results will be analyzed by an accredited statistician.

A novel link between mitochondrial function and oxidative stress mediated by the ubiquitin conj

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Biological Sciences

Oxidative stress, where cells accumulate reactive oxygen species (ROS), is among the most prominent types of harmful environment, damaging cellular biomolecules, fostering cell death, and contributing to neurodegeneration. Eukaryotic cells experience many endogenous and exogenous sources of ROS, with the mitochondria being one of the largest generators. However, many details of the mechanisms regulating mitochondria in response to oxidative stress are not fully understood. The goal of this project is to elucidate and characterize the mechanisms by which cells mitigate the damaging effects of ROS while keeping mitochondria functional during cellular exposure to stress. Among enzymes known to be important for the stress response and clearance of defective mitochondria is the ubiquitin conjugase Rad6. Previously, we showed through western blotting that rad6delta has more porin, an osmolarity protein correlated with the number of mitochondria, than the WT strain. Furthermore, fluorescence microscopy revealed that the deletion of Rad6 increases the amount of total and ROS-producing mitochondria, suggesting that Rad6 acts as a regulator of the levels and function of mitochondria in yeast. To further investigate Rad6's role in mitochondrial function, we incubated WT and rad6delta in mitochondrial-inhibiting drugs, CCCP and antimycin, and found that rad6delta was more susceptible to both. This suggests that rad6delta relies more heavily on mitochondrial function to grow. Due to these findings as well as literature on the mitophagic role of the human homolog of Rad6 (Ube2a), we hypothesized that Rad6 is involved in mitophagy, the selective degradation of mitochondria in order to maintain homeostasis. We created rad6delta and WT cells expressing GFP-tagged Idh1 and Om45, proteins localized in the mitochondrial matrix and outer mitochondrial membrane respectively, and subjected these cells to starvation to induce mitophagy. Western blotting revealed higher levels of degradation of both Idh1 and Om45 in the rad6delta strain during starvation induced by growth to stationary phase in respiratory media and nitrogen-deficient conditions, suggesting increased mitophagy in the rad6delta strain. Our results provide the first evidence that Rad6 regulates the quantity and activity of yeast mitochondria via a mitophagic role. Future work aims to uncover a mechanism by which cells balance the quantity and activity of the mitochondria to mitigate the harmful effects of ROS.

Location Bias of G Protein-Coupled Receptor Kinases Promotes Biased Signaling at CXCR3

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Biological Sciences

G protein-coupled receptors (GPCRs) are the largest family of cell-surface receptors and are known to canonically signal through two main effector proteins: G proteins and β-arrestins. Biased agonism at GPCRs refers to the functional selectivity of a ligand towards certain signaling pathways over others. Other forms of bias that influence GPCR signaling are receptor and location bias, which affect the downstream pathways of receptor activation, leading to disproportionate levels of signaling between the G protein and β-arrestin pathway. A mechanism underlying signaling bias is the phosphorylation barcode hypothesis, referring to ligand induced receptor phosphorylation promotes differential engagement with downstream transducers. GPCR kinases (GRKs) are known to be responsible for receptor phosphorylation. GRK2, GRK3, GRK5, and GRK6 are expressed ubiquitously. Although there is supportive evidence demonstrating that differential phosphorylation promotes biased signaling, there is little known about the role of GRKs in directing GPCR functional selectivity. To study the role of GRKs in biased signaling, we studied the GPCR CXCR3, a chemokine receptor involved in leukocyte migration which has three endogenous biased ligands, CXCL9, CXCL10, and CXCL11, and two synthetic biased ligands, VUF10661 and VUF11418. We found that, upon ligand stimulation, GRK2 and GRK3 are recruited to CXCR3, with VUF10661 and CXCL11 being the most efficacious ligands. We also observed that GRK3 and GRK5 are able to traffic to endosomes following ligand stimulation with VUF10661 even though all ligands are able to promote receptor internalization. Given biased engagement of the GRKs observed at both the receptor and in endosomes, we next studied the functional consequences of ligand and location bias by creating GRK 2, 3, 5 and 6 constructs that are specifically targeted to the plasma membrane or the endosome. Using GRK2/3/5/6 KO cells, we determined that localization of the GRKs impacts receptor internalization, ERK activity, and transcription in a ligand specific manner. We observed that the endogenous and synthetic ligands of CXCR3 demonstrate biased engagement of the GRKs at different subcellular locations and highlight the functional significance of these findings on downstream receptor signaling. Together, these data provide evidence that the functional selectivity of biased signaling extends beyond β-arrestins and G-proteins to other effector proteins that interact with GPCRs.

Title: Identification of a covalent inhibitor that targets Ubc13 in the malaria parasite

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Biological Sciences

Malaria was responsible for over 600,000 deaths in 2020, of which more than 90% was caused by the protozoan parasite Plasmodium falciparum. The increase in resistance to current therapeutics demonstrates the necessity for new antimalarial drugs with novel targets and mechanisms to fully eradicate malaria. The *P. falciparum* enzymes that mediate protein ubiquitination, an essential post-translation modification in the parasite, serve as potential but under-explored malaria drug targets. Ubiquitin is a small 8 kDa protein, and its attachment to substrate proteins is mediated by an enzymatic cascade consisting of a ubiquitin-activating (E1), conjugating (E2), and ligase (E3) enzyme. Interestingly, the E2 PfUbc13 is a central mediator of Lys63-linked polyubiquitin (K63-Ub), a noncanonical ubiquitin linkage type that is associated with essential proteasome-independent processes including DNA repair, protein localization, and signaling pathways. However, there have been no identified chemical probes that target PfUbc13, and the enzyme partners of PfUbc13 are unknown. Previous studies discovered that the compound NSC697923 is a covalent inhibitor of HsUbc13, a homolog of PfUbc13 (80% sequence similarity). Thus, it was hypothesized that NSC697923 also covalently binds to PfUbc13, specifically at its active site Cys86 residue by Michael addition. Through trypsin digestion of PfUbc13 treated with the vehicle control DMSO followed by mass spectrometry analysis, a peptide at 817 Da was detected, corresponding to the expected mass of the Cys86-containing peptide. When PfUbc13 was treated with NSC697923, a peptide at 929 Da was detected, corresponding to the covalently modified peptide. Moreover, previous studies have suggested that the E1 PfUBA1 and PfUbc13 are enzyme partners. This interaction was validated using in vitro ubiquitin transfer assays monitored by anti-Ub western blot. Furthermore, addition of NSC697923 to the assay reactions resulted in dose-dependent inhibition of PfUbc13~ubiquitin conjugate formation, further indicating that NSC697923 targets PfUbc13. Overall, this work has identified a chemical probe that covalently binds to PfUbc13 and inhibits ubiquitin transfer from PfUBA1, thus providing a tool that can be used to interrogate PfUbc13 function.

Reproducible research and visualization techniques in support of the Atlantic BRS

Nick Kaney
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Biological Sciences

Conducting a Behavioral Response Study is difficult and multi-faceted. To parameterize the relationship between exposure and response, we need to ascertain the 3D position of Cuvier's beaked whales (Ziphius cavirostris) in relation to the sound source and the modeled sound field. Data are collected on the tagged animals using satellite-linked transmitters, and on the boat(s) with handheld GPS, goniometers and acoustic devices. Sound propagation fields are modeled separately and need to be integrated with the observed data to estimate received level. All these data sources vary in temporal and spatial resolution. Here we describe a reproducible research workflow we developed as part of an undergraduate research program at Duke University (MUSER) to help visualize and analyze the data collected during the 2019 Atlantic BRS Season. We developed four components: 1) an R data package containing all of the observed and ancillary data on animal movements and diving patterns, as well as the timing and location of each of 4 controlled exposure experiments; 2) a dashboard that summarizes the data at a high-level, 3) an R Shiny app that provides detailed information on the location, movements, and diving patterns of beaked whales; and 4) an R Shiny app that allows the user to visualize the 3D position of the animals in relation to the modeled sound propagation. We demonstrate each of these 4 components during an interactive session and document how reproducible research approaches are critical in complex and multivariate projects typical of marine mammal research.

Determining Requirements for Gαi:β-arrestin complex formation at G Protein-Coupled Receptors

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Biological Sciences

G protein-coupled receptors (GPCRs) comprise the largest class of transmembrane receptors and are targeted by nearly a third of prescription drugs. Canonical GPCR signaling involves pathways mediated by G proteins and β-arrestins (βarrs). While these have historically been thought of as separate, our lab has demonstrated that the inhibitory G protein Gα subunit (Gαi) and βarr form complexes following receptor activation. We have also shown that Gαi:βarr complex formation occurs even if the receptor signals through other classes of G proteins or lacks G protein activity altogether, as in the case of endogenously βarr-biased Atypical Chemokine Receptor 3 (ACKR3). To expand on this work, we used a split nano-luciferase assay to examine Gαi:βarr complex formation at βarr-biased mutants of two 'balanced' receptors, which exhibit both G protein and βarr signaling—the type 1 Angiotensin II Receptor (AT1R) and D2 dopamine Receptor (D2R). Consistent with our results with ACKR3, we found that mutation of these other receptors to abrogate G protein-mediated signaling did not eliminate Gαi:βarr association. Observing this at multiple additional receptors further supported the idea that G protein activity is not required for Gαi:βarr complex formation. To add to our characterization of the determinants of Gαi:βarr association, we then sought to explore how different mechanisms of βarr activation may contribute to complex formation at ACKR3. To accomplish this, we utilized previously described polar core, proximal finger loop, and lipid mutant βarr constructs. The polar core and proximal finger loop mutants achieve constitutive activation by different mechanisms, while the lipid mutant's ability to be recruited to and bind at the plasma membrane is deficient; these result in decreased Gαi:βarr association at Vasopressin Receptor 2 (V2R). We compared these mutants' ability to associate with Gαi at ACKR3. Our lipid mutant βarr construct showed a diminished ability to associate with Gαi, suggesting that βarr recruitment is important in complex formation. Additional insights into the determinants of Gαi:βarr association in the context of receptor, G protein, and βarr activity will enhance our understanding of the Gαi:βarr complex and its importance in GPCR signaling, potentially informing future development of therapeutic agents targeting GPCRs.

Getting Funky with CRISPR-Cas9: Engineering a METTL3 Catalytic-Dead Mutant

Ashwin Kulshrestha
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Biological Sciences

N6-methyladenosine (m6A) is an abundant post-transcriptional RNA modification present throughout the body that is enriched under both physiological and pathological states. m6A methylation is being investigated in a number of important biological contexts, such as RNA metabolism and neurological function and disease. Studies into effects of m6A methylation, however, are limited by currently poor techniques for detection of modified nucleotides. To improve m6A detection techniques, it is necessary to compare a technique in development with a control cell line which does not express m6A. Prior attempts to generate this target cell line in mammalian systems have been largely unsuccessful in producing the desired decrease in m6A abundance. The goal of this project is to produce an m6A-depleted cell line through Cas9-homology directed repair of METTL3: the primary writer protein that deposits m6A on mRNA transcripts. Targeted mutations (D395A and W398A) were to be generated in the methyltransferase domain of METTL3 to eliminate its ability to create m6A. Over 100 candidate cell lines (final count pending) were cultured and screened to identify a successfully modified line expressing the desired mutations in the METTL3 sequence. Successful generation of such a cell line may aid m6A research by providing a mammalian cell line with depleted methylation, thereby enabling potential improvement of m6A detection techniques by acting as a negative control. More generally, studies into m6A biology may be furthered by novel comparisons of biological processes between wild type and m6A-depleted cells.

Crystalline silica inhalation modulates BXSB murine lupus

Advika Kumar
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Biological Sciences

Anti-myeloperoxidase (MPO) vasculitis and systemic lupus erythematosus (SLE) are devastating autoimmune diseases where the body's immune system attacks itself. Genetic susceptibility and inhalational exposure to crystalline silica (Si), a common mineral, have been linked to disease development, but the etiology remains poorly understood. Our lab reported elevated anti-MPO antibodies (Ab) in the blood and lungs of silica-exposed female BXSB mice, an established model of SLE and ancestral strain for SCG/Kj mice with anti-MPO Ab and severe glomerulonephritis. Here, I conducted studies to determine if an anti-MPO Ab response is amplified in BXSB male mice that express accelerated SLE due to a y-chromosome-linked autoimmune accelerator locus. I examined the harvested tissues from a cohort of 8 male BXSB mice that we exposed to silica or vehicle for anti-MPO and anti-DNA Ab. Injury was assessed using enzyme-linked immunosorbent assays, immunohistochemistry, and immunopathology. Key findings were extrapolated from lung staining, including the presence of 1) severe lung hemorrhage and macrophages in Si-exposed mice; 2) abundant lymphoid structures in Si-exposed BXSB mice; and 3) numerous lymphocytes dispersed throughout the lung tissue. Quantitation of serum autoantibodies revealed no difference in levels of circulating anti-ssDNA IgG, but a significant difference in anti-MPO IgG autoantibody levels, with surprisingly lower levels detected in Si-exposed mice. Understanding the basis and consequences of these altered anti-MPO Ab responses will enrich our understanding of the origins of autoimmunity and expose mechanisms of disease.

Elucidating the role of submucosal gland-derived reserve stem cells in long-term airway repair

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Biological Sciences

Chronic lower respiratory diseases impact hundreds of thousands of people in the United States each year. In many of these diseases, incomplete repair of the surface airway epithelium (SAE) contributes to disease progression. SAE repair is partially facilitated by reserve stem cell populations from submucosal glands (SMGs), which are branched tubular glandular tissues located beneath the pseudostratified mucociliary epithelium of the conducting airways in humans. SMGs are typically thought to be comprised of 3 major cell types: serous, mucous, and myoepithelial cells. Using 10X Genomics single cell RNA sequencing technology to analyze cells from a healthy human SMG, I characterized epithelial cell populations in the SMGs at significantly higher resolution, identifying six serous cell subclusters distinguished by expression of key cell markers, some of which have not been identified in the literature to date. I also demonstrated that expression of SOX9, a critical transcription factor and canonical pan-SMG epithelial cell marker, is unevenly distributed across SMG epithelial cell subtypes. It has previously been shown that SOX9+ myoepithelial cells from the SMGs can serve as reserve stem cells and migrate to the SAE after injury. To track the fate of SOX9+ gland-derived cells at late time points after injury, I used Sox9-creER:R26R-tdTomato mice to lineage-label Sox9-expressing SMG cells and track their fate at 50, 80, 90, and 105 days after migrating to the SAE. I confirmed previous findings and found that SMG-derived, Sox9-lineage labeled cells differentiate into SCGB1A1-expressing club cells and acetylated tubulin-expressing ciliated cells, proportion of lineage-labeled cells that differentiate into these airway cell types does not show a clear trend over time. Some lineage-labeled cells on the SAE express none of these known airway cell markers, thus their fate is currently unknown. Altogether, this work offers a more in-depth picture of the cellular organization of SMGs and the role that SMG-derived reserve stem cells play in airway repair. Understanding these mechanisms will lend new insight into the incomplete repair processes at work in chronic human lung diseases.

The contributions of distinct cell types in regulating orientation in the superior colliculus

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Biological Sciences

The superior colliculus (SC) is involved in orienting vertebrates towards or away from behaviorally-relevant stimuli of interest. The contributions of distinct cellular populations in this circuit to orienting behavior are not well understood. We used optogenetic stimulation to map the contributions of two distinct cell types, parvalbumin-positive (PV+) neurons and vesicular glutamate transporter 2-positive (VGlut2+) glutamatergic neurons, in the SC to orienting behavior in freely-moving mice. Using high-speed video tracking, we quantified the impact that stimulation of these populations had on kinematic parameters of orienting movement. By varying the location of stimulation within the SC, we mapped the topographic relationships between these neural populations and behavior. We found that stimulation of VGlut2-expressing neurons elicited two distinct contraversive turning behaviors: whole-body turning or inward turning towards the body. In contrast, stimulation of PV-expressing neurons only slightly elicited contraversive turning but produced large bursts of locomotion. In both cell types, degree of turning varied robustly with frequency of stimulation. Under head fixation, stimulation of both populations produced contraversive licking. Number of licks, tongue extension, and latency of licking varied with frequency of stimulation. We found that both populations produced long-range ascending and descending projections to the thalamus and brainstem respectively. These results demonstrate that the SC contains distinct output populations that may mediate different types of orienting behavior. This research will help us better understand the circuit mechanisms in the SC and the recruitment of the SC by other regions, such as the basal ganglia, in the control of orienting during goal-directed actions.

Targeting XIAP Suppresses Formation of Inflammatory Breast Cancer Tumor Organoids

Seayoung Lee
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Biological Sciences

Inflammatory breast cancer (IBC) is the most aggressive subtype of breast cancer. Despite accounting for 1-5% of breast cancer diagnoses, IBC is responsible for over 10% of breast cancer mortality. The lower survival rates of IBC patients in comparison to those of other breast cancers may be attributed to the nature of its unique presentation in the breast. Unlike the palpable tumor mass found in other breast cancer subtypes, IBC presents as circulating tumor cell clusters, termed tumor organoids or tumor emboli. Our previous findings show high expression of the X-linked Inhibitor of Apoptosis Protein (XIAP) in both IBC pre-clinical models and tumor organoids. XIAP is the most potent inhibitor of apoptosis and is known to drive proliferative and immunosuppressive activity in IBC specifically. The smac mimetic Birinapant has high affinity for XIAP and two other IAP proteins, cIAP1 and cIAP2, and can disrupt their apoptotic interaction with caspases. However, the effect of XIAP inhibition on IBC tumor organoid formation remains to be investigated. In this study, IBC-derived tumor organoids cultured in a simulated dermal lymphatic microenvironment were treated with the XIAP inhibitor Birinapant before formation. The results show that Birinapant inhibited the formation of tumor organoids at both dosages 300 nM and 1000 nM, demonstrating that XIAP activity is necessary for IBC cells to aggregate into circulating tumor clusters. Thus, these findings demonstrate the importance of XIAP in IBC progression and metastatic dissemination as well as the therapeutic potential of using XIAP inhibitors to suppress tumor organoid formation.

Implications of CD155 on the Migratory Abilities of Medulloblastoma

Sean Li
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Biological Sciences

Brain cancers are the leading cause of death in pediatric populations. Medulloblastoma, a type of solid brain tumor, appears the most frequently and is considered a Grade IV tumor, characterizing it as being malignant and having a high growth rate. Thus, despite medulloblastoma patients having high initial five-year survival rates (~85% and 70% between average-risk and high-risk disease) using current treatment options, recurrence occurs in approximately 30% of patients. Within this group, 80% will present with metastasis, leading to a one-year survival rate of less than 40%. Thus, there is a need for additional therapeutic options that can improve these outcomes.

CD155, the poliovirus receptor, has garnered interest as a potential therapeutic target for medulloblastoma. CD155 has been found to positively impact tumor immunosuppression, proliferation, and migration for multiple types of cancers, and our lab has found that the overexpression of CD155 mRNA is seen in medulloblastoma models alongside other pediatric brain tumors. Therefore, to build further evidence for using CD155 as a therapeutic target, the effect of CD155 on medulloblastoma model migration was tested.

To test medulloblastoma migration on a three-dimensional scale, Corning Matrigel assays were used. 283 wildtype medulloblastoma models were exposed to either a monoclonal CD155 antibody (blocking the receptor) or a PBS/glycerol control solution prior to being seeded in wells containing a Matrigel matrix. After the models were exposed to their respective conditions and added to the Matrigel-containing wells, the cells were allowed to migrate into the Matrigel for 24 hours. Once the 24-hour period has finished, the Matrigel membranes were removed and imaged under 5x magnification.

Fiji was used to analyze the number of cells invading each Matrigel membrane, and it was found that the models exposed to the CD155 monoclonal antibody experienced less migration through the Matrigel matrix ($p = 0.006$). These results support the idea that CD155 may have a role in the high malignancy and recurrence rate of medulloblastoma. More migration assays, like a wound-healing assay, will be conducted to further build upon this initial evidence.

Investigating the Role Maf Proteins Play in Enterocyte Physiology Using Mouse Models

Celina Ma
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Biological Sciences

Enterocytes are cells in the intestine that are responsible for secreting digestive enzymes and absorbing nutrients. Distinct from the adult, neonatal enterocytes rely on bulk intake of nutrients via phagocytosis and endocytosis and most nutrient degradation occurs intracellularly in lysosomes rather than in the intestinal lumen. The transcriptional regulators of this bulk uptake activity are unknown. Work from our lab has identified enterocyte specific transcription factors MafB and cMaf. To determine the function of Maf proteins in the intestinal epithelium, we deleted Maf proteins, MafB and cMaf, in the embryonic mouse intestine (Maf DKO). Starting early in postnatal development and persisting throughout life, Maf DKO mice are approximately 75% the body weight of control mice. Subsequent RNA sequencing of P7 intestinal epithelium showed down-regulation of genes related to lysosomes, phagocytosis, and endocytosis in Maf DKO mice. Using western blotting, I have shown that legumain and cathepsin, lysosome-specific proteases, as well as Gulp1, a phagocytic gene, have significantly decreased expression in neonatal Maf DKO mice. Further, I compared protein level changes in different regions of the small intestine. The decreased expression of leguamin was specific to distal regions of the intestine which are known to have high lysosomal activity. Through uptake assays involving gavaging fluorescently labeled carbohydrates and proteins, we have identified functional deficits in the capacity of neonatal Maf DKO enterocytes to take in nutrients. Additionally, the RNA sequencing also revealed mis-regulation of several metabolic genes. Arg2 and trehalase, genes important for urea and carbohydrate metabolism, had significantly increased protein expression in neonatal Maf DKO mice, showing mis-regulation of metabolic genes. This work has identified transcription factors important for bulk uptake and metabolism of nutrients in the neonatal intestine. Further characterization of genes mis-regulated in Maf DKO mice will provide mechanistic insight on novel genes important for nutrient uptake in neonatal enterocytes.

Using HACK conversion to improve CRISPR knock-out efficiency of spastin in Drosophila

Shibani Mallik
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Biological Sciences

Spastin is a microtubule-severing protein implicated in microtubule growth and degradation. In humans, mutations in Spastin are linked to Autosomal Dominant Hereditary Spastic Paraplegia, a neurodegenerative motor disorder. Ubiquitous deletion of spastin in Drosophila revealed similar motor defects in adult flies and a mutant phenotype in synaptic bouton morphology and function in larvae. However, it remains unknown if tissue-specific spastin deletion, specifically in neurons or glial cells, may cause similar phenotypes. CRISPR-mediated deletion of spastin was attempted indirectly using the UAS-Gal4 system to express promoters and Cas9, but it was hypothesized that the direct coupling of tissue-specific promoters to the Cas9 transgene may be more effective in producing the desired deletion. However, promoter-Cas9 stocks are scarce. To take advantage of the large number of existing promoter-Gal4 lines, this study used the HACK (homology-assisted CRISPR knock-in) method to convert promoter-Gal4 lines to promoter-Cas9 lines through a series of genetic crosses and visual analysis using a fluorescence microscope. The conversions of four promoter-Gal4 lines were attempted with no detectable success. This study also involved crossing existing promoter-Cas9 lines to spastin guide RNAs to determine if the Cas9 lines or guide RNAs were defective. Following the completion of these genetic crosses, larval dissection was performed and immunofluorescence microscopy was used to visualize and quantify boutons at the neuromuscular junction. These crosses and subsequent analysis of synaptic bouton phenotype showed no significant results. These results suggest that further research should focus on the creation of new guide RNAs to effectively knock out spastin.

Lentiviral delivery of fluorescent biosensors to induced pluripotent stem cell cardiomyocytes

Alexandra Markunas
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Biological Sciences

Sudden cardiac death of children and adolescents occurs principally due to variants in genes encoding cardiac ion channels that can manifest as lethal arrhythmias. Human-induced pluripotent stem cells (hiPSCs) can be used to derive cardiac myocytes (hiPSC-CMs), which serve as a valuable tool for modeling cardiac diseases in vitro. I aimed to develop a lentiviral delivery system to genetically encode both voltage and calcium fluorescent biosensors within hiPSC-CMs derived from patients with cardiac conditions to analyze their arrhythmias in vitro. HEK293T cells were transfected with two lentiviral packaging constructs and the recombinant plasmid DNA of Arclight, ArchD95N, GCaMP5G, coding for a GFP-fused voltage indicator, red-shifted voltage indicator, and GFP-fused calcium indicator, respectively. Biosensing lentivirus was recovered from media, titered, and then used to transduce ostensibly healthy control line 8BH hiPSCs and hiPSC-CMs. GFP or RFP positivity through confocal microscopy confirmed successful delivery of the appropriate fluorescent biosensor to the iPSC-CMs. Action potentials and calcium transient tracings were generated from line scans of lentivirus infected hiPSC-CMs that exhibited cyclic changes in fluorescence intensity, thus demonstrating the ability of this delivery system to allow for dual modeling of transmembrane potential changes and calcium handling in hiPSC-CMs. I was then able to apply traditional in vitro and in silico methods in addition to this biosensor delivery system to characterize the pathogenicity of a T223M variant in the TBX5 gene that is associated with prolonged electrical repolarization of the heart, finding the variant to be pathogenic. Enrichment efforts will be continued to develop stable lines of biosensor-expressing hiPSCs that can be readily differentiated into hiPSC-CMs for optical analysis. This capability to phenotype the same cells long-term is significant in providing the capacity to monitor arrhythmias in cardiomyocytes derived from patients with cardiac conduction and calcium-handling abnormalities, alongside the ability to monitor the arrhythmia-induction capabilities of pharmaceuticals or the impact of therapeutic candidates in vitro.

Investigating ERRα in the eye as a function of age and disease

Fatima Massare Somers

Faculty Mentor(s): Goldis Malek, Tanu Parmar, Vipul Parmar, Mike Lekwuwa and Mayur Choudhary

Biological Sciences

Age-related Macular Degeneration (AMD) is a major cause of vision loss in the elderly, for which there are limited treatments available. Estrogen Related Receptor alpha (ERRα) is an orphan nuclear receptor, previously shown to be important in a myriad of physiological processes including regulation of metabolic genes. Our preliminary data has demonstrated an increase in the expression of ERR with age in human ocular tissue. Herein we propose to begin investigating the mechanisms of action of ERR in cells vulnerable to AMD as a function of age. Our goal is to determine the extent to which this receptor supports ocular cell homeostasis in the eye, using gain of- and loss of-function in vitro assays. Our results will ultimately guide us in discovering if ERRα may play a role in AMD development and progression.

Using an AAV-4 vector to express human-CXCL13 chemokine in cell culture and in vivo

Madison McMichael
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Biological Sciences

Humanized mouse models allow us to gain greater insight into the mechanisms underlying human immune-mediated diseases. Specific targeting of mouse lungs allows for the study of lung diseases through closer inspection of human genes and immune cells. In this study, I sought to create a model in which the human CXCL13 chemokine is expressed in a mouse lung. I planned to infect the mouse lung cells with an Adeno-associated virus-4 (AAV-4) serotype vector, such that it attracts human lymphocytes in a humanized immune system model. Preliminary data using flow cytometry demonstrates that the AAV-4-eGFP control vector transduces African green monkey cos-7 cells in culture. In Aim 1, I tested if cos-7 cells infected with an AAV-4-human-CXCL13-eGFP vector express the human CXCL13 chemokine. Flow cytometry confirmed GFP expression by infected cells, and using PCR, I amplified human CXCL13 from cDNA generated from vector-infected cells. The goal of aim 2 was to confirm that the AAV-4 serotype can infect mouse respiratory tract cells in vivo. C57BL/6 and NOD-scid-gamma mice were intravenously injected with the AAV-4-eGFP control vector and organs were harvested 1 month later. Flow cytometry of isolated lung cells did not detect differences in spontaneous GFP expression between the uninjected control mice and the mice that received an intravenously administered AAV-4 vector. In ongoing experiments, we will measure human CXCL13 gene expression in lung cells using the PCR assay and GFP expression using immunohistochemistry. Ultimately, we hope to use this new model in future experiments with humanized mouse subjects to study autoimmunity and other immune-mediated diseases that affect the lungs.

Differential Efficacy of Mutant IDH Inhibition Among Gliomas

Emily Miller

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Biological Sciences

Gliomas are difficult to treat tumors of the central nervous system that have few options for treatment or therapies. However, a current clinical trial (NCT02481154) on AG-881, an inhibitor of mutant IDH, has shown some potential. Mutant IDH contributes to the growth and survival of tumor cells in various ways, with a suspected pathway involving the overproduction of the oncometabolite D-2-hydroxyglutarate (D-2HG), which is suspected of promoting an immunosuppressive microenvironment and inducing differentiation defects. For this reason, inhibitors of mutant IDH, such as AG-881, which prevent the production of D-2HG through the selective inhibition of mutant IDH, are being investigated for therapeutic purposes. However, the full potential of IDH inhibition for therapeutic purposes remains unknown. Here, we seek to investigate the therapeutic benefit of mutant IDH inhibition among low- and high-grade gliomas. It was hypothesized that AG-881 would significantly decrease the growth potential of low-grade glioma cells in vitro but would diminish in efficacy as tumor stage and grade increased because the role of mutant IDH becomes unclear as tumors progress. To test the effect of AG-881 among gliomas of various grades, cell proliferation was monitored by real time cell imaging. Immunocytochemistry characterized the cells and assessed extent of differentiation, with liquid chromatography mass spectrometry measuring levels of D-2HG in the supernatant to ensure efficacy of mutant IDH inhibition and D-2HG abrogation. Western blots and qPCR were also used for protein profiling biomarker assessment of proliferation and differentiation. Based on the proliferation data to date, and the impact on self-renewal capacity via the extreme limiting dilution assay (ELDA), we are beginning to see trends of a differential impact of AG881 on various cell lines. Additionally, optimizations for live cell imaging will be performed to quantitate these differences and confirm these findings.

Characterization of SCA48-linked CHIP mutants

Andrew Nguyen
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Biological Sciences

Mutations in C-terminus of Hsc70 Interacting Protein (CHIP) have recently been implicated in spinocerebellar ataxia type 48 (SCA48), a neurodegenerative disease characterized by gait ataxia and/or cognitive-affective symptoms. Impaired proteostasis is associated with a wide array of neurodegenerative diseases, and CHIP, through the proteosome-ubiquitin pathway, is involved in proteostasis. Interestingly, SCA-48-associated mutations in CHIP lie in its two functional domains (TPR and U-box), suggesting that these disease-related mutations may be related to specific defects in CHIP protein function. Preliminary data suggest that interactions with the molecular chaperone HSP70 may be impaired in SCA-48-associated CHIP mutant protein. However, before this can be examined further, we first investigated how the SCA-48-associated CHIP mutations affect their relative protein stabilities.

Engineering MAP Hydrogel Scaffolds to Modulate Molecular Transport and Bioactivity

Ethan Nicklow
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Additional Mentor(s): Alexa Anderson
Biological Sciences

Tissue engineering researchers often use biocompatible polymer materials to create scaffolds that support cell growth and regeneration. Microporous annealed particle (MAP) gels are particle-based scaffolds, first described by the Segura lab, comprised of hydrogel microparticles (HMPs) crosslinked together to form a bulk, porous scaffold. In this study, we examine methods of tuning MAP scaffold properties at both the local and bulk scales to improve the modularity of our material. We hypothesize that decoupling bulk and local properties can help tailor our material towards specific cell responses and applications. In addition to biocompatibility and material properties, diffusive properties are especially important as diffusion is essential for nutrient delivery and waste removal in cells. Therefore, we also aim to develop methods for assessing diffusion through MAP scaffolds to better our understanding of mass transport in MAP. HMPs were produced via microfluidics, lyophilized in 70% EtOH, then rehydrated at varying weight percentages (wt%). Cell viability was assessed via culturing murine cells with lyoHMPs and mechanical properties were evaluated through rheological testing. To study the effects of lyoHMP wt% on mass transport, MAP gels were cast around a spacer to create a cavity in the gel. Radial diffusion was evaluated using a fluorescent marker imaged over time. Diffusion was evaluated in nonporous (NP) gels, MAP gels with varying wt% lyoHMPs, and MAP gels with lyoHMPs of different sizes. We were able to produce HMPs with varied wt% of HA that, once lyophilized, showed no impact on cell viability in scaffolds. Tuning lyoHMP wt% in MAP scaffolds allowed us to control the particle fraction in the bulk scaffolds. Our analysis of diffusion showed that MAP scaffolds support increased molecular diffusion compared to NP gels. For MAP scaffolds, increasing the particle fraction of the scaffolds or decreasing the HMP size hindered diffusion. We were also able to modulate local stiffness by tuning the polymer content of the HMPs. MAP gels comprised of lower polymer content HMPs yielded softer gels and NP gels of the same composition showed similar trends. In this work, we described methods for tuning bulk MAP scaffold properties by changing local HMP stiffness as well as the HMP particle fraction. A new method for evaluating diffusion in granular materials was established and will be implemented for future MAP scaffold characterization.

Metabolic Response to Influenza Vaccination

Claire Parker
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Biological Sciences

Chronic immune activation and severe inflammatory states are positively associated with resting metabolic rate (RMR), but the impacts of mild immune stimuli on metabolism are poorly understood. This study investigates the within-individual association between the immune response to influenza vaccination and RMR in young adults. We evaluated C-reactive protein (CRP) levels as a proxy measure of immune system activation and RMR in 17 individuals at baseline (pre-vaccine), 2 days post-vaccine, and 7 days post-vaccine. We implemented Wilcoxon matched-pairs signed-rank tests and Type II Wald chi-square tests of linear mixed-effect models were implemented to test the hypothesis that influenza vaccination was energetically costly and immune system response to vaccination was significantly associated with metabolic rate. CRP significantly increased from baseline to day 2 by an average of 1.94 ± 2.35 mg/L (p< 0.0001). There were no statistically significant changes in RMR from baseline to day 2 (p=0.98) or day 7 (p=0.21). Log(CRP) did not significantly predict log(RMR) (p= 0.46). When including systolic blood pressure was included in the model, it was a nearly significant predictor of RMR (p=0.066. There is no evidence that influenza vaccination results in a corresponding increase in RMR. In the context of prior studies, these results suggest that the energetic cost of an influenza vaccine's the mild immune system stimulus of an influenza vaccine is either too small to measure by indirect calorimetry or is fully compensated for by temporary downregulation of energy allocated to other body systems.

Comparative Neuroendocrinology of Lemur Pregnancies

Jonathan Pertile
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Biological Sciences

The neuropeptide oxytocin, OT, sometimes known as the “love hormone,” regulates a variety of social behavior, such as aggression, mating, and parental care. Despite its role in the mother-infant bond, information on OT concentrations across reproductive phases is limited and largely restricted to rodents and haplorhine primates. Strepsirrhine primates, including lemurs, display great variation in life-history traits and parental care styles. Ring-tailed lemurs (*Lemur catta*) provide intensive maternal care, carry their infants, usually give birth to singletons, and have relatively slow life histories. Conversely, ruffed lemurs (*Varecia* spp.) park their infants in nests rather than carrying them, give birth to litters, and have relatively fast life histories. Here we examine the relationship between behavioral aspects of these life-history differences and changes in OT concentrations across pregnancy and lactation. We collected behavioral data and urine samples from female ring-tailed and ruffed lemurs before and during their pregnancies at the Duke Lemur Center. Urinary OT concentrations are being quantified with an enzyme immunoassay previously validated in other mammalian species and now used in lemurs for the first time. We will relate differences in behavior and OT concentrations to reproductive phase and species.

Understanding the loading and release mechanisms of Polyketide Synthase enzyme complexes in the Apicomplexan parasite Toxoplasma gondii

Danielle Pitchon
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Biological Sciences

Polyketide synthases (PKS) are large enzyme assembly lines that generate complex molecules known as polyketide natural products that are released and utilized by the producing organism. While most polyketide synthases are composed of different combinations of the same enzymes, some contain enzymes with more unique mechanisms. In *Toxoplasma gondii*, the parasite that causes toxoplasmosis, there are two predicted PKSs that contain unusual loading and release mechanisms, a fatty acyl-adenylate ligase (FAAL) and a reductase (R) domain. This study explores these two enzymes using cloning, protein expression, purification, and analysis techniques to characterize their respective substrate selectivity and mechanism. For the fatty acyl-adenylate ligase of the first polyketide synthase, modeling and docking data suggested that fatty acids with 13 and 14 carbons are the substrates that bind in the most energetically favorably manner, while mass spectrometry analyses suggest a potentially inactive domain. For the second polyketide synthase, the reductase enzyme is predicted to perform two 2-electron reductions, creating an alcohol in the final metabolite. This work represents the first study characterizing these types of domains from an Apicomplexan parasite and lays the groundwork for future investigation into metabolite identity.

Investigating circadian rhythms and metabolism in mouse models of Alzheimer's Disease

Grace Qi
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Biological Sciences

The traditional view of Alzheimer's disease (AD) pathology has focused on the accumulation of abnormal proteins such as amyloid plaques and hyperphosphorylated tau throughout the brains of individuals with this disease. However, sleep wake patterns, commonly known as circadian rhythms, and metabolic pathways are also altered as part of the disease process. While not as noticeable as these overt and typical pathologies, novel factors like circadian rhythms and brain metabolism may provide new insights into the underlying pathology of AD and contribute to the development of novel therapeutics. These two factors are of interest since they precede the development of memory loss and cognitive decline. In studying these novel factors, we demonstrate that AD involves a widespread change in brain chemistry that is not limited to a single pathology.

Previous work has shown that alterations in the methionine metabolic pathway directly affect the methylation of protein phosphatases and kinases that control tau phosphorylation. Circadian rhythm disruptions are also associated with tau hyperphosphorylation in the context of hibernation and are often observed in AD patients who display increased wakefulness and excessive nocturnal activity. We used quantitative RT-PCR to analyze the changes in expression of genes involved in methionine metabolism, circadian rhythms, and microtubule maintenance in mouse models of AD. We also used western blots to determine and compare the levels of various kinases, methyltransferases, and microtubule-associated proteins in brains and retinas of AD vs. control mice. In addition, we focused on one novel protein in this pathway, radical s-adenosyl-l-methionine containing (RSAD1), a mitochondrial protein that is responsible for methylation within neurons and has been shown to be expressed at higher levels in brains of patients with AD when compared to cognitively normal controls. We found key differences in the expression patterns of RSAD1 and other proteins of interest in the suprachiasmatic nucleus of AD mice as compared to control mice. We also localized expression of RSAD1 within mouse retinas, though it is still unclear what the function of this protein within the visual system is. In sum, we demonstrate that circadian rhythms and metabolic pathways are altered in AD and may be useful targets for therapeutics.

Mapping Distinct Behaviors and Neural Ensembles Evoked by Social and Nonsocial Odor Cues

Brinda Raghavendra
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Biological Sciences

Dysregulated social and nonsocial behavior is a debilitating issue across a range of neuropsychiatric disorders, including anxiety disorders, major depression, and autism spectrum disorders. Reward processing and mesolimbic dopamine (DA) systems are commonly disrupted among these disorders and affective impairments can appear in both social and nonsocial forms of motivated behavior (e.g., social, eating behavior). However, it is largely unknown whether social motivation deficits are due to a generalized disruption of positive valence systems that control a range of motivated behaviors, or due to perturbations in more specialized social processing networks. Thus, a key question is how are social processing neurons intertwined with or embedded into positive valence systems? Previous studies have primarily focused on either social or nonsocial natural reward functions in single brain regions. Less is known about the functional circuitry that underlies this processing and whether subsets of neurons within social and positive valence systems are tuned towards specific types of motivational stimuli (e.g., social, nonsocial) within the VTA and its upstream regulators. Here we employ a novel behavioral paradigm to screen for naturally attractive stimuli within social and food domains. Once behavioral attraction is established, we utilize a genetic mouse line to permanently tag brain-wide neural activation in response to social versus nonsocial food odor cues. In combination with traditional Fos immunohistochemistry, we examine whether social and nonsocial ensembles are intertwined or separate within specific brains regions. Together, these and ongoing efforts will elucidate neural subcircuits that govern distinct forms of motivated behavior.

Evaluating NK Cell and Monocyte Contribution to ADCC elicited by Infant Candidate HIV Vaccines

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Biological Sciences

In this non-human primate (NHP) study, MIV06, we attempted to measure vaccine-elicited natural killer cell-mediated ADCC using samples collected from a candidate infant vaccine study recently performed using the infant macaque animal model. To test vaccine efficacy, vaccinated animals and control animals were challenged with SHIV-1. In the end, the vaccine was not effective in preventing acquisition, however there were differences in the number of challenges needed for individual animals to be infected, and in the peak viral load measured in the infected animals. This generated the questions: 1) Was there a difference in NK cell ADCC as contributing to the overall antibody response in animals that took longer to be infected compared to those that did not? and 2) Was there a relationship between NK cell ADCC and peak viral load after infection?

To answer this question, the overarching goal of this study was to measure the contribution of NK cells and monocytes to the ADCC response elicited by this vaccine. ADCC was measured by a flow-cytometry based assay, and the contribution of NK cells and monocytes was determined by applying a recently descripted analysis technique to these flow cytometry data sets known as area scaling analysis (ASA). Applying ASA to this dataset helped evaluate the individual contributions of NK cells and monocytes in the elimination of target cells. Such an analysis increased our understanding on how vaccine-elicited antibodies recruit different populations of effector cells and induce a more effective response against HIV or SIV infected cells to better design the next infant vaccine trial.

Density Dependent Control of Oyster Parasites by Generalist Consumers

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Biological Sciences

Parasites remain understudied within the context of trophic ecology, despite being shown to affect food web stability, interactions, and energy flow. Consumers similarly exert strong controls on their communities through consumptive effects (direct predation), and non-consumptive effects (physical and chemical cues). Here we examine the extent of consumer control over parasite-host interactions. Mud crabs (Panopeus herbstii) are abundant generalist consumers on oyster reefs which primarily feed on juvenile oysters. Their prey may also include Boonea impressa, small, ectoparasitic snails which feed on oysters in high densities (~ 20 individuals per oyster) and significantly decrease oyster filtration and growth. We administered chemical cues of mud crabs to oysters under three parasite loads and compared chlorophyll-a concentrations of water samples to measure filtration capacity. Oysters under low and medium (n=<10) parasite loads had higher chlorophyll-a concentrations (indicative of less filtration) after mud crab cues were administered. However, oysters under high (n=20) parasite loads had lower chlorophyll-a concentrations (indicative of more filtration) after mud crab cues were administered. Parasite feeding activity decreased across all cue treatments, and was significantly reduced at high loads after cues were administered. These results suggest that at lower parasite densities generalist crab cues increase oyster stress resulting in reduced filtration capacity, while at higher parasite densities crab cues reduce parasite feeding activity alleviating stress and increasing filtration capacity. Our results are illustrative of the role and strength of non-consumptive consumer effects in regulating communities, and the importance of species interactions in driving community disease ecology and health.

Characterizing the Mosquito Microbiome's Role in Regulating the Aryl Hydrocarbon Receptor

Daniel Ryan
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Biological Sciences

The Anopheles gambiae mosquito is Sub-Saharan Africa's primary vector for malaria, which remains a public health crisis in that region. While deployment of insecticides against mosquitoes has curbed the spread of malaria, increasingly widespread insecticide resistance in A. gambiae is a barrier to malaria eradication. One factor contributing to mosquito insecticide resistance is the A. gambiae aryl hydrocarbon receptor (AhR), an intracellular receptor and transcription factor. However, the source and identity of ligands that activate AhR remain unclear. In humans, AhR can bind catabolites of the amino acid tryptophan (trp-catabolites) produced by bacteria of the human microbiota. Hence, I hypothesized that the mosquito microbiota produces trp-catabolites that regulate the A. gambiae AhR. To assess whether the A. gambiae microbiota has the capacity for trp-catabolism, I bioinformatically searched the A. gambiae microbiome for genes for tryptophanase and phenyllactate dehydratase subunit C (FldC), which are trp-catabolizing enzymes. I found that tryptophanase genes were widespread amongst bacteria of the phyla Bacteroidetes and Fusobacteria, while the FldC gene was conserved in one genus belonging to Fusobacteria. To determine whether trp-catabolites are potential ligands for the A. gambiae AhR, I aimed to test the binding affinity of trp-catabolites for AhR through differential scanning fluorimetry but was unable to purify the protein to test its affinity for potential ligands. My work entails progress in identifying potential microbiota-produced ligands for AhR and provides groundwork for future binding studies to identify ligands of the A. gambiae AhR.

Sequence homology predicts E1-E2 ubiquitination interaction in the malaria parasite

Sabrina Sebastian-San Miguel
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Biological Sciences

The protozoan parasite *Plasmodium falciparum* is responsible for the deadliest form of malaria and is continually developing resistance to current drug therapies. Targeting the *Plasmodium* ubiquitin enzymatic machinery offers a novel avenue for disease control. Ubiquitin, a small 8 kDa protein, is attached to substrate proteins via the three-enzyme cascade of a ubiquitin-activating (E1), -conjugating (E2), and ligase (E3) enzyme. Ubiquitin attachment is a post-translational modification that serves as an important protein signal involved in various cellular processes such as protein degradation, DNA repair, and endocytosis, and is essential to malaria parasite survival within the human host. However, ubiquitination in malaria parasites, particularly in the context of drug targeting, is an underexplored area. Specifically, little is known about Lys 63-linked polyubiquitin (K63-Ub) in *Plasmodium falciparum*. The E2, PfUbc13, is key to mediating K63-Ub, but its enzymatic partners are unknown. It has been shown that HsUBA1 and HsUbc13 are E1-E2 partners, and studies have suggested that the E1, PfUBA1, transfers ubiquitin to PfUbc13. I sought to biochemically validate this interaction by performing ubiquitin transfer assays with PfUBA1 and the PfUbc13 homolog HsUbc13 (80% sequence similarity). Anti-ubiquitin western blots used to monitor ubiquitin transfer show that PfUBA1 transfers ubiquitin to HsUbc13 *in vitro*, further suggesting that PfUBA1 and PfUbc13 are enzyme partners. Additionally, treatment with the chemical probe NSC697923 resulted in dose-dependent inhibition of HsUbc13~ubiquitin conjugate formation, indicating that this E1-E2 activity can be chemically targeted. Moreover, concurrent work in the lab validated ubiquitin transfer between PfUBA1 and PfUbc13. These results provide important insight into the mediation of K63-Ub in the malaria parasite and demonstrate that sequence homology can be used as a predictive tool for E1-E2 interactions.

Assessing anti-B7-H3 antibody and gp70 cancer vaccine therapy for TNBC

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Biological Sciences

The combination of a tumor-targeting, immune-stimulating antibody and cancer vaccine has recently demonstrated compelling efficacy for HER2+ breast cancers in the clinic, achieving 100% disease-free survival for 8 years post-treatment (NCT00524277). We hypothesize that the efficacy of this combination stems from the ability of the antibody to augment vaccine-induced tumor antigen presentation to cytotoxic T lymphocytes (CTLs), a potent mechanism by which antitumor immunity is incited. Additionally, we hypothesize that this combination strategy provides therapeutic benefits for TNBC, an aggressive breast cancer subtype, if the regimen is adapted to target TNBC-specific antigens (i.e., B7-H3 and gp70). To this end, we first adapted NCT00524277's treatment components for murine preclinical studies; we created M-m276, a B7-H3-targeting, immune-stimulating antibody, and a gp70-targeting cancer vaccine. Next, we assessed M-m276's ability to promote cross-presentation and trogocytosis, two mechanisms by which antibody therapy could increase tumor antigen presentation to CTLs. Finally, we administered M-m276 with gp70 vaccine in B7-H3+ and B7-H3- models of the murine TNBC 4T1. We found that M-m276 drastically increases the trogocytosis of tumor membrane by antigen-presenting cells (APCs) in vitro, which enables these APCs to present greater amounts of tumor antigen to CTLs. Additionally, we found that M-m276 with cancer vaccine provides increased therapeutic benefits in vivo compared to antibody and vaccine alone when treating 4T1 that is B7-H3+, not B7-H3-.

Probing the role of PfUbc13 in antimalarial activity

Keying Sun
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Biological Sciences

Malaria, the most life-threatening infectious disease of the 21st century, continues to thwart its host and current medicine, causing 627,000 deaths worldwide in 2020. This global health burden is caused by protozoan parasites of the genus Plasmodium, of which the deadliest species is *P. falciparum*. Malaria parasites undergo a complex lifecycle where the sporozoite form is first deposited in the human host through the bite of an Anopheles mosquito. These parasites travel to the liver to undergo an obligatory developmental stage. Then, thousands of merozoites are released from the liver to invade red blood cells (RBCs). In the RBCs, the parasites asexually reproduce and develop into ring, trophozoite, and schizont forms. Schizonts cause the infected RBC to rupture, releasing merozoites into the blood stream to reinvade surrounding RBCs. This cyclical invasion of RBCs causes the clinical symptoms of malaria. Artemisinin-based combination therapies (ACTs) target the blood stage of the parasite and serve as the frontline antimalarial treatment. However, the emergence of drug resistance threatens the efficacy of ACTs. Thus, there is an urgent need to better understand the mechanism of action of ACTs and to identify novel drug targets to combat malaria drug resistance. Potential, underexplored drug targets in *P. falciparum* are the enzymes that mediate ubiquitin attachment to substrate proteins. Interestingly, previous studies have shown that deletion of the ubiquitin-conjugating enzyme PfUbc13 increases parasite sensitivity to dihydroartemisinin (DHA), suggesting that PfUbc13 has a role in ACT antimalarial activity. PfUbc13 is an essential gene and a central mediator of Lys63-linked polyubiquitination (K63-Ub) of substrate proteins, which also implicates this protein modification in ACT activity. To probe the role of PfUbc13 in the DHA mechanism of action, the EC¬50 values of DHA and the PfUbc13 probe NSC697923 against blood stage parasites were determined to be 0.17 ± 0.04 nM and 6.8 ±0.4 µM, respectively. These values will be used in future studies to evaluate the synergistic relationship between DHA and NSC697923. Moreover, treatment of blood stage parasites with NSC697923 resulted in decreased K63-Ub levels compared to untreated parasites. Overall, these results lay the groundwork for additional investigations that will further probe the role of PfUbc13 and K63-Ub in the antimalarial mechanism of ACTs.

Characterizing an in vivo Mice Model for Studying Senescence in Hepatocytes

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Biological Sciences

Senescence is a stress-induced durable cell cycle arrest and increases with aging. Researchers suggested that the accumulation of senescent cells in tissues such as white adipose, pancreas and liver contribute to the progression of non-alcoholic fatty liver disease (NAFLD). Previous studies about senescence in the liver have generated models by overexpressing or deleting senescence-related genes in the whole organ. Although some of this published data appear to be contradictory, this discrepancy in results could be addressed by targeting each specific liver cell type separately. Considering that the impact of senescence specifically on hepatocyte function remains unclear, we constructed an in vivo senescence mice model by overexpressing CDKN2A (P16) specifically in Hepatocytes. To do that the transgene construct AAV8-TBG-eGFP-P16 or its control, AAV8-TBG-eGFP, were administered in C57BL/6 wild-type mice through tail vein injection. One week after the AAV injection, blood and liver tissue were harvested and molecular analysis were performed. We confirmed by GFP immunohistochemistry (IHC) that the vast majority of hepatocytes were infected one week after the injection. P16 mRNA and protein levels were significantly increased in the P16 group compared to its control. RNA-sequencing analysis confirmed that senescence-related pathways were significantly upregulated in our model, suggesting that we successfully induced senescence in hepatocytes. Next, to understand how Hepatocyte senescence affects liver regeneration, we performed 70% partial hepatectomy (PH)one week after administering the AAV. Liver mass recovery after PH was significantly delayed in the P16 animals, suggesting an impaired regenerative capacity. RNA seq analysis revealed significant up-regulation of TNFa signaling via NF-kB, unfolded protein response, UV response, and hypoxia pathways, and down-regulation of peroxisome, bile acid metabolism, beta-catenin signaling, myogenesis and mitotic spindle pathways in the P16 group. Thus, livers that were excessively populated by senescent hepatocytes at the time of PH developed increased cellular stress, dysregulated metabolism and reduced proliferative activity after PH. We plan to further leverage our model to improve molecular understanding of how hepatocyte senescence impacts recovery from NAFLD and other types of liver injury that increase hepatocyte senescence.

Mapping Drosophila pigmentation mutations: su(b) phenotype

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Biological Sciences

The pathway of creating pigment is important and complex across a wide evolutionary scale. Drosophila pigmentation uses melanin as its primary pigment and two types of sclerotin contribute as well. All of which are created via a shared biosynthetic pathway. This shared pathway contains mutations and genes dating back to the early 20th century with names like yellow, speck, ebony, and black. In 1981, Allen F. Sherald screened for mutations that suppressed the black phenotype in Drosophila and named them "suppressor of black", or sub(b). The black gene converts L-aspartic acid into beta alanine which is used as a substrate by the ebony protein to make NBAD sclerotin. Mutants in ebony or black do not make this type of sclerotin, and the unused dopamine is sent into melanin biosynthesis resulting in the dark body color. In this research project, we will isolate su(b) and genetically map it through genetic complementation and identify the genomic lesion by DNA sequencing. Additionally, we will determine how Beta-alanine metabolism can be affected through alanine feeding experiments and quantitatively characterize the su(b) phenotype.

Characterizing Mechanisms of Innate Immunity: Role of GBPs in Defending Against F. philomiragia

Arthi Vaidyanathan
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Biological Sciences

Understanding the mechanisms through which the innate immune system fights against bacteria contributes to our fundamental knowledge of the pathology of infection. While guanylate binding proteins (GBPs) have been known to play an important role in host defense to bacterial, viral, and protozoan pathogens for some time, their specific methods of action are still contested. To further explore the role of GBPs, the bacterial genus of Francisella was selected due to some known species requiring GBPs to produce a sufficient immune defense. The most well-known species of this genus, F. tularensis, is extremely deadly and seems to best all immune responses it encounters in humans and mice. Less virulent F. novicida has been shown to require GBPs to trigger a pyroptotic cell-death response, killing both host cell and bacteria to protect the organism. Finally, F. philomirgia, the most harmless of the 3 species, has yet to be characterized in relation to its GBP dependencies. The primary goal of this study is to utilize 2 strains of F. philomiragia (Muskrat and CGD) as a probe to explore the pathways through which GBPs defend against intracellular pathogens in macrophages. Through in-vitro infections of murine macrophages and observing the changes in bacterial burdens of the cells over time, I determined that GBPs are required in the killing of F. philomiragia Muskrat, but not F. philomiragia CGD. For F. philomiragia Muskrat, results show that within the GBP cluster, GBP2 specifically is required for functionality. Furthermore, preliminary mechanistic analyses demonstrate that F. philomiragia Muskrat does not require the same pyroptosis triggering sensors as cousin F. novicida. Hence, a key difference is identified in the downstream action of GBP between the species. Continued analysis shows that reactive oxygen species are also not involved downstream of GBPs in the defense against F. philomiragia Muskrat and conclusions regarding the role of autophagy, the cell's way of recycling, are unclear from my results. Further study exploring if GBPs directly kill F. philomiragia Muskrat bacteria, recruit autophagy proteins, or serve another yet uncharacterized role would contribute valuable insight into the nuances and structure of the body's immune response to bacterial infection.

Serological Profiling of COVID-19 at the Point-of-Care from Blood

Simone Wall
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Biological Sciences

Highly sensitive, specific, and point-of-care (POC) serological assays are essential to manage the COVID-19 pandemic. Serological assays, which detect antibodies (Abs) against SARS-CoV-2, can provide information about past SARS-CoV-2 infection and assess patient humoral response. While many assays have been developed to detect anti-SARS-CoV-2 Abs, most rely on centralized laboratories to conduct the assay or are not quantitative, limiting their applicability for widespread use. In this study, we developed a microfluidic serological assay built upon a "nonfouling" polymer brush coating, enabling sensitive detection directly from undiluted biological samples. This multiplexed assay detects Abs against nucleocapsid, spike S1, and spike receptor-binding domain. To deploy this assay at the POC, we developed a self-contained, automated microfluidic flow cell that only requires the addition of the sample and a drop of wash buffer to run for 1 hour. Next, the flow cells are imaged with an inexpensive, handheld fluorescent detector. The fluorescent intensity of the capture antigens scales with Ab concentration. We first validated that the assay was responsive and specific to anti-SARS-CoV-2 recombinant Abs spiked into undiluted pooled human serum. Next, we tested 34 COVID-19 positive, 41 negative, and 18 samples from patients with other coronavirus infections to assess clinical performance. Our test was able to detect all positive samples 2 weeks after symptom onset and achieved a specificity of 100%. Using our assay, we were also able to track seroconversion in six individual patients. Additionally, we found that our assay was concordant with a live virus microneutralization assay. Finally, we conducted a proof-of-concept test at the POC on whole blood samples. No coagulation of blood in the channels was observed and the assay accurately identified both negative and positive samples with 100% sensitivity and specificity. Collectively, these results demonstrate that our assay can detect Abs directly from convalescent COVID-19 patient whole blood at the POC with high sensitivity and specificity. Our quantitative, portable, user-friendly platform can be a valuable tool to manage the COVID-19 pandemic. Our test could be used at the individual-level to assess Ab dynamics over time from natural infections or after immunization. Further, because of the high sensitivity and specificity, it could be used at the population-level to perform epidemiological studies.

Caspases 3 and 7 Suppress mtRNA-Dependent MAVS Activation During Apoptosis

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Biological Sciences

Many traditional chemo and targeted therapy treatments have also been shown to enhance anti-tumor outcomes through the activation of the adaptive immune system. This occurs through the release of defined immunogenic factors during death that can recruit and activate the host immune system. Mitochondrial nucleic acids are one capable endogenous ligand for cytosolic innate immune sensor activation and induction of these immunogenic factors, including Type I Interferons (IFNs). IFNs play a role in the activation of antigen-presenting cells and recruitment of CD8+ T-cells to the site of release. To avoid unwanted inflammation during cell death, apoptotic caspases prevent mitochondrial nucleic acid recognition through proteolytic inhibition of innate immune sensor proteins. Interestingly, restricting caspase activity during apoptosis has been utilized as a cancer immunotherapeutic tool by allowing for mitochondrial DNA (mtDNA) recognition via the cGAS/STING cytosolic DNA sensor pathway. Previous work has shown that inhibition of caspase 9 (an initiator caspase) paired with radiation can release mtDNA that is recognized by cGAS to produce IFNs and significantly improve tumor progression in mouse models. Based on these findings, our group sought to investigate if the recently discovered endogenous ligand of mitochondrial RNA (mtRNA) would stimulate an anti-tumor immune response during caspase-independent cell death (CICD). Using CRISPR/Cas9 gene-editing technology, we show that caspases 3 and 7 act redundantly to suppress mtRNA cytosolic sensing pathways during apoptosis. Caspase-inhibition paired with cytotoxic therapies leads to mitochondrial outer-membrane permeabilization (MOMP) and the spillage of mtRNA into the cytosol that is then recognized by pattern recognition receptors to initiate the production of IFNs and immune cytokines in vitro, as well as a host immune response in vivo. Our work provides the first evidence for mtRNA as an immune stimulatory endogenous ligand in cancer cells through the MAVS-TBK1-IRF3 signaling axis.

The role of the TOMM40 poly-T variant on APOE-TOMM40 gene cluster regulation in humanized mice

Angela Wei
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Biological Sciences

Genome-wide association studies (GWAS) have consistently implicated polymorphisms in the human TOMM40-APOE genomic region to be associated with late-onset Alzheimer's disease (LOAD). While the apolipoprotein e4 (APOE e4) allele is the strongest and most reproducible genetic risk factor for LOAD, sequence variants in the TOMM40 '523' poly-T region have also been suggested to contribute to LOAD risk, independent of their proximity to APOE. However, the functional roles of these elements in LOAD pathology, as well as the molecular mechanisms underlying LOAD at large, remain unknown, underscoring the need for a physiologically relevant modeling system able to capture all features of the disease. Towards this end, this study employs a mouse model humanized via targeted replacement for the TOMM40-APOE genomic region and their promoter regions to investigate the impact of TOMM40 poly-T length on APOE and TOMM40 expression within the liver and brain tissue, along with their possible interactions in LOAD pathogenesis. Most significantly, factoring for mice's sex, age, and variant length yielded different regulatory patterns in brain and liver tissue, signaling the importance of considering these variables when refining the effect of TOMM40 poly-T variant length. Critically, this is the first study using humanized mouse models for the TOMM40-APOE region to study the TOMM40 poly-T, both addressing the fundamental flaw of species differences for in vivo mouse models while retaining the physiological relevance provided by animal models' organ systems.

Bass Connections Project

RNA-seq analysis of mRNA export from the nucleus

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Biological Sciences

mRNAs that are exported from the nucleus are thought to first enter the cytosol to be translated. It is also thought that the mRNA that code for secretory and membrane proteins are then localized from the cytosol to the endoplasmic reticulum through the signal recognition particle (SRP) pathway. However, using a CRISPR/Cas9 gene editing strategy, the Nicchitta Lab recently discovered that mRNAs localize to the ER in gene-edited HeLa or HEK293 cells lacking two key gene products of the SRP pathway: the a and b subunits of the SRP receptor. They also observed that newly exported mRNAs predominantly localize to the ER and not the cytosol. The identity of the SRP-independent mRNA localization pathway is currently unknown. To determine if an alternative pathway was up-regulated following disruption of the SRP pathway, RNA-seq analyses were performed on the parental Cas9 cell lines, SRPRB knockout cell lines, non-targeting siRNA-transfected cell lines, and lastly SRPRB knockout cell lines transfected with an siRNA targeting SRPRA. My project's goal is to determine whether there is evidence of adaptation in SRP KO. To do this, I will process RNA-seq data sets noted above via the Basepair informatics pipeline using the differential gene analysis tool DSeq2 and examine for up- and down-regulated genes. In addition, I will perform GO analysis of up- and down-regulated gene lists to further examine for evidence of adaptation to the KO/KD genotype. Adaptation, if it is occurring, would be apparent through significant up or down-regulation of genes whose products can be linked to aspects of RNA biology. Currently, the RNAseq analysis and DSeq2 studies are complete and from these data, and we have identified modest changes in gene transcription in the SRPR KO condition.

Exploring the immune response in obesity-associated colorectal cancer

Catherine Yao
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Biological Sciences

Longitudinal epidemiologic data reveals growing incidence of diet-based obesity and colorectal cancer (CRC). However, the regulatory mechanism behind pro-obesity high-fat diets (HFDs) on increased CRC risk are not well understood. Research has shown that HFD-induced obesity augments the population of Lgr5+ intestinal stem cells (ISCs). Further research revealed that when Lgr5+ISCs are co-cultured with T-helper (Th) cells, they may act as non-conventional antigen-presenting cells that influence intestinal epithelial cell differentiation and fate. Thus, this ISC-Th axis may play a critical role in shaping the immune microenvironment of colon cancer. We hypothesize that antigen presentation by Lgr5+ cancer ISCs is impaired in obesity. To measure and compare T cell recruitment in lean versus obese murine tumors, we performed immunohistochemistry to stain CD3+, CD4+, and CD8+ T cells in intestinal crypts where ISCs lie. To measure and compare the prevalent MHC-II gene expression of H2AB1 in lean versus obese murine tumors, we performed in situ hybridization to probe the RNA of H2AB1 in intestinal crypts. For in vitro studies, we injected lean versus obese mice with sham or toll-like receptor agonist treatments; we measured and compared the gene expression of MHC-II genes (H2Aa, H2AB1, Ciita), anti-microbial response/inflammation genes (Reg3g, Nfk2b), and co-stimulation genes (IcosI, Sectm1a, Sectm1b). We expect Lgr5+ ISCs to interact with and activate Th cells in an antigen-specific manner. These findings will help our broad understanding of the immune surveillance in obesity-associated colorectal cancer, which may hold therapeutic potential.

Investigating Substrate Specificity of Ramoplanin Family Fatty Acyl-AMP Ligases

Jeffrey Zheng
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Biological Sciences

Ramoplanin is a non-ribosomally synthesized peptide with broad-spectrum antibiotic activity. However, ramoplanin suffers from low solubility and poor tolerance in the bloodstream which previous studies have attributed to length and structure of the fatty acid tail. Hence, modifications to the lipid tail can be used to alleviate many of these shortcomings. Within the biosynthetic machinery of ramoplanin, fatty acyl-AMP ligases (FAALs) are the enzymes responsible for activation of the fatty acids. Following activation, the acyl-AMP is then attached to an acyl carrier protein (ACP) for subsequent acylation to the peptide. Holistic understanding of the biosynthesis pathway could enable creation of mutant strains with modified fatty acid tails for improved solubility and tolerance. Thus, specificity of FAAL active sites for non-native fatty acids was analyzed to understand the mechanism that controls fatty acid activation. To characterize the FAALs, computational modeling, FAAL activation assays, FAAL/ACP acylation assays, and generation of FAAL inhibitors were utilized. In silico models observed that the protein favorably bound fatty acids both longer and shorter than the native length. However, experiments indicated that binding affinities were lower for substrates with branching. In contrast, in vitro experiments demonstrated that FAAL activity for branched fatty acids were increased relative to their straight chain counterparts. Additionally, FAAL activity increased when activating fatty acids up to three carbons longer than the native length substrate while shorter fatty acids reduced activity. When incubated with ACP, acylation of the ACP was limited to mid-length fatty acids and controlled by the identity of the ACP. These results inspired the synthesis of an acyl-AMS mimetic designed to inhibit the FAAL machinery. Thus, these results enable further work to be carried out in obtaining crystal structures and modifying the biosynthetic machinery for new ramoplanin derivatives.

Creative Arts

Rural Medicine: A Photographic Depiction

Aaron Zhao
Faculty Mentor(s): Jules Odendahl-James
Creative Arts

Rural America is disproportionately burdened with worse health outcomes, more chronic illnesses, and fewer doctors to treat such maladies. One in five Americans have diabetes in Navajo Nation, 82% of rural Mississippi requires medication for chronic hypertension, and more young doctors are choosing to leave rural countryside practices for larger metropolitan cities. With approximately 4 million North Carolinians (40%) living in rural communities, these medical issues hit the state of North Carolina harder than many other states. W. Eugene Smith's "Country Doctor" photo series masterfully depicts the life of a mid-20th century country doctor in the Rocky Mountains. The lurid photographs are intimate yet respectful, providing the viewer with a glimpse into the unvarnished world of a medical doctor. Doctors were forced to be frugal with resources and flexible with their responsibilities. Many aspects of rural medicine have changed since then, but which values still hold true today? To fully understand the state of rural medicine in America, one must first see rural medicine from the perspective of a rural doctor. This visual art presentation aims to viscerally capture the state of medicine and health care in six rural North Carolina towns. Accompanied by text collated from interviews of rural physicians and patients, this collection of artistic-documentary photographs depicts the unforeseen triumphs and challenges of practicing 21st century rural medicine in North Carolina.

Health / Clinical Research

Uncovering the imprint of chronic disease: Perspectives from rheumatoid arthritis patients

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Health / Clinical Research

Rheumatoid arthritis (RA) patients face psychological hardship due to physical discomfort, disabilities, and anxieties. Previous research indicated a bidirectional relationship and patient desire for emotional support from providers. This study examined lesser-understood RA experiences across the psychological and social contexts in relation to self-perception through the patients' expression of their struggles with these burdens. We conducted four semistructured focus groups and eleven interviews (total n = 31). A codebook was developed and refined through iterative transcript coding via NVivo-12. Four emerging themes were identified by inductive, thematic analysis: (1) the patients' healthy appearances were a myth, with subthemes revealing a conflict between an inclination to hide the disease and a desire for validation, while feeling embarrassed by symptom manifestations and disappointment at withdrawal from social interactions; (2) an identity crisis due to diminished functionality, autonomy, and sense of self; (3) RA constantly occupied the mind, as its unpredictability dictated daily schedules and altered plans; and (4) the disease's chronic nature influenced personal outlook to worry about or accept the uncertainty. Even with effective treatment, the invisibility of the disease, the fear and anticipation of flare-ups, and identity clashes caused emotional distress. The insights offer a different perspective on personalized medicine, complementing clinical treatments based on genetic or biomarker profile. For patient-centered holistic care, education is needed to prompt both patients and providers to discuss psychological issues for more customized, integrated interventions. The findings can help inform healthcare teams and families in recognizing and supporting these physical-psychological intertwined experiences, thereby ameliorating patients' wellbeing.

Low-Cost Gastroschisis Silo for Sub-Saharan Africa: Testing the safety profile in porcine model

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Co-Author(s): Caroline Salzman
Health / Clinical Research

Gastroschisis mortality in sub-Saharan Africa remains high at 59-100%. Silo inaccessibility contributes to this extreme disparity, as standard of care (SOC) silos cost $280, and most median family incomes in sub-Saharan Africa are <$200 per month. Our team of surgeons, engineers, and neonatologists from the U.S. and Uganda have previously described design and bench-testing of a low-cost (LC) silo that costs <$5 and is constructed from a urine collection bag and female condom ring, which are locally available in sub-Saharan Africa. Here we describe in vivo testing of the LC silos. A piglet model of gastroschisis was achieved by eviscerating the intestines through a 2cm midline abdominal wall incision. The bowel was placed into either a LC or SOC silo, maintained for one hour and then reduced. Procedure times for placement, intestinal reduction and silo removal were recorded. Tissue injury was assessed by protocol-blinded histological examination of the abdominal wall and intestine. Quantitative assessment of bacterial and fungal growth on the silos were performed using standard culture techniques. Data were analyzed using the Mann-Whitney test (alpha=0.05). Six piglets (2.0-2.6 Kg) were block randomized to LC (n=3) or SOC (n=3) silo application. There was no visible gross injury to abdominal wall or intestine specimens in either the LC or SOC silos. Concurrently, there was no difference in minor bleeding (bruising) between the LC and SOC silos (Table 1). The differences in silo application times (LC 42 ± 48 seconds; SOC 47 ± 18 seconds; p=1.0), bowel reduction times (LC 6 ± 3.5 seconds, SOC 1 ± 2 seconds; p=0.40) and silo removal times (LC 2 ± 0.5 seconds, SOC 3 ± 1.5 second; p=0.40) between groups were not statistically or clinically significant, indicating similar ease of use. Microbiology analysis revealed bacterial growth on all samples, but the bacterial density was below the standard inoculum in vitro acceptability of 10^5 CFU/g for both the LC and SOC silos (Fig 1). There was no statistically significant difference in bacterial or fungal growth between the LC and SOC silos (anaerobic p=0.34; aerobic p=0.06; fungal p=0.80). In conclusion, a low-cost silo, designed for manufacturing and clinical use in sub-Saharan Africa, demonstrated similar ease of use, absence of tissue injury, and acceptable microbiology profile similar to SOC silos. Data from this porcine model will allow our team to proceed with a pilot clinical study in Uganda.

Pregnancy-Associated Breast Cancer: A Retrospective, Single-institution Case Series

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Health / Clinical Research

Pregnancy-associated breast cancer (PABC) is associated with worse survival vs non-PABC. Given advances in systemic therapy and recent efforts to de-escalate morbid locoregional treatment, we sought to examine presentation and management of PABC in a contemporary cohort of women. We identified women≥18 years old diagnosed with Stage 0-IV breast cancer during pregnancy or within one year of delivery between 2014 and 2020 in our institutional database. Patient demographics, tumor data, and treatment are reported. 15 patients were included in the study (median age 36 years, range 23-47). Most patients were White (53.3%, n=8), and smaller proportions were Black (33.3%, n=5) and Asian (13.3%, n=2). Of the 15 patients reviewed, 11 (73.3%) were privately insured, and 4 (26.7%) had government-issued insurance (i.e., Medicaid or TriCare). The vast majority of patients were diagnosed with invasive ductal carcinoma (93.3%, n=14). None had metastatic disease (M1) at presentation but, notably more than half (53.3%, N=8) were diagnosed with HER2+ breast cancer. 14 of 15 patients received breast surgery, and the only patient who did not had her scheduled surgery canceled due to an issue with her insurance. 71.4% (n=10) of those who received surgery ultimately elected for mastectomy. Notably, all patients in our cohort received chemotherapy; 53.3% (n=8) received endocrine therapy, and 66.7% (n=10) received radiation therapy. Two of the 15 patients died within 20 months of their diagnoses following progression to metastatic disease; both had triple-negative breast cancer. Our results indicate a high prevalence of locally advanced and biologically aggressive disease. Further research is needed to better understand the most effective treatment options for patients who are diagnosed with breast cancer while pregnant or postpartum.

Exploring the Role of G Protein-Coupled Receptor Kinase 3 in Paget's Disease of Bone

Arindam Ghosh
Faculty Mentor(s): Teresa Tarrant and Rishi Rampersad
Health / Clinical Research

Paget's Disease of Bone (PDB) typically afflicts patients above the age of 55 and presents itself with various clinical symptoms, such as bowing of the legs, increased fractures, or bone pain. Mechanistically, this is caused by an imbalance between osteoclast and osteoblast activity. Multinucleated osteoclasts are derived from macrophages and are responsible for breaking down bone, while osteoblasts help build new bone. In PDB, osteoclast overactivity alters bone remodeling homeostasis and forces osteoblasts to compensate by laying down bone in a disorganized manner, creating a woven appearance of bone and reducing the strength of bone. The Tarrant Lab is currently studying a G Protein Receptor Protein Kinase 3 deficient (Grk3-/-) mouse as a potential animal model of human PDB. Preliminary data from the Tarrant lab revealed that aged Grk3-/- female mice had lesions in their femurs that were similar to those of human PDB. We hypothesize that both genders will be equally affected. To evaluate this, we performed microcomputed tomography on the femurs of male and female aged (22 months) Grk3-/- to identify potential lesions, which appear as small holes in cortical bone. After images were obtained, we fixed and embedded the femurs in paraffin, sectioned them with a microtome into five micron sections, and stained for tartrate resistant alkaline phosphatase (TRAP), which specifically identifies osteoclasts that are prevalent in PDB lesions. Analysis of femurs confirmed that Grk3-/- male and female mice developed lesions resembling those found in PDB. 34 of the 38 femurs analyzed contained lesions. By gender, prevalence was 89% in femurs from female mice and 90% in femurs from male mice. Thus, the Grk3-/- mouse model seems to replicate facets of human PDB, and gender did not significantly impact penetrance. Dovetailing with our exploration of Grk3 expression in different cell types and differentiation of Grk3 deficient osteoclast precursors, this data suggests that Grk3 deserves further study in the context of PDB.

Facilitating Flourishing for Youth w/IDD in Healthcare Transitions Using Human-Centered Design

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Co-Authors: Cameron Love, Riddhi Patel and Rashi Wadhwani
Health / Clinical Research

Transitions of care to adulthood are especially challenging for youth with intellectual and developmental disabilities (IDD) and their families. Existing guidelines for transitions to adulthood are not tailored to meet the needs of youth with IDD. Understanding system-level gaps that impact patients and result in inadequate transitions for youth with IDD can be addressed by employing a human-centered design (HCD) approach that invites individuals who are directly affected to participate at every stage of a structured problem-solving process. The objective of this study is to describe how we applied HCD methods to explore how health systems can facilitate flourishing for youth with IDD during transitions to adulthood. This project is part of a broader, multidisciplinary initiative called the Open Design Studio which aims to promote flourishing across the Durham community. Our work to-date has focused on empathizing and understanding, two stages foundational to the HCD process. To facilitate these HCD stages, we applied qualitative descriptive methods to conduct and analyze semi-structured interviews. Using snowball sampling, we recruited and interviewed 20 key informants— 8 family members/caregivers of youth with IDD, 7 community partners, 1 student advocate, 10 health professionals, and 3 individuals with IDD. Each 45-60 minute interview was conducted virtually via Zoom. Detailed notes were taken and interviews were recorded and transcribed. We applied thematic content analysis to identify key themes from interviews. We organized interview data using Mural, a virtual brainstorming platform for HCD teams. Five themes emerged from key informant interviews: healthcare navigation, gaps between healthcare and non-healthcare settings, communication, access to resources and acknowledgement of socioeconomic factors, and cultural understanding (Table 1). HCD methods were successfully implemented to identify recommendations about how to facilitate flourishing through healthcare transitions for youth with IDD. We will use these findings to co-design potential solutions with stakeholders during subsequent ideating and prototyping HCD stages. HCD offers a promising approach to design better systems and support flourishing for youth with IDD and their families during health transitions.

Bass Connections Project

Association between CMS Price Transparency Compliance and Hospital Characteristics

Xinshi Ma

Faculty Mentor(s): Yuqi Zhang and Marcelo Cerullo

Health / Clinical Research

Research Objective: Hospitals' compliance with the Centers for Medicare and Medicaid Services' 2021 price transparency regulations vary considerably. However, the rationale behind the decision to not disclose price reports remains unclear. Our study aims to examine how federal funding and financial performance characteristics are associated with compliance.

Study Design: We used logistic regression with state fixed effects to examine the association between compliance and financial performance controlling for teaching status, ownership type, and volume-adjusted CARES Act funding. Stratified analyses by ownership type were also conducted.

Population Studied: Of 4,525 short term acute care hospitals with 2019 HCRIS filings, 3,329 received CARES Act payments. 3,287 hospitals were ultimately analyzed after excluding outliers (total margins < -100% and CARES Act Payment per Patient Days > $10,000 [or >3 standard deviations]).

Principal Findings: Overall compliance among hospitals was, 62.5% (n= 2,055). After adjustment, the likelihood of compliance was lower among non-profit (odds ratio [OR] 0.58, 95% CI: 0.47-0.72; p<0.001) and government (OR 0.79, 95% CI: 0.61-1.03; p=0.004) hospitals, compared to for-profit hospitals. Holding employee FTEs constant, higher margins were associated with lower probability of compliance: for a 100% increase in total margins, probability of compliance decreased by 24.5 percentage points (PP) (95% confidence interval [CI] -0.40 to -0.09; p=0.002) among all hospitals, decreased by 31.7 PP (95% CI: -0.60 to -0.03; p=0.029) among for-profit hospitals, and decreased by 21.8 PP (95% CI: -0.43 to -0.01; p=0.040) among non-profit hospitals. Teaching status was associated with greater likelihood of compliance among government hospitals (OR 2.37, 95% CI: 1.18-4.48; p=0.014) but not among non-profit or for-profit hospitals. Higher CARES Act payment per patient days was associated with higher probability of compliance among for-profit hospitals only (3.20 PP for each dollar per patient days, 95% CI: 0.008-0.057; p=0.008).

Conclusions: There is a strong association between higher margins and lower compliance with CMS price transparency regulations among non-profit and for-profit hospitals. However, the association between compliance and the level of federal funding is unclear. Finally, accounting for hospital financial status, for-profit hospitals are more compliant than non-profit and government hospitals.

Bass Connections Project

Hospital Characteristics Associated with Observed Transcatheter Aortic Valve Replacement Prices

Roni Ochakovski

Faculty Mentor(s): Yuqi Zhang and Marcelo Cerullo

Health / Clinical Research

Transcatheter aortic valve replacement (TAVR) prices vary considerably. To address price transparency, the Center for Medicare and Medicaid Services (CMS) recently required hospitals to post "accessible pricing information" for shoppable procedures. Our study aims to describe the variation in TAVR prices as a factor of hospital financial performance among hospitals ranked by The U.S. News and World Report (USNWR). Payer-specific prices for TAVR were extracted from the Turquoise Health Price Transparency Dataset and linked to each hospital's Medicare cost report using the 2019 Hospital Cost Report Information System to determine facility-specific profit margins, markup values, bed days available, amount of government appropriations received, and total hospital capital. A modified two-part model was used to assign an "expected" TAVR price at the hospital level, adjusted for each hospital's financial and operational characteristics as listed above. Using the observed median TAVR prices for each hospital (calculated from all listed payer prices), an observed-to-expected (O:E) TAVR price ratio was calculated for each hospital. Hospitals were then categorized into quintiles of their O:E ratio. Seven hospital characteristics (USNWR TAVR performance scores, median all-payer within-hospital TAVR price, net hospital profit margin, hospital markups [i.e., charge-to-cost ratio], bed days available, and CMS wage index) were examined across O:E ratio quintiles using the Wilcoxon Rank Sum Test. The within-hospital variation of TAVR price (given by interquartile range [IQR]) was examined using weighted least squared regression. 640 TAVR-performing hospitals ranked by USNWR were studied. Overall, price disclosure was 48.6% (n=311). Between the lowest and highest O:E ratio quintiles, median hospital markup (4.75 vs 5.33;p=0.41) and median net hospital margin (1.76 vs 3.15;p=0.12) were not statistically significant. The highest O:E ratio quintile had lower median TAVR prices compared to the lowest O:E ratio quintile ($72,129.12 vs $49,022.03;p<0.001). Most significantly, TAVR price IQR's within hospitals had a linear decline from the lowest to the highest O:E ratio quintiles ($119,043 vs $27,240;p<0.001). USNWR ranking scores had no significant variation across the quintiles (p=0.95). We found that hospitals that charge more than expected for TAVRs do not have higher profit margins nor markups and are not higher ranked by USNWR as those that charge less than expected.

Bass Connections Project

LowCostomy

Joanna Peng
Faculty Mentor(s): Ann Saterbak, Dorothy Dow and Amy Barto
Health / Clinical Research

There is an immense need for low-cost colostomy appliances among ostomy patients living in low-income countries in Sub-Saharan Africa. There are an estimated 1.2 million new cases globally of colorectal cancer annually (1). Additionally, instances of colorectal cancer and the need for colostomies is increasing in Sub-Saharan Africa at an annual rate of up to 6.8% in certain countries (2). Without access to colostomy products, low-income patients often resort to handmade solutions, such as taping a plastic bag to one's stoma. However, these makeshift solutions are not safe, odor-proof, or leak-proof, which lead to a decreased quality of life for these individuals. To combat this problem, we have developed LowCostomy: a highly affordable (<5 cent) colostomy appliance that capitalizes on utilizing low-cost, recycled materials in addition to a novel, all-natural formula of beeswax and pine resin that replaces the expensive adhesive material found in traditional adhesives for colostomy appliances. Existing skin-care products that separately contain beeswax and pine resin have been proven to be safe, non-toxic, and non-corrosive (7, 8,9, 10); however, there are still potential risks for rash, irritation, infection and other topical reactions upon the combination of these two substances. The purpose of this study is to investigate the safety of a beeswax-pine resin mixture as it acts as a buffer between the patient's peristomal skin and the colostomy appliance. Rather than testing the entire LowCostomy system, this study will focus solely on the safety of the beeswax-pine resin buffer. The effect of having the beeswax-pine resin buffer adhered to the skin on the abdomen over a one-week period will be analyzed independently by two separate licensed physicians, with an emphasize on assessing changes in color, odor, texture, and overall skin health. Subject surveys will also be utilized to record subject experiences and will include quantitative sections like the 5-D itch scale. Compiled results will be analyzed by an accredited statistician.

Deep Learning Based Cervical Cancer Detection

Megan Richards
Faculty Mentor(s): Nimmi Ramanujam
Health / Clinical Research

Cervical cancer results in over 300,000 deaths annually, with the majority occurring in countries with low or middle Human Development Indices (HDI). For cervical cancer screening in low-HDI countries, the World Health Organization recommends a “see-and-treat” paradigm, in which a diagnosis is immediately made upon visualization of the cervix and application of a liquid contrast agent to highlight any potential cervical lesions. This paradigm faces limitations due to the low prevalence of trained professionals along with diagnostic variability in provider visual interpretations, motivating efforts to use machine learning to classify cervical images.. In this work, I evaluate several deep learning approaches in a global cervical cancer dataset and evaluating the robustness and interpretability of these methods.

Low-Cost Gastroschisis Silo for Sub-Saharan Africa: Testing the safety profile in porcine model

Caroline Salzman
Faculty Mentor(s): Ann Saterbak
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Health / Clinical Research

Gastroschisis mortality in sub-Saharan Africa remains high at 59-100%. Silo inaccessibility contributes to this extreme disparity, as standard of care (SOC) silos cost $280, and most median family incomes in sub-Saharan Africa are <$200 per month. Our team of surgeons, engineers, and neonatologists from the U.S. and Uganda have previously described design and bench-testing of a low-cost (LC) silo that costs <$5 and is constructed from a urine collection bag and female condom ring, which are locally available in sub-Saharan Africa. Here we describe in vivo testing of the LC silos. A piglet model of gastroschisis was achieved by eviscerating the intestines through a 2cm midline abdominal wall incision. The bowel was placed into either a LC or SOC silo, maintained for one hour and then reduced. Procedure times for placement, intestinal reduction and silo removal were recorded. Tissue injury was assessed by protocol-blinded histological examination of the abdominal wall and intestine. Quantitative assessment of bacterial and fungal growth on the silos were performed using standard culture techniques. Data were analyzed using the Mann-Whitney test (alpha=0.05). Six piglets (2.0-2.6 Kg) were block randomized to LC (n=3) or SOC (n=3) silo application. There was no visible gross injury to abdominal wall or intestine specimens in either the LC or SOC silos. Concurrently, there was no difference in minor bleeding (bruising) between the LC and SOC silos (Table 1). The differences in silo application times (LC 42 ± 48 seconds; SOC 47 ± 18 seconds; p=1.0), bowel reduction times (LC 6 ± 3.5 seconds, SOC 1 ± 2 seconds; p=0.40) and silo removal times (LC 2 ± 0.5 seconds, SOC 3 ± 1.5 second; p=0.40) between groups were not statistically or clinically significant, indicating similar ease of use. Microbiology analysis revealed bacterial growth on all samples, but the bacterial density was below the standard inoculum in vitro acceptability of 10^5 CFU/g for both the LC and SOC silos (Fig 1). There was no statistically significant difference in bacterial or fungal growth between the LC and SOC silos (anaerobic p=0.34; aerobic p=0.06; fungal p=0.80). In conclusion, a low-cost silo, designed for manufacturing and clinical use in sub-Saharan Africa, demonstrated similar ease of use, absence of tissue injury, and acceptable microbiology profile similar to SOC silos. Data from this porcine model will allow our team to proceed with a pilot clinical study in Uganda.

Resection for Hepatocellular Carcinoma with Macrovascular Invasion: A Meta-Analysis

Andrew Tran
Faculty Mentor(s): Amy Bejsovec and Mindie Nguyen
Health / Clinical Research

Objective: To estimate pooled overall survival (OS), recurrence-free survival (RFS) and complication rates in hepatocellular carcinoma (HCC) patients with macrovascular invasion (MVI) following surgical resection.

Summary Background Data: Guidelines recommend that patients with portal vein tumor thrombosis (PVTT) and/or hepatic vein tumor thrombosis (HVTT) should undergo systemic therapy. However, recent data suggest that surgical resection may be beneficial in selected cases, but outcomes are heterogenous.

Methods: In this systematic review and meta-analysis, we searched Pubmed, Embase, and Cochrane databases from inception to Nov 10, 2020, without language restrictions, for studies reporting outcomes of adult HCC patients with MVI who underwent liver resection with curative intent.

Results: We screened 8,598 articles and included 40 studies involving 8,218 patients. Among all patients with MVI, the pooled median OS was 14.39 months (95% CI 10.99 18.84), 1-year OS was 54.47% (95% CI 46.12 – 62.58) and 3-year OS was 23.20% (95% CI 16.61 – 31.42). Overall, 1-year and 3-year RFS were 27.70% (95% CI 21.00 – 35.57) and 10.06% (95% CI 6.62 – 15.01), respectively. Among patients with PVTT, median OS was 20.41 months in those with segmental/2nd order involvement compared to 12.91 months if 1st order branch was involved and 6.41 months if the main trunk was involved. The pooled rate of major complications was 6.17% (95% CI 3.53 – 10.56).

Conclusions: Overall median survival was 14.39 months for HCC patients with MVI following resection. Median survival was higher in PVTT with segmental/2nd order involvement at 20.41 months versus 6.41 months if the main trunk was involved.

Development of a handheld retinal imaging device for diagnosing diabetic retinopathy

Franklin Wei
Faculty Mentor(s): Hafeez Dhalla and Joseph Izatt
Health / Clinical Research

Diabetic retinopathy (DR) is the leading cause of blindness in working-age adults worldwide. Although there are effective treatments available, many cases of DR go undetected due to low compliance with screening protocols. Diagnosis of DR requires quality images of the retina of the eye, which can be acquired in a clinic through standard tabletop fundus cameras, or in the field with handheld fundus cameras, or HFCs. However, current HFCs suffer from poor image gradability due to glare and poor contrast resulting from the highly reflective cornea in the anterior eye, so there is a need for a handheld retinal imaging device with superior image quality compared to HFCs. This project has developed a handheld scanning laser ophthalmoscope (SLO) which uses low-power laser beam in conjunction with a high-speed optical detector (a photomultiplier tube) to collect reflectance data at points on the retina. The laser beam is scanned by galvanometer mirrors to paint an image of the entire retina. We have developed a novel spiral scan pattern which scans the laser beam across the retina in a spiral curve to improve the speed of the scan. The spiral scan pattern is visible to the patient, and its visually attractive shape acts as a fixation target which draws the patient's gaze towards the center, reducing motion artifacts during the scanning process. High-performance GPU algorithms process millions of data points each second to produce images of the retina for real-time display to the operator during the imaging session. The entire device is packaged into a custom-fabricated ergonomic housing which is tethered to an optical engine mounted on a portable cart. A small-scale clinical study (n=8) has validated the functionality of the device across patient demographics.

Low-Cost Gastroschisis Silo for Sub-Saharan Africa: Testing the safety profile in porcine model

Patrick Wilson

Faculty Mentor(s): Ann Saterbak and Tamara Fitzgerald

Health / Clinical Research

Gastroschisis mortality in sub-Saharan Africa remains high at 59-100%. Silo inaccessibility contributes to this extreme disparity, as standard of care (SOC) silos cost $280, and most median family incomes in sub-Saharan Africa are <$200 per month. Our team of surgeons, engineers, and neonatologists from the U.S. and Uganda have previously described design and bench-testing of a low-cost (LC) silo that costs <$5 and is constructed from a urine collection bag and female condom ring, which are locally available in sub-Saharan Africa. Here we describe in vivo testing of the LC silos. A piglet model of gastroschisis was achieved by eviscerating the intestines through a 2cm midline abdominal wall incision. The bowel was placed into either a LC or SOC silo, maintained for one hour and then reduced. Procedure times for placement, intestinal reduction and silo removal were recorded. Tissue injury was assessed by protocol-blinded histological examination of the abdominal wall and intestine. Quantitative assessment of bacterial and fungal growth on the silos were performed using standard culture techniques. Data were analyzed using the Mann-Whitney test (alpha=0.05). Six piglets (2.0-2.6 Kg) were block randomized to LC (n=3) or SOC (n=3) silo application. There was no visible gross injury to abdominal wall or intestine specimens in either the LC or SOC silos. Concurrently, there was no difference in minor bleeding (bruising) between the LC and SOC silos (Table 1). The differences in silo application times (LC 42 ± 48 seconds; SOC 47 ± 18 seconds; p=1.0), bowel reduction times (LC 6 ± 3.5 seconds, SOC 1 ± 2 seconds; p=0.40) and silo removal times (LC 2 ± 0.5 seconds, SOC 3 ± 1.5 second; p=0.40) between groups were not statistically or clinically significant, indicating similar ease of use. Microbiology analysis revealed bacterial growth on all samples, but the bacterial density was below the standard inoculum in vitro acceptability of 10^5 CFU/g for both the LC and SOC silos (Fig 1). There was no statistically significant difference in bacterial or fungal growth between the LC and SOC silos (anaerobic p=0.34; aerobic p=0.06; fungal p=0.80). In conclusion, a low-cost silo, designed for manufacturing and clinical use in sub-Saharan Africa, demonstrated similar ease of use, absence of tissue injury, and acceptable microbiology profile similar to SOC silos. Data from this porcine model will allow our team to proceed with a pilot clinical study in Uganda.

Humanities

AT THE CROSSROADS, BLACK DISABLED LIVES DO MATTER: Race and disability in special education

Dakota Douglas
Faculty Mentor(s): Jules Odendahl-James
Humanities

Recent flourishing social movements have exposed the masses to the widespread discrimination which affects social minorities in the American landscape. However, narratives of racial and cultural violence are nothing new for vulnerable communities. The Black American community historically, and presently, has called for increased visibility and equity in policy and practice.

Viewing hate through a lens of intersectionality highlights the compounded impacts of discrimination. The social determinants that are attached the burden of Black folk in America are oftentimes shared with members of the disability community. Interactions between race and disability are highlighted by America's public special education system. Understanding why racism and ableism are intimately integrated provides context for the unique struggles of Black students with disabilities from their testing process, to their experiences in the classroom. Bias in testing leads to higher placement of social minorities into special education classes, creating an overrepresentation of Black students in these segregated courses that is 2-3x higher than that of their White counterparts. To understand the mechanisms driving the intersections between these identities, this paper crafts a narrative that defines the origins of race and disability, and explores how intrinsic American ideals like neoliberalism and capitalism concurrently delegitimize Black people and people with disabilities. Next, the process of testing and instances of testing discrimination are highlighted. In its conclusion, this paper explores the important role of special educators and their responsibility to be activists for Black visibility in the classroom. As our education system, schools, and teachers are brought the opportunity to increase visibility of minority struggles in this new era of activism, now more than ever they must address racism and ableism, and work to address their explicit and implicit roles in sustaining both.

Even if the Institution Forgets, We Remember: How Activist Memory Drives Student Organizing

Shania Khoo
Faculty Mentor(s): Calvin Cheung-Miawe, Jessica Namakkal and Deonte Harris
Humanities

Student organizing—and thus, this thesis—is about memory: about how past efforts and movements persist and (re-)emerge in the present, and about how the past is used to (re)imagine and (re)build the future. At Duke University, Asian/American students since the early 2000s have found their ways backwards into the archives, into the long histories of campus organizing, and into strategies of the past in order to advocate for the institutionalization of Asian American Studies and Ethnic Studies. I refer to this through this thesis as activist memory to understand how students recover, interact, and build on the archives to ground their place within campus history and to make sense of the feelings and emotions associated with campus organizing. Using Duke University as a case study, this thesis is guided by the question: What is the role of activist memory in sustaining contemporary Asian/American student organizing in a predominantly white institution? Through the dual use of oral history interviews of current and graduated Asian/American students and archival materials of Asian/American organizing spaces, I un/recover activist memory of the movement for Asian American Studies at Duke. In doing so, I interrogate how students have engaged with memory to understand their positionalities, maintain and pass it on, and learn from it to inform their organizing. This thesis reveals that students, through organizing for Asian American Studies and learning about its past, are negotiating and shifting the future of the field.

Sustainability in Hawai'i: Caring for 'Āina, Culture, and People

Kate Leonard
Faculty Mentor(s): Anne Allison and Katya Wesolowski
Humanities

This thesis seeks to explain how the black boxing of sustainability by the local and state governments has made Hawaii's efforts for sustainability—as defined by the 2050 Sustainability Task Force—inefficient and ineffective. By solely focusing on the environmental dimension of sustainability, the government neglects the economic and cultural dimensions of sustainability, and therefore does not create programs that make Hawai'i truly sustainable. Furthermore, their work and definitions inform their constituents—the residents of Hawai'i—as to how sustainability should be understood. I explore this in areas such as fishing, farming, and Agricole rum distilling where the foregrounding of the environment has social and political implications for the residents of Hawai'i and even more so, the individuals positioned within Hawaii's food production system. We also see how this black boxing is contested by the works of chefs. Furthermore, I explain how those positioned in Hawaii's food system use their work to bring more attention to these neglected cultural and economic dimensions. This reveals how the work of the local and state government does not always address the problem it seeks to fix and fails to serve its constituents situated within Hawaii's food production system. Importantly, I also show how the three dimensions of sustainability are interconnected in a way that means that sustainability cannot be solved by only working towards the environmental component.

Caring for a Corrupt Corpus: Ethical and Legal Standpoints on English Consumption (1660-1714)

Charlotte Lim
Faculty Mentor(s): Astrid Giugni
Humanities

Did the monopoly of the English East India Company benefit or corrupt England? The literature of the English Restoration (1660-1688) both influenced and was influenced by the growing political and economic relevance of joint-stock companies and international trade. The legislative changes of this period reflected and further shaped normative attitudes concerning consumption and commerce. Early modern commentators on the political economy integrated these attitudes in their writings by borrowing medical language like "consumption" and the "body politic." For instance, writers like Gerard de Malynes (fl. 1586-1623) used this lens of physical and mental health to diagnose economic problems and propose remedies. We examine the rhetorical devices that writers used to describe "monopoly" and their relationship to the care of the national body. Further study of "monopoly" comes from a close analysis of court cases regarding the legality of the East India Company's trade. We compare the contemporary legal status of monopolies to the ambiguous ethical discussion surrounding them. Our inquiry is informed by the economic theories of the physician Bernard Mandeville (1670-1733) and his predecessors; we contrast the language, arguments, and rulings of the cases with these philosophers' work in an effort to understand the evolving ethical landscape inspired by these jurists. Finally, we apply "distant reading" techniques to the Early English Books Online (https://quod.lib.umich.edu/e/eebogroup/) database to uncover patterns within early modern discourses of monopolies. We investigate whether there were any changes in the attitudes toward the East India Company's monopoly before and after the Restoration. While acknowledging the limitations of quantitative research on archival materials, we use computational methods to track frequent words, topics, and sentiments. Using this interdisciplinary approach, we hope to uncover the dynamics of the relationship of early capitalism with ethics.

Bass Connections Project

17th-century Ephemeral Literature: Machine Learning in Understanding Ordinary Lives in London

Yuchen Lu
Faculty Mentor(s): Astrid Giugni
Humanities

The fire of 1666 destroyed most of London's print production. With print shops burned and the book trade declined, London focused on the restoration of the city and the Stuart monarchy. Under such political and religious turmoil, we see a democratizing movement of print culture in London. Despite government censorship, anonymous and ephemeral literature boomed during the early 1660s. Lay authorship, including broadsides and controversial literature, fulfills the increased intellectual curiosity of ordinary people in this era.

Because print records from the 17th century are partial, can machine learning, which relies on data completeness, serve as a valuable tool to study ephemeral literature? This research explores to what extent machine learning models can be used to study humanities, where primary sources are commonly incomplete. I combine close reading with machine learning models on textual data to track the evolution of identities in London communities. For instance, I find that with the increase in lexical richness, the text is targeted toward a more general audience. I use close reading to classify the topics and sentiment of documents and scale the classifier using machine learning models. In addition, the study also addresses possible solutions to common text analysis challenges, including how to decrease entities duplications, missing data, and spelling variations. Using statistical inferences, including Entity Resolution and Latent Allocation, the study investigates how to improve the performances of machine learning models in ephemeral literature.

Women in Art History: 1930-1950: Intellectual Realities

Kerry Rork
Faculty Mentor(s): Lee Sorensen
Humanities

Despite its relative inclusivity compared to other disciplines, art history has long since been a male-dominated field, and the discoveries of women are only recently beginning to be evaluated. Recognizing this disparity and hoping to better understand its reality, since summer of 2020, I have worked to create a database of female-identifying art historians from English-language art journals such as The Metropolitan Museum of Art Bulletin and the Burlington Magazine from the years 1930 to 1950 to answer questions like what the most common topics or specialities, what time periods saw the greatest increase in women writers, and what factors lead to an increase in women writers. Institution, journal, year, continent and country of the subject matter, subject, and artistic medium were folded into a database of over 750+ entries, examining and detailing each woman's article in their respective publications with some areas of interest in mind. Since my database's inception, I have used visualization tools to provide another dimension of uncovering the gender disparity within art history.

An Unexpected Discovery: Analogizing Fifteen Female Artists of the Mid-20th Century

Abby Shlesinger
Faculty Mentor(s): Kristine Stiles
Humanities

Hans Hofmann was a German-born artist and teacher. He first established his eponymous school in Munich in the 1910s, later moving and founding The Hofmann School in New York in the early 1930s. At his schools, he taught hundreds of aspiring artists, many of whom later became professional artists. These artists included dozens of professional female artists working in figuration, abstraction, surrealism, and sculpture. This thesis investigates the artwork of fifteen of these female artists and considers the connections between their lives, artworks, and legacies. The fifteen female artists are classified into three groups: figurative artists, abstract artists, and sculptural artists. Each grouping, or chapter, revolves around a central theme shared by the artists within it. Yet, all the artists shared a common teacher, primary place of work, and period spent working. By examining their artwork and personal statements, we come to understand how each artist incorporated Hofmann's concepts of "push and pull" and "plasticity" into their paintings and sculptures, using these theories to supplement their aesthetic and thematic interests.

Caring for a Corrupt Corpus: Ethical and Legal Standpoints on English Consumption (1660-1714)

Heidi Smith
Faculty Mentor(s): Astrid Giugni
Humanities

Did the monopoly of the English East India Company benefit or corrupt England? The literature of the English Restoration (1660-1688) both influenced and was influenced by the growing political and economic relevance of joint-stock companies and international trade. The legislative changes of this period reflected and further shaped normative attitudes concerning consumption and commerce.

Early modern commentators on the political economy integrated these attitudes in their writings by borrowing medical language like "consumption" and the "body politic." For instance, writers like Gerard de Malynes (fl. 1586-1623) used this lens of physical and mental health to diagnose economic problems and propose remedies. We examine the rhetorical devices that writers used to describe "monopoly" and their relationship to the care of the national body.

Further study of "monopoly" comes from a close analysis of court cases regarding the legality of the East India Company's trade. We compare the contemporary legal status of monopolies to the ambiguous ethical discussion surrounding them. Our inquiry is informed by the economic theories of the physician Bernard Mandeville (1670-1733) and his predecessors; we contrast the language, arguments, and rulings of the cases with these philosophers' work in an effort to understand the evolving ethical landscape inspired by these jurists.

Finally, we apply "distant reading" techniques to the Early English Books Online (https://quod.lib.umich.edu/e/eebogroup/) database to uncover patterns within early modern discourses of monopolies. We investigate whether there were any changes in the attitudes toward the East India Company's monopoly before and after the Restoration. While acknowledging the limitations of quantitative research on archival materials, we use computational methods to track frequent words, topics, and sentiments. Using this interdisciplinary approach, we hope to uncover the dynamics of the relationship of early capitalism with ethics.

Bass Connections Project

Caring for a Corrupt Corpus: Ethical and Legal Standpoints on English Consumption (1660-1714)

Amy Weng
Faculty Mentor(s): Astrid Giugni
Humanities

Did the monopoly of the English East India Company benefit or corrupt England? The literature of the English Restoration (1660-1688) both influenced and was influenced by the growing political and economic relevance of joint-stock companies and international trade. The legislative changes of this period reflected and further shaped normative attitudes concerning consumption and commerce. Early modern commentators on the political economy integrated these attitudes in their writings by borrowing medical language like "consumption" and the "body politic." For instance, writers like Gerard de Malynes (fl. 1586-1623) used this lens of physical and mental health to diagnose economic problems and propose remedies. We examine the rhetorical devices that writers used to describe "monopoly" and their relationship to the care of the national body. Further study of "monopoly" comes from a close analysis of court cases regarding the legality of the East India Company's trade. We compare the contemporary legal status of monopolies to the ambiguous ethical discussion surrounding them. Our inquiry is informed by the economic theories of the physician Bernard Mandeville (1670-1733) and his predecessors; we contrast the language, arguments, and rulings of the cases with these philosophers' work in an effort to understand the evolving ethical landscape inspired by these jurists. Finally, we apply "distant reading" techniques to the Early English Books Online (https://quod.lib.umich.edu/e/eebogroup/) database to uncover patterns within early modern discourses of monopolies. We investigate whether there were any changes in the attitudes toward the East India Company's monopoly before and after the Restoration. While acknowledging the limitations of quantitative research on archival materials, we use computational methods to track frequent words, topics, and sentiments. Using this interdisciplinary approach, we hope to uncover the dynamics of the relationship of early capitalism with ethics.

Bass Connections Project

Uncovering, Resisting, Remembering: African American Labors at Duke and in Durham

Huiyin Zhou
Faculty Mentor(s): Robert Korstad and Adam Rosenblatt
Additional Mentor(s): Orilonise Yarborough
Humanities

This project started as a Story+ summer research project. I worked with Friends of Geer Cemetery in hopes of uncovering the lives of Black ancestors buried in Geer, Durham's first Black cemetery. The final product was organized into a StoryMaps digital exhibit: tinyurl.com/uncover-black-labors-geer. Now, I am expanding this project with Professor Emeritus of History, Robert Korstad. My research objective is to unsettle the University's "official" narrative and focus on the lives of non-academic workers of color and injustices they faced, many of which persist today. I mainly work with Duke University archives, state records, city directories, student publications, and public newspapers. For the Story+ exhibit, I focused on five African American laborers, including Caroline Barnes (b.1844-d.1924), who was enslaved by Washington Duke and became a community philanthropist later in life, and Albert Armstrong, the coachman for Washington Duke and an important fundraiser for Lincoln Hospital which served for Black Durhamites during the era of segregation. Now, I am extending this work and creating an alternative archive of African American janitors who worked for Trinity College from in the years before it became Duke University. Supplementing archival and genealogical research with sociohistorical and narrative analysis, I seek to uncover stories of dignified and whole individuals whose stories are often erased and distorted in official, white supremacist archives. When applicable, I enrich the details of these counter-narratives through descendant interviews and memoirs. Under an intersectional framework, I reconstruct life histories that shed light on racialized and gendered labors in lower-class African American communities and the importance of unearthing community-oriented memory against white supremacy. As a Chinese international student organizer, I treat this project as part of a larger political movement in building multiracial coalitions and resisting structural erasure.

Physical Sciences

Investigating Antimicrobial Metallopeptide Mechanisms: Hist-5

Rithik Castelino
Faculty Mentor(s): Katherine Franz and Annastassia Gallo
Physical Sciences

C. Albicans is an opportunistic yeast, fungal species that has been found to be responsible for candidemia, one of the most common type of blood-stream infections in the United States. Histatin-5 (Hist-5) is an antifungal peptide found in human saliva and is known to be particularly effective against C. Albicans. Exposing Hist-5 to Cu2+ has also been shown to enhance its antifungal properties. The mechanism by which Hist-5 acts upon C. Albicans, is uncertain but it has been shown to be intracellular, specifically in the fungal mitochondria. The proposed mechanism which this project explores, is that Hist-5 inhibits C. albicans growth through the production of reactive oxygen species (ROS), driving lipid peroxidation of the mitochondrial lipid membranes. Hist-5 was successfully synthesized. Successful synthesis has been verified through HPLC and mass spectrometry. The presence of malondialdehyde (MDA), a natural byproduct of lipid peroxidation will be checked by utilizing a Thiobarbituric Acid Reactive Substances (TBARS) assay. This project is currently a work in progress, and work so far have been focused on creating a complete and reliable methodology for verification of the proposed mechanism.

Identification and characterization of the NAB2 binding site on Nedd4 E3 ubiquitin ligase

Nicholas Doak
Faculty Mentor(s): Dewey McCafferty
Physical Sciences

A cellular hallmark of the neurodegenerative disorders Parkinson's disease and amyotrophic lateral sclerosis (ALS) is the dysregulation of protein transport and trafficking within the neuron. The N-aryl benzimidazole (NAB) family of small molecules has been shown to interact with Nedd4, an E3 ubiquitin ligase, and Rab1, a GTPase regulator of intercellular protein trafficking, to reverse the alpha synuclein or TDP-associated neurotoxicity of these disease states. The current study aims to evaluate the binding and mechanism of action of NAB family member NAB2 with Nedd4 and Rab1 to better understand the binding-associated conformational changes and structure-activity relationships which allow NAB2 to restore normative protein trafficking. Our work thus far has been centered on the expression and purification of a stabilized preparation of the Nedd4 isoform 4 version of the E3 ubiquitin ligase. This will be used along with a newly synthesized diazirine-NAB2 conjugate to identify the NAB2 binding site on Nedd4 through photoaffinity labeling. Oxford Nanopore sequencing has confirmed the construction and successful transformation of the Nedd4 isoform 4 encoding pGEX3-TEV-Nedd4 vector into a BL21(DE3) E. coli cell line. Following expression, glutathione-agarose affinity purification and SDS-PAGE gel analysis has indicated the successful production of the desired 136 kDa Nedd4 GST-tagged fusion, with optimization of the proteolytic cleavage of its fusion protein and final purification currently underway. Following incubation of Nedd4 with a diazirine-NAB2 adduct and cross-linking upon exposure to 350 nm light, the binding site will be confirmed by tandem mass spectrometry and validated by substrate protection assays.

Investigating the buffer-independent thermodynamics for zinc binding to histatin 5

Sean Gao
Faculty Mentor(s): Katherine Franz and Terrance Oas
Additional Mentor(s): Joanna Campbell
Physical Sciences

Histatin 5 (Hist5) is an antimicrobial peptide found in human saliva and it serves as a first line of defense against pathogens, including the opportunistic fungal pathogen Candida albicans. The recent rise in antifungal resistance has led scientists to study Hist5 as a potential therapeutic agent. Hist5 can bind several metal ions in vitro, a property that has been shown to modulate its biological activity. Here, we use isothermal titration calorimetry to quantify the binding stoichiometry and thermodynamics between Hist5 and Zn(II) in multiple buffers across a temperature range. From these experiments, we can extract various buffer-independent thermodynamic values. By correlating these values with spectroscopic data and results from biological assays, we are working toward a deeper understanding of the biophysical basis for Hist5's Zn(II)-dependent mechanism of action.

Multi-Assay Profiling to Reveal the Modularity of the MALAT1 Triple-Helix Stability in vitro

Dhanasheel Muralidharan
Faculty Mentor(s): Amanda Hargrove and Martina Zafferani
Physical Sciences

The rapidly increasing characterization of RNA tertiary structures has revealed their pervasiveness and active roles in human diseases. Therefore, small molecule-mediated modulation of RNA tertiary structures constitutes an attractive avenue for the development of tools for both therapeutically targeting and/or uncovering the pathways associated with these RNA motifs. This potential has been highlighted by preliminary targeting of the triple helix present at the 3'-end of the non-coding RNA MALAT1, a transcript implicated in several human diseases. This triplex has been reported to decrease the transcript susceptibility to degradation and, ultimately, promote its cellular accumulation. While small molecules have been shown to bind and impact the stability of the MALAT1 triple helix, the molecular recognition properties behind these structural modulations are not well understood. To elucidate these properties, we designed a focused library utilizing the diminazene scaffold, which is under-explored but precedented for nucleic acid binding, to target the MALAT1 triple helix. To gain a holistic perspective on small molecule recognition, we evaluated this library through a multi-dimensional approach to assess what parameters, if any, could predict small molecule affinity and effect on triplex stability. We designed and/or optimized competition, calorimetry, and thermal shift assays as well as a novel quantitative enzymatic degradation assay, which led to the discovery of bidirectional modulators of triple helix stability within the scaffold-centric library. Furthermore, employment of quantitative structure activity relationship (QSAR) afforded predictive models for stability- and affinity-based assays. Together, this work provides novel biophysical tools for the evaluation of small molecule:RNA triplex interactions and predictive models that can be applied to small molecule interrogation of the growing body of disease-associated RNA triple helices.

Identification of E. Coli Protein-Copper Binding Sites Using HDX-Mass Spectrometry

Aaron Petty
Faculty Mentor(s): Michael Fitzgerald
Physical Sciences

Metal ions have a diverse set of functions and interactions in biology, many of which are deleterious. Copper, in particular, has been shown to have strong antimicrobial activity due to its cytotoxicity, such that cells have evolved mechanisms to strictly regulate intracellular levels of copper. Previous work in E. coli has indicated that copper binds and differentially stabilizes specific proteins and in high concentrations can cause specific proteins to precipitate out of solution. Here, histidine hydrogen/deuterium exchange-mass spectrometry is used to investigate the binding sites of protein-copper binding in the E. coli proteome. As part of this work we are also adapting the HDX Workbench software to facilitate the high throughput analysis of histidine HDX-MS data on the proteomic scale.

1,2-Amino Alcohol Syntheses via CuH-Catalyzed Reductive Couplings of 2-Azatrienes with Ketones

Faraan Rahim
Faculty Mentor(s): Steven Malcolmson
Physical Sciences

Small molecules are a major therapeutic avenue for the treatment of human diseases. As new and enhanced medications are needed to target diseases and more efficient methods are desired for drug manufacturing, novel chemical transformations are needed to facilitate the discovery of new drugs and streamline their synthesis. An additional level of complexity is that many pharmaceuticals are chiral, and often only particular stereoisomers exert the desired therapeutic effects. To address these challenges, chemists invent new reagents and discover new catalysts and catalytic processes to devise strategies for chemical synthesis, permitting access to complex chemical landscapes. Nitrogen-containing compounds have tremendous importance and are widespread as pharmaceuticals. Nitrogen is often bound to stereogenic carbon atoms in these molecules. Such chiral amines come in many forms, including 1,2-amino tertiary alcohols, which contain two adjacent stereogenic carbon atoms bound to nitrogen and oxygen. We have developed the CuH-catalyzed reductive coupling of 2-azatrienes with ketones as an effective, stereoselective strategy to access 1,2-amino tertiary alcohols. This strategy is a one-step carbon-carbon bond-forming reaction that sets both carbon atoms in the correct stereochemical configuration, demonstrating high degrees of enantio- and diastereoselectivity.

Nonlinear dynamics of trapped Leidenfrost drop

Tianrui Wu

Faculty Mentor(s): Ronen Plesser, Jenny Magnes and Harold Hastings

Physical Sciences

We present our experimental investigation of the dynamics of trapped ultra-mobile Leidenfrost water drop on heated spherical dishes. We use Leidenfrost drops to investigate non-linear motion of underdamped macroscopic objects in a two-dimensional Hookean field under thermal forces. Leidenfrost drops are released into aluminum containers with a spherical surface. Drops are recorded using high-speed camera and their position time-series are obtained with video-processing methods. The motion of drops is then examined with frequency spectra analysis and recurrence methods. We found negative slope of spectral density in the frequency spectrum, positive maximum Lyapunov exponent (MLE) and embedding dimension of 3. We identified multiple repeating box structures with diagonal and orthogonal lines in the recurrence plot. We identified two major stages of Leidenfrost drop motion in spherical dishes. A dissipative quasi-periodic behavior is identified in the transient stage. Noise-induced nonlinear motion is discovered in the steady-state stage with apparent transitions between two modes of motion.

The self-calibration of galaxy intrinsic alignment

Junzhe Zhou
Faculty Mentor(s): Michael Troxel
Physical Sciences

The structure and growth of the matter distribution in the universe over cosmological distances and timescales encode crucial information about the fundamental forces and the nature of space-time. The recent extragalactic photometric observations, such as the Dark Energy Survey (DES), have produced a wealth of data on the matter distribution, and are hopeful to constrain key cosmological parameters to percent-level accuracy through galaxy clustering and weak gravitational lensing. The intrinsic alignment (IA) of galaxies is a significant systematic bias for weak lensing surveys and encodes valuable information concerning cosmic evolution. However, past research in IA mainly attempted to eliminate it as a bias in the weak lensing survey through phenomenological modeling. Here, we aim to measure IA directly and in a model-free manner using the DES Year 3 source catalog. This objective is realized in two steps. We first present a new measurement scheme that leverages machine learning and survey simulations to infer galaxy clustering statistics from galaxy samples that are sensitive to survey properties. We then implement a newly developed self-calibration method to reconstruct the IA signal using the auto- and cross-correlations of galaxy positions and shapes. This result will further our understanding of both the systematics in current weak lensing measurements and the physics of structural formation on the cosmological scales.

Quantitative Sciences

Time series analysis using multitaper method

Yijia Liu
Faculty Mentor(s): Hau-tieng Wu
Quantitative Sciences

In time series analysis one decomposes the signal into sine waves of different frequencies, which can be estimated via the Fourier transformation. As we only have finite amount of data, the estimates will not be precise, and it is important to study the statistical properties of these estimates. Due to the manifestation of the Heisenberg's uncertainty principle, it is not possible to construct a perfect estimator that can simultaneously achieve all the desirable properties. The multitaper estimate is a cornerstone of modern-day spectral analysis. It trades resolution for wide-band bias, while reducing the variance by taking the mean of several orthogonal estimates. In applications, medical practitioners use the multitaper estimate to study sleep by analyzing the EEG of the subjects and obtaining a classification of different types of sleep stages. In astronomy it has been helpful in the detection of gravitational waves at LIGO. Despite the widespread applications, the mathematical properties of the multitaper estimate are not well-studied for general stationary processes. In our work, we fill this gap by proving properties of the multitaper estimate for a class of stationary processes and derive a way to detect oscillatory wave-shape function via a Gaussian multiplier bootstrap.

Pose Analysis for Room-Scale VR

Sasamon Omoma
Faculty Mentor(s): Maria Gorlatova and Ying Chen
Quantitative Sciences

The goal of this research is to better understand how people move in virtual spaces. This task involved analyzing existing user positional and orientation data, and also collecting our own data. For our user study, I created several virtual environments where people may navigate via 'real walking', that is, their motions in the physical world are mapped directly to movement in the virtual world rather than navigating via a controller. By understanding mobility patterns, we may create VR frame prediction algorithms to improve the currently resource-intensive task of rendering the VR headset visuals.

Automated Signal Quality Assessment of Electroencephalography Data in Preclinical Mouse Models

Eric Qi
Faculty Mentor(s): Boyla Mainsah and Leslie Collins
Quantitative Sciences

In this work, we investigate the use of machine learning as an alternative to manual review and annotation of electroencephalography (EEG) data in preclinical mouse models of brain injury. Preliminary results from classifiers trained and tested on independent datasets from two preclinical mouse models of brain injury show high performance in predicting labels of signal patterns of interest. These results demonstrate the potential utility of an automated tool to assess the quality of EEG data in preclinical models of brain injury.

Social Sciences

Black Health in America: Effects of Trust on Health Care Utilization in the African Diaspora

Natalie Ezem
Faculty Mentor(s): Maria Febbo
Social Sciences

Historically, there has been a complicated and understudied dynamic between those that identify with the African Diaspora and the United States medical system. This complex relationship has introduced cultural, social, political, and economic factors influencing the perception and usage of resources within the health system for people within those communities. Furthermore, available literature often groups different populations across the African Diaspora together because they fall under the same racial classification, "black." However, this similarity overshadows key differences, such as religious beliefs, cultural differences, and financial costs, that determine the way each group perceives and uses treatment for their health. Consequently, this qualitative study investigated the impact of trust on health care utilization between black foreign-born African immigrants, including first and second-generation immigrants, compared to black Americans born in the United States. There were six U.S.-born black American participants and five foreign-born African immigrants, resulting in a total of eleven in-depth interviews. For U.S.-born black Americans, the study revealed a high level of trust in individual providers but not the healthcare system as an institution. On the other hand, foreign-born African immigrants from West Africa had a significantly higher level of trust in the United States healthcare system compared to those from the Central, Eastern, and Southern regions of Africa. When evaluating the role of trust in using resources within the healthcare system across both study populations, there seemed to be no clear conclusion on its impact. Some participants considered trust as one of their top three factors influencing how they utilized health care resources, while others indicated that it was not important to them at all. Some participants noted that trust interacted with their personal identifiers – such as gender, race, and socioeconomic status – and their health care utilization. While there is variability in trust between the sample populations, participants mentioned that their level of trust in the United States healthcare system and their usage of resources would increase if there was more staff diversity, improvements in cultural competency surrounding different medical practices, and greater accessibility to quality care, especially in low-income communities.

Experience of Diabetes in Young Adults in Mysore District

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Diabetes is a global epidemic -- by 2045, it is predicted that over 700 million adults will be living with type 2 diabetes (T2D). As T2D becomes more prevalent around the world, it is crucial to develop cost-effective, community-driven, and sustainable methods to promote healthier physical and mental disease outcomes. Applying the biopsychosocial framework, I will conduct individual interviews to examine the relationships between diabetes management, experience, and overall quality of life in young adults living with diabetes in Mysore district. I am leading this project in collaboration with the Public Health Research Institute of India (PHRII), a non-governmental organization based in Mysore, Karnataka in South India. With PHRII staff and community health workers, I will recruit study participants, administer a demographic survey and depression screening tool, conduct interviews, and analyze the resulting data. The results of the study will be used to identify key pathways that negatively impact physical and mental health in this community. This data will inform next steps in related research, prioritizing areas of need that will lead to more successful interventions. There are limited existing studies on this subject in low and middle-income countries; this study would contribute to the understanding of the T2D patient experience and key barriers to health management in an underserved region of India. This qualitative study will provide information on the experience of living with T2D in Mysore district and new understanding of how social determinants of health contribute to the comorbidity of T2D and depression or other mental health issues.

Understanding CEnR Partnerships from Institutional and Community Stakeholder Perspectives

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Collaborative research partnerships between institutions of higher education like Duke and local communities offer a valuable form of scholarship and a transformative approach to teaching and learning in a manner that can benefit local practice and scholarship. Yet, ethical and effective university-community research collaboration is complicated by numerous factors: university incentive structures, rigid research processes, lack of community clarity on academic processes, and power dynamics that challenge partners' willingness to voice concerns, and more. These concerns are amplified when considering ethics of university partnerships and research involving community organizations engaging with members of historically excluded and exploited populations.

The goal of our research project is to examine and document both the practices and experiences of community-engaged scholarship from the perspective of community partners and of the researchers. Our research questions included: How are partnerships initiated and structured, and what prompts these processes? What challenges are experienced in the partnership process, and what are key indicators and facilitators of fruitful partnerships? What are current practices and processes of research partnership at Duke University and in the Durham community? How do current and best practices differ, at all, among different institutes and departments of institutes of higher education and/or types of community organizations?

With a diverse research team membership spanning undergraduate and graduate students and staff and faculty within the Nicholas School of the Environment , Office of Civic Engagement, Sanford School of Public Policy, School of Law, Service-Learning, Social Science Research Institute, and Trinity College of Arts and Sciences, our unique two-pronged approach utilizes distinct

surveys and interviews of both institutional and community stakeholders and researchers collected over the course of the academic year in order to uncover perceptions of the successes, challenges, and solutions from both perspectives; this is core to our equity lens, as it moves away from prioritizing university perspective and instead allows for an approach driven directly by a community lens. Our intended outcomes include deliverables for university and community stakeholders on recommendations for best practices for countering power dynamics and considering diverse perspectives in community engaged research.

Bass Connections Project

Overcoming the Fear of Destruction through Technological Advancement and Political Obligation

Maria Morrison
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Social Sciences

This thesis finds that political obligations were increasingly discussed by the British Prime Minister from 1933 to 1939, leading to the conclusion that these obligations were the force driving the British entry into World War II. A well-founded fear that London would be destroyed by German bombing has long been considered the primary fear keeping Britain out of the war and continuing the appeasement of Nazi Germany. This paper provides an analysis of speeches from the British Prime Minister from Hitler's power increase in March 1933 to the declaration of war in September 1939, comparing the relative frequency of words about that fear versus those about technological advancement and political obligation. Understanding the influences behind this landmark decision helps scholars understand what motivates politicians in wartime and what risks they are willing to take in order to create the type of world they want to live in.

Revisiting CA Proposition 209: Changes in Science Persistence Rates & Overall Graduation Rates

Anh-Huy Nguyen
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Social Sciences

I evaluate changes in the science persistence rate and overall graduation rate of all applicants to the University of California system when racial preferences are no longer in place. Using a general equilibrium framework to estimate different admissions and allocation rules, I find that the removal of racial preferences leads to a cascade of minority enrollees into less selective campuses and a surge of non-minority enrollees into more selective campuses. The improved matching between campuses and students results in higher science persistence rates and graduation rates across the pool of all applicants. In particular, the gains are driven by minority students who are originally admitted in the baseline and non-minority students who are originally rejected in the baseline but are induced into the system in the counterfactual. I also investigate claims that applicants may have strategically gamed during the admissions process by misrepresenting their interest in the sciences in order to maximize their admissions probability. While there exist incentives to apply in different majors across the campuses, I find evidence that applicants often fail to game optimally, suggesting that they may not be fully informed of their relative admissions probabilities in the sciences and non-sciences.

Beliefs About Life After Death Predict Prejudice in This Life

Gwyn Reece
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Social Sciences

Religious people show prejudice against members of groups that threaten their values, such as atheists. Still, little research has explored how specific religious beliefs, such as afterlife beliefs, relate to prejudice against these groups. This research investigates the relationship between religious, spiritual, and secular afterlife beliefs (e.g., heaven, hell, reincarnation) and attitudes toward members of religious and political groups. Across three studies, one drawing data from a national survey (N = 1429), and two using data from original surveys (N = 905, N = 862), we find that both religious and secular afterlife beliefs predict attitudes toward these groups even after controlling for religiosity. Religious afterlife beliefs predict stronger prejudice against value-threatening groups like Democrats and atheists even when controlling for religiosity. This prejudice is mediated by defensive theology and buffered by peace of mind. Similarly, secular afterlife beliefs predict stronger prejudice against Republicans and religious people. This prejudice is also buffered by peace of mind. Finally, religious afterlife beliefs mediate the association between religiosity and prejudice against value-threatening groups. These findings suggest that afterlife beliefs contribute to prejudice independently of the broader religious and nonreligious identities associated with them.

Psychology- Graduation with Distinction candidate

Note-taking mediums, expectations and learning outcomes

Sihan(Jen) Wang
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Social Sciences

Note-taking by hand has been a common practice among college students. As the role of technology (e.g., laptops) grows in the classroom, the controversies over note-taking mediums remain. How do different mediums (laptop, longhand) impact student learning outcomes? The present study explored whether note-taking mediums influenced students' test performances when paired with different studying expectations (expecting to review, expecting not to review). Test scores showed that image-related and text-related test performance did not differ significantly across conditions, revealing no significant interactions between note-taking mediums and expectations. Additionally, the present study explored the potential role of metacognitive awareness as a proxy for note-taking skill levels and studying strategies. However, no significant correlations were found between metacognitive awareness and learning outcomes.

Psychology- Graduation with Distinction candidate

Meeting of the Minds

The Atlantic Coast Conference (ACC) Meeting of the Minds conference is held each spring and is hosted by one of the 15 ACC member schools. It is funded in part by revenue from athletic events. The conference celebrates undergraduate research and provides an opportunity for sharing ideas and collaboration. Every spring, outstanding undergraduate researchers and a faculty mentor from all ACC universities are invited to present their original research.

ACC member schools include:

- Boston College
- Clemson University
- Duke University
- Florida State University
- Georgia Tech
- NC State University
- Syracuse University
- University of Louisville

This year's ACC Meeting of the Minds conference took place at the University of Virginia from April 1-3, 2022. Below you will find presentation titles for the 5 Duke undergraduates selected to present at the conference:

Drew Greene
Title: *Visualizing Durham Public School Communities*
Anna Greenleaf
Title: *Paying It Forward: How Unequal Access to Resources Reduces Generosity*
David Hugo
Title: *The Dangers of Going with the Flow: Using Autonomous Transmitters to Document Downstream Passage Conditions and Risk of Fish Injury at Dams*
Raia Lockerman
Title: *Tracing the Evolution of Black Internationalism: Contemporary Renewals of Black – Palestinian Solidarities.*
Kerry Rork
Title: *Colonization and the University: Exploring Settler Colonialism at Trinity College, Dublin*

Index

Behura, 24
Bier, 74
Biswas, 75
Boger, 25
Brandt, 8
Capoot, 9
Castelino, 99
Choi, 26
Costanzo, 27
Crawford, 28
Cyrus, 29
Daniels, 76
Doak, 100
Douglas, 88
Ezem, 112
Gao, 101
Gardner, 30
Gerges, 10
Ghosh, 77
Gopisetty, 113
Guan, 31
Guerrero, 32
Han, 11
He, 12
Hicks, 33
Hu, 34
Kaney, 35
Kapustin, 78
Khoo, 89
Kohlmann, 36
Kulshrestha, 37
Kumar, 13, 38
Langan, 39
Lapay, 114
Lee, 40, 41
Leonard, 90
Li, 42
Lim, 91
Liu, 14, 108
Lu, 92
Lusk, 15
Ma, 43, 79
Mallik, 44
Markunas, 45
Massare Somers, 46
Mawyer, 16
McMichael, 47
Miller, 48
Morrison, 116
Muralidharan, 102
Nguyen, 49, 117
Nicklow, 50
Ochakovski, 80
Omoma, 109
Parker, 51
Peng, 81
Pertile, 52
Petty, 103
Pitchon, 53
Qi, 54, 110
Raghavendra, 55
Rahim, 104
Raich, 17
Ramasubramanian, 56
Reece, 118
Richards, 82
Rogers, 57
Rork, 93
Ryan, 58
Salzman, 83
Sebastian-San Miguel, 59
Sevchik, 18
Shead, 19
Sheu, 60
Shlesinger, 94
Smith, 95
Sun, 61
Tang, 62
Tran, 84
Tucker, 20
Ulak, 63
Vaidyanathan, 64
Victor, 21
Wall, 65
Wang, 119
Washart, 66
Wei, 67, 85
Weng, 96
Wilson, 86
Wu, 105
Yang, 68
Yao, 69
Zhao, 72
Zheng, 70
Zhou, 97, 106
Zucker, 22