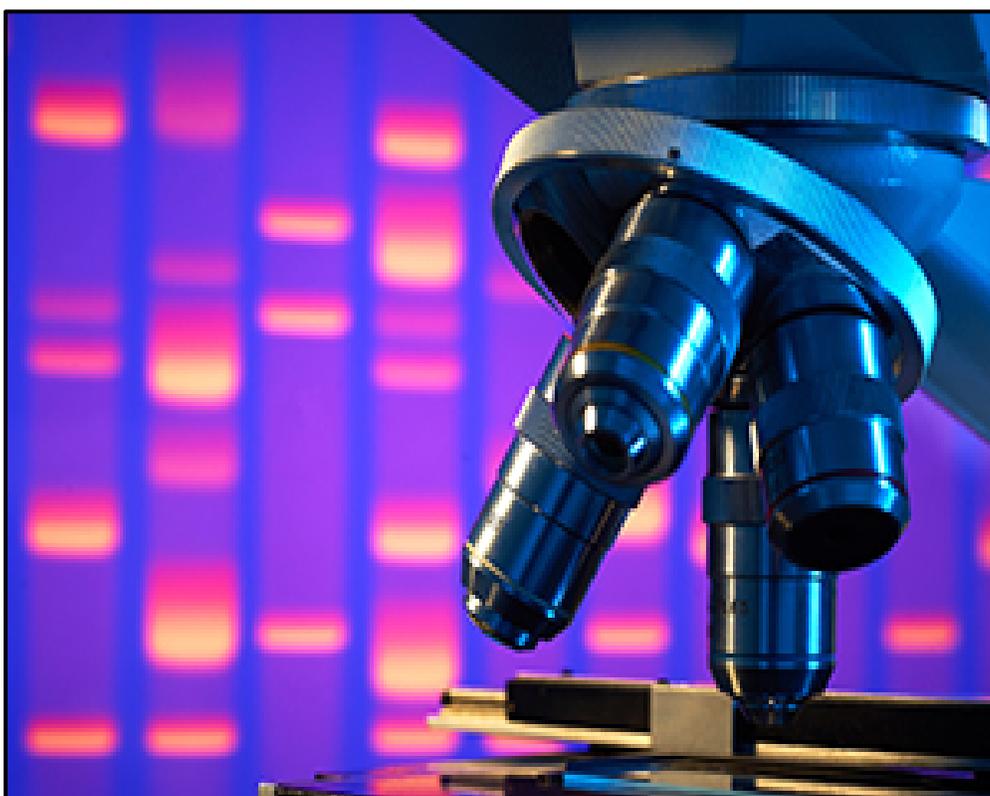


Summer Research Program

Poster Abstracts



Friday, July 26, 2019

Medical Education Building
1st Floor Lobby

9:00 – 10:30 am, Judges and Students Only

10:30 – 11:30 am, Open to the Public

11:30 – 12:00 pm, Award Ceremony

2019 Summer Research Internship Program

The Summer Research Internship Program provides research opportunities for medical students, undergraduates and high school students. The program directors, Dr. Paula Gregory and Dr. Fern Tsien, match high school and undergraduate students with mentors in laboratories or clinics at LSU Health Sciences Center, University Medical Center, and the Louisiana Cancer Research Consortium (LCRC). The Summer Research Internship Program allows students to cultivate their interest in pursuing careers in either basic or clinical sciences. During the program students conduct their own research project or work on part of an ongoing research project. Funding support for this program comes from:

- **Entergy Corporation**
- **LSUHSC School of Medicine, Office of the Dean**
- **Louisiana Cancer Research Center (LCRC)**
- **National Institutes of Health (NIH), National Cancer Institute (NCI)**
- **National Science Foundation (NSF), Research Experiences for Undergraduates (REU) Program**

Drs. Gregory and Tsien would like to extend their special appreciation to mentors, laboratory and administrative personnel, and poster session judges who helped make the Summer Research Internship Program a success! Their assistance with this project affords each student a chance to be part of a bigger, ongoing research project and allows them an opportunity to pursue their career goals.



Hanna Almoaswes
Undergraduate
Xavier University, New Orleans, Louisiana

Dean Smith
Dean and Professor
LSU Health Science Center School of Public Health

“Addressing the Complexity of Orphan Drug Pricing”

Witnessing her son's face twitching, unable to control his own arms, let alone feed himself, Abbey Meyers could not be more relieved to put a name to the illness afflicting her child: Tourette's Syndrome.

Ms. Meyers joined the Tourette's Syndrome Association, which started off small in 1972, and through lobbying with influential people, ended up playing a huge role in promoting drugs for rare diseases like Tourette's through the Orphan Drug Act of 1983.

A Rare Disease according to rarediseaseday.org is defined in the United States as a disease that, "... affects fewer than 200,000 Americans at any given time." The Orphan Drug Act was created to spur innovation in rare disease treatment through incentives: market exclusivity for seven years; instead of the usual five, or even twelve for biologics; tax benefits; clinical research subsidiaries; and exemptions for usual drug application fees charged by the Food and Drug Administration, lowering the cost of drug development. Despite these incentives, out of the 7000 rare diseases that exist, only about 5% have treatments. Many available drugs are very expensive. Orphan drug pricing is complex and in need of explanation and new solutions.

My research examined drug development narratives, created typologies based on patterns of how drugs came to market, and analyzed whether pricing was justified. Numerous narratives justify pricing. The media's focus tends to be on unjustifiable pricing, creating distrustful tension that results in unfair scrutiny for all companies. Reasons for high prices of orphan drugs include the expense of developing new drugs, along with pricey failures encountered before the successful drug. Companies may charge high prices as a precaution to assure a sufficient profit before potential competition arises, or they may raise prices after acquisition of a drug when they seek to continue research and potentially create new, innovative drugs.

A key finding is that some drugs fit justified typologies; however, there are also a few that fit typologies for unjustified high prices. Therefore, we need policy changes. Transparency may be necessary to hold companies accountable and allow the public to learn when prices compromise accessibility. Other reforms include prohibiting "pay for delay" tactics that stagnate the introduction of generics into the market and limiting patent extensions strictly due to an improvement or additional benefit in an altered form of a drug. Defining what constitutes as a rare disease more rigidly and decreasing coverage under the Orphan Drug Act could also result in lower prices. The ultimate solution to inaccessibility of high price drugs is unclear due to the complexity of rare disease pricing. However, there are steps that could be taken to improve the situation.

Joussette I. Alvarado

Undergraduate

Loyola University New Orleans, New Orleans, LA

Mentor: Jovanny Zabaleta

Department of Pediatrics-Stanley S. Cancer Center, LSUHSC, New Orleans, LA

“Immune profiles of colorectal cancer in African American and Caucasian individuals”**Abstract**

Colorectal cancer (CRC) is one of the leading causes of cancer related deaths worldwide for both genders, but the prevalence of the disease in the U.S. is disproportionately higher in African American (AA) than in Caucasian (CA) individuals. Inflammation is considered a hallmark of cancer. The activity of inflammatory cells may lead not only to genetic instability but also to mechanisms such as tissue remodeling, angiogenesis, and treatment resistance. Recent data suggest a differential role played by immune cells in the development of CRC. Understanding how these cells are differentially associated with immune surveillance and how they can be modified to enhance future therapies, i.e. immunotherapy, is essential to reduce CRC disparities. This is particularly important in the context of the genetic differences that underlie higher inflammatory responses in some individuals. These responses may lead to changes in tumor cell transcriptome to adapt to the challenge of the host's immune system. We have previously shown, in a highly admixed population, that European genetic ancestry is associated with adenomatous polyps (AP) and that African genetic ancestry is associated with CRC, even after adjusting for many non-genetic risk factors. We have recently shown that a correlation of the African ancestry and the presence of a pro-inflammatory haplotype in the *ILB* gene, -3737C/-1464G/-511T/-31C, increases the risk of CRC. Interestingly, our replication of the study in AA individuals with CRC showed an increased prevalence of the same haplotype. This haplotype has been associated with increased levels of transcription of the *IL1B* gene promoter. In addition, suppression of IL1- β responses decreases CRC burden in mouse models. This action seems to be carried out through changes in the type of cellular infiltration into the tumors. Taken together, these results suggest a significant role of the immune system in mediating the disparities of CRC observed the U.S. between AA and CA. Our long-term objective is to establish a correlation between the genomics of CRC with the immune response and cellular infiltration in African American individuals. The immediate goal of this study is to validate by real-time PCR recent findings in the lab showing a differential immune infiltration of CRC tissues from AA and CA. Similarly, our goal is to set up the conditions for the immunohistochemistry validation of these biomarkers as well as to determine the effect that secreted products from CRC cell lines may have on the expression of those markers in peripheral blood mononuclear cells (PBMC). ***We hypothesize that the degree and type of immune infiltration in colorectal cancer (CRC) tissues of AA individuals is different from those of CA.***

Sara, I, Beaulieu

Undergraduate

Louisiana State University, Baton Rouge, Louisiana

Mentor's Name: Patrick Greiffenstein (MD)

LSU Health Science Center, UMC, Department of Trauma Surgery

“Evaluation of Makeshift Tourniquet Efficacy on a Simulated Model of an Exsanguinating Limb”

Background: Tourniquets are a device used in emergency situations to stop profuse bleeding. Tourniquets have been used for centuries using various techniques to achieve circumferential compression of the limb tissue in order to occlude the arterial flow upstream from the bleeding site. This simple technique is part of the Stop the Bleed (STB) campaign curriculum to educate the lay public as well as medical professionals in basic intervention skills to mitigate life-threatening exsanguination. Although many different commercial tourniquets exist, these are often not available in emergency situations before professional first responders (EMS, Fire, Police) arrive. Creating a makeshift tourniquet from available material is theoretically easy and could help prevent further blood loss until that happens. However, it is unclear if a makeshift tourniquet might work as effectively or if the basic technique for making a tourniquet is able to be taught effectively to lay persons taking STB courses. The purpose of this study was to test whether a makeshift tourniquet is as effective and easy to use as the commercial army grade Combat Application Tourniquet (CAT).

Methods: A simulated model of a thigh was designed and developed to test the effectiveness of makeshift tourniquets. The thigh model was built using materials with similar densities, size and consistencies of human limb tissue. Different types of commercially available material were used to construct the thigh model. The flow pressure through the simulated vessel was set to 150 mm/Hg, the systolic blood pressure of an average adult. The model was validated by professional emergency medical providers with experience treating patients with exsanguinating limb injuries (two Trauma surgeons and one Navy special operations medic with combat experience). Subjects were asked to apply each tourniquet type on the leg model to achieve cessation of flow (COF) through the simulated vessel in the model emptying into a reservoir that was visible. Each application was timed from the moment of picking up the material/tourniquet to cessation of flow of water into the reservoir. Pressure within the proximal vessel was maintained at 150mmHg using an IV pressure bag with a manometer. Each application was timed and recorded and the participants were given a five point questionnaire to fill out. First the CAT was tested in this manner. Next, a brief video was shown demonstrating the technique for making and applying a tourniquet from household materials and the participants were asked to perform the maneuver in a timed fashion on the ELS. Eight trained surgeons with trauma experience were tested in this manner. Data was collected on the time to COF and amount of fluid lost; basic comparative statistics were performed.

Caroline F Bickerton
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Dr. Francesca Peruzzi
Louisiana State University Health Sciences Center, Stanley S. Scott Cancer Center

Role of 4E-BP1 and the Unfolded Protein Response in Triple Negative Breast Cancer Cell Survival

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype characterized by a lack of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). This disease is associated with high metastasis and poor prognosis and constitutes 15-20% of all breast cancers. As a heterogeneous subtype lacking normal hormone receptors, TNBC proves difficult to treat. Extensive research is now being conducted to identify biomarkers and novel therapeutic treatments for this invasive cancer.

Previous research has demonstrated that the unfolded protein response (UPR) triggered by endoplasmic reticulum (ER) stress plays a crucial role in TNBC cell survival and metastasis. The UPR is responsible for reducing the protein translational load on the ER under cellular stresses such as nutrient deprivation, which is common in the tumor microenvironment. While the UPR enables cell survival for a period of time, prolonged ER stress prompts the UPR to activate apoptotic signaling. TNBC, however, manipulates the UPR to evade cell death and continue cell growth even under sustained ER stress.

A key regulator of protein synthesis that is differentially expressed in TNBC is the eukaryotic initiation factor 4E (eIF4E)-binding protein 1 (4E-BP1), which binds to eIF4E in order to prevent translation at the 5' cap of an mRNA transcript. Although 4E-BP1 functions as a negative regulator of eIF4E and therefore as a tumor suppressor, cancers like TNBC utilize deregulation of 4E-BP1 to promote internal ribosome entry site (IRES) translation of specific oncogenic proteins. Furthermore, ATF4, a transcription factor involved in UPR signaling, has been shown to activate 4E-BP1 production. Recent findings suggest that 4E-BP1 may also regulate ATF4 expression in a positive feedback loop.

We set out to determine the role of 4E-BP1 and the UPR in the IRES-dependent translation of oncogenic proteins in TNBC cells. In this study, we found that 4E-BP1 is overexpressed and more active under cellular stress, correlating to an increase in ATF4 expression. Knockdown of 4E-BP1 prompts a decrease in ATF4 expression, suggesting that 4E-BP1 is both regulated by and mediates expression of ATF4. Expression of c-MYC, a protein containing an IRES sequence, also strongly decreases when 4E-BP1 is silenced, which suggests that 4E-BP1 may regulate IRES translation activation. We aim to apply these findings to the development of potential therapeutic targets of this deadly cancer.

Angelle L. Brown
High School Student
Louise S. McGehee School, New Orleans, LA

Tung-Sung Tseng and Hui -Yi Lin
LSUHSC School of Public Health

Health Behavior Differences between African-American and White Breast Cancer Survivors

Background: Breast cancer is the most commonly diagnosed cancer among women in the United States, and the second most common cause of cancer deaths in women. African-American women with breast cancer suffer worse clinical outcomes than White women with breast cancer. For example, the median age of breast cancer diagnosis in the US is younger for African-American women than White women, with a median age of 59 and 63, respectively. Additionally, in 2015, breast cancer death rates were 39% higher in African-American women compared to White women. In order to reduce the racial disparity of breast cancer mortality, it is essential to understand demographic and health behavior differences between different racial groups. The primary objective of this study is to evaluate differences in selected health behaviors (physical activity, alcohol consumption, and smoking status) and demographic factors between African-American and White breast cancer survivors.

Methodology: In this study, we analyzed data from African-American and White breast cancer survivors over the age of 18 in the National Health Interview Survey (NHIS) dataset from 2012-2017. NHIS is a national dataset that routinely investigates a broad range of health topics through personal household interviews. The health behaviors of interest included physical activity (none or moderate/vigorous), alcohol consumption (never, former, current infrequent, current light/moderate, or heavy), and smoking status (never, former, or current). Other selected demographic and clinical factors included age at interview, age at breast cancer diagnosis, education status, and income status. Chi-square analysis and t-tests were used to test differences of selected demographic and health behaviors between African-American and White women for categorical and continuous variables, respectively.

Results: This study included 3,397 breast cancer survivors, of which 375 (11.0%) were African-American and 3,022 (88.9%) were White. African-American women were more likely than White women to have lower levels of physical activity (73.5% vs. 61.4% with no physical activity, respectively, chi-square test $p < 0.001$), lower levels of alcohol consumption (1.4% vs. 5.5% heavy alcohol consumers, respectively, $p < 0.001$), lower education levels ($p < 0.001$), and lower income levels ($p = 0.027$). 24% of African American women had less than a high school education. 31% had a high school education and 45% had more than a high school education. However, differences in smoking status were insignificant (10.2% vs. 9.8% current smokers, respectively, $p = 0.388$). African-American women were younger than White women (t-test $p < 0.001$). At the time of the interview, the average age for African American women was 66. The average age for White women was 69, so White women tended to be older. There was no statistically significant differences in age at breast cancer diagnosis were found ($p = 0.198$).

Conclusion: Our study findings showed that African American breast cancer survivors tended to have worse health behaviors and lower socioeconomic status than White breast cancer survivors. Understanding the factors that are associated with breast cancer health disparities are necessary to decrease the morbidity rate in African-American women.

Ann M. Byerley

Undergraduate

Rochester Institute of Technology, Rochester, NY

Bruce Bunelle and Frank Lau

Center for Stem Cell Research and Regenerative Medicine, Tulane University, New Orleans, LA
LSUHSC Surgery Department, New Orleans, LA**CHARACTERIZATION OF HUMAN AMNIOTIC FLUID STEM CELLS (hAFSCs)**

Background: Amniotic fluid (AF) is an inexpensive and easily obtainable source of adult stem cells collected during third trimester elected cesarean sections. Human amniotic fluid stem cells (hAFSCs) are now considered a new source for therapy because of their ability to differentiated into multiple cell lineages.

Aim: The aim of the present study is to characterize stem cells isolated from hAFSCs.

Methods: Flow cytometry was performed on AF and hAFSCs. Colony forming unit (CFU) for day 14 and proliferation assays for day 1, 7, 14 and 21 were done for hAFSCs.

Results: Flow cytometry data show that AF consists of a heterogeneous cell *population* of hAFSC that express the mesenchymal stem markers (CD90, CD73, and CD105), epithelial cells (CD326) and immune cell makers (CD14, CD3, CD45, CD117, MAC1). CFU data indicated the self-renew ability of hAFSCs. Proliferation data shows the exponential cell growth over a period of 21 days.

Conclusion: The AF provides a novel source of *stem cells with* potential use in cellular therapy and regenerative medicine.

Chelsea N. Callicotte

Post-baccalaureate

California State University Northridge, Northridge, CA

Scott Edwards, Ph.D.:

LSU Health Sciences Center, Alcohol & Drug Abuse Center of Excellence, Neuroscience Center of Excellence

“Dopamine Signaling as a Non-Opioid Analgesic Strategy for Chronic Pain”

Prescription opioids are a critical first-line treatment for chronic pain. However, chronic treatment with opioids can lead to paradoxical increases in pain sensitivity, termed hyperalgesia, which may promote use of opioids to manage worsening pain symptoms. Decreases in ventral striatal dopamine have been reported in chronic pain states. In this study, we used a rodent model of chronic inflammatory pain to investigate the therapeutic efficacy of dopamine receptor agonist medication, pramipexole, as a non-opioid analgesic strategy for chronic pain. Adult male Long-Evans rats received Complete Freund's Adjuvant (CFA) or saline injections into the left hindpaw. CFA animals demonstrated a persistent decrease in paw withdrawal thresholds in the left paw, indicating mechanical hyperalgesia. Animals received injections of pramipexole (1mg/kg, s.c.) or vehicle (saline) 1 hour prior to measuring von Frey thresholds. Our findings indicate that systemic administration of pramipexole attenuated pain in animals with chronic inflammatory pain by increasing paw withdrawal thresholds following acute and repeated pramipexole treatment. Currently, we are investigating the neurobiological mechanisms underlying the anti-hyperalgesic efficacy of pramipexole using Western blotting. We expect that this efficacy will be based on the drug's ability to normalize dopamine signaling and reduce neuroinflammation and ROS in the ventral striatum.

Taylor K. Collins

Undergraduate

University of Southern California, Los Angeles, California

Dr. Tiffany Wills, PhD:

Department of Cell Biology and Anatomy, LSU Health Sciences Center

“The Effects of Adolescent Alcohol Exposure on Stress Circuitry and Behaviors”

Individuals who consume alcohol as adolescents are known to have an increased risk of developing an alcohol use disorder. However, the mechanisms leading to this increased vulnerability are unknown. To help uncover these mechanisms, we utilize a mouse model of adolescent intermittent alcohol vapor exposure (AIE) and examine its effects on the adolescent brain. One brain region of particular interest is the bed nucleus of the stria terminalis (BNST) because of its role in stress and negative affect induced alcohol relapse. Previous work in the lab has demonstrated that adolescent alcohol enhances glutamate release and plasticity in the BNST. The current work set out to test the hypothesis that adolescent alcohol exposure activates BNST inputs involved in stress/fear-related circuitry and produces long-term changes in contextual-fear conditioning, a behavior that is in part mediated by the BNST. To do this, male C57BL/6J mice were exposed to two four-day cycles of 16hr in ethanol vapor chambers with 8hr of withdrawal, separated by three undisturbed days. A subset of mice were injected with Green Retrobeads into the BNST prior to AIE, which tags neurons in brain regions that project to the BNST. These injected mice were then perfused 4-6hrs after acute withdrawal from AIE. Immunohistochemistry was then performed to identify co-labeling of the immediate early gene *c-fos* and cells labeled with tracer. Specifically, we quantified *c-fos* and tracer co-labeling in the locus coeruleus, paraventricular nucleus, dorsal raphe nucleus, and central and basolateral amygdala to identify stress sensitive regions that project to the BNST and are activated by AIE withdrawal. A separate cohort of mice were also exposed to AIE and then allowed to voluntarily consume alcohol in an intermittent two bottle choice paradigm (escalating to 20% ethanol for 4 weeks). After a 6-7 day water only withdrawal period, mice were tested in contextual fear conditioning. Mice receive 6 low-intensity (0.4 mA, 1 sec) electric shocks paired with a context. The amount of time spent freezing before and after the shocks, as well as anxiety-like activity in the center of the open field prior to shock, is recorded. Although we have previously found that an AIE history increases post-shock freezing compared to air vapor in adult mice, our mice exposed to AIE in conjunction with voluntary alcohol drinking did not demonstrate increased freezing compared to air vapor-alcohol drinking mice ($p > 0.05$). However, we did find that alcohol drinking increased anxiety-like behavior regardless of adolescent vapor exposure when compared to mice exposed to vapor without drinking history ($p = 0.013$). Average alcohol intake over the four weeks was not correlated with fear or anxiety-like activity (p 's > 0.05). Voluntary alcohol consumption attenuated the enhancement in fear conditioning previously seen in AIE-exposed mice, but increased anxiety-like activity. These data suggest that altered activation of stress circuitry by adolescent alcohol promotes an altered fear and anxiety-like behavioral state that is sensitive to voluntary alcohol intake.

***Michael J. Constans**

Undergraduate Louisiana State University, Baton Rouge, LA

Mentor's Name: Dr. Nicholas Gilpin
LSUHSC, Department of Physiology

“A heterogeneous population of ventral tegmental area neurons project to the central amygdala”

Alcohol Use Disorder (AUD) is a disorder characterized by alcohol dependence that affects around 16 million Americans. While the neural adaptations that occur in individuals with AUD are not entirely understood, they may involve alterations in stress and reward circuitry. Here, we focused on a connection between the ventral tegmental area (VTA) and the central amygdala (CeA), a circuit that possibly plays a role in alcohol dependence-associated behaviors. The CeA is a region of the brain that is primarily associated with feelings of stress and alcohol dependence-associated behaviors. The VTA, however, is associated with more positive emotions and is sometimes referred to as the “reward center” of the brain. Previous work from our lab has demonstrated increased activity of CeA-projecting VTA neurons in the alcohol dependent rat relative to naïve controls. Our overarching hypotheses are that following the induction of alcohol dependence, this circuit becomes activated and contributes to alcohol dependence-associated behaviors.

However, this circuit is largely under-characterized, even in the naïve brain. Previous work in our lab has determined that only about 30% of CeA-projecting VTA neurons are dopaminergic, suggesting a substantial population of putative glutamatergic or GABAergic neurons. Electrophysiological studies from our lab indicate that stimulation of VTA terminals in the CeA can elicit excitatory postsynaptic currents, suggesting that at least some of these neurons are capable of glutamate release. The goal of this research is to better characterize this circuit in alcohol-naïve rats by examining the expression profiles of CeA-projecting VTA neurons. We hypothesized that there would be a substantial portion of glutamatergic CeA-projecting VTA neurons.

This hypothesis was tested using retrograde tracing and *in situ* hybridization on tissue sections of alcohol-naïve. VTA-containing tissue sections were imaged and analyzed to determine if the CeA-projecting neurons contained vesicular glutamate transporter 2 (vGluT2), which would indicate that neuron is glutamatergic, or tyrosine hydroxylase (TH), which would indicate that neuron is dopaminergic.

Of the CeA-projecting neurons analyzed, some were found to have projected from the substantia nigra (SNc), a neighboring brain region to the VTA. In both the CeA-projecting VTA neurons and the CeA-projecting SNc neurons, there was a substantial amount of neurons that contained neither vGluT2 or TH, indicating they were neither glutamatergic or dopaminergic (putative GABAergic). In the substantia nigra, a large percent of the neurons were found to be only glutamatergic while a smaller percent were found to be able to express both vGluT2 and TH. In the VTA, there was an even amount of dopaminergic and glutamatergic neurons while none were able to co-express vGluT2 and TH. These findings are important for better characterizing the expression profile of CeA-projecting neurons and will help to show how they affect the CeA downstream. Future research will extend this analysis to include expression profiles of GABAergic CeA-projecting neurons, as well as determining how these neurons are altered in the alcohol dependent brain. Ultimately, characterization of this circuit could provide useful targets in the treatment of individuals with AUD.

Dean John Hendrix T. Dante
Undergraduate
Tulane University, New Orleans, LA

Lothar Lauterboeck and Qinglin Yang
LSUHSC: Cardiovascular Center of Excellence

“Direct evidence IF1 preserves mitochondrial ATP during hypoxia”

Abstract

Adenosine triphosphate (ATP) is an organic chemical that provides energy in living cells. Mitochondria generate over 90% of ATP via oxidative phosphorylation driven by proton motive force (PMF). The PMF drives the forward rotation of ATP synthase to synthesize ATP. Under hypoxic conditions, ATP is hydrolyzed, driving the backward rotation of ATP synthase, which pumps back proton to maintain mitochondrial membrane potential. A naturally occurring inhibitor (ATPase inhibiting factor 1, IF1) has long been shown to be an ATP synthase regulator, but it remains unclear that IF1 inhibits the backward rotation or the entire rotation of ATP synthase. An upregulation of IF1 has been found in many cancer cells, failing hearts and diabetic skeletal muscle. However, the exact role of IF1 on ATP synthase activities remains controversial, due in part to the lack of tools to directly monitor the spatiotemporal ATP contents in cells.

In this work, we used an imaging approach to investigate how IF1 determines the changes of mitochondrial ATP in normoxia and hypoxia to understand the role of IF1 in regulating ATP synthase. We transfected mouse embryonic stem cells (MEF) derived from IF1 knock-out, overexpression, and wild-type mice with a plasmid carrying a mitochondria-specific dye that visualizes mitochondrial ATP production (mitoMaLionR). The plasmid was delivered by the lipid-base transfection system (Lipofectamine). Cells were incubated for 24h with the DNA/Lipofectamine followed by a medium change and recovery for 24h. Images were taken using Cytation 5 with 20x and 40x objective lens. To determine if mitoMaLionR is localized in the mitochondria, cells were double-stained with MitoTracker Green FM. The double staining showed that mitoMaLionR was localized in the mitochondria with minimal cytosolic fluorescence. No difference in mitoMaLionR was found among the three experimental groups under normoxia. After switching to hypoxia (2% O₂) for 3h, MEF with IF1 overexpression exhibited higher fluorescent intensity than that of control cells, indicating that ATP was preserved. In the IF1 knock-out cells, the fluorescent intensity was significantly reduced compared to the control.

In conclusion, the used mitochondrial detection probe is a powerful tool to visualize the mitochondrial ATP. The mitochondrial ATP imaging results support that IF1 inhibits the hydrolysis of the ATP synthesis to preserve ATP under the hypoxic condition.

Jasmin E Elnaggar
High School
Benjamin Franklin High School, New Orleans, Louisiana

Mentor's Name: Dr. Levitt and Dr. Simon
LSUHSC

“Alcohol-Mediated Dysregulation of Mitochondrial Protein Expression in Skeletal Muscle of SIV-Infected Female Rhesus Macaques”

Rates of heavy drinking in people living with human immunodeficiency virus (PLWH) is almost twice that found in the non-HIV-infected population. At-risk alcohol use in PLWH disrupts skeletal muscle regulation, which is the major metabolic tissue regulating whole-body energy homeostasis. Mitochondria are essential for skeletal muscle metabolic health. As people are living longer with HIV due to antiretroviral therapy (ART), age-related comorbidities are increasing in PLWH including insulin resistance and cardiometabolic risk. One mechanism contributing to these comorbidities is mitochondrial dysfunction. Proteins implicated in mitochondrial biogenesis and function are peroxisome proliferated-activated receptor (PPAR) gamma coactivator (PGC)-1 α , PGC-1 β , PPAR α , and mitochondrial transcription factor A (TFAM). PGC-1 α and PGC-1 β are transcriptional coactivators considered master regulators of mitochondrial biogenesis, PPAR α is a transcription factor for fatty acid oxidation enzymes, and TFAM is a mitochondrial transcription factor. Previous work has shown that chronic binge alcohol (CBA) dysregulates expression of genes implicated in mitochondrial function in skeletal muscle of SIV-infected female rhesus macaques. CBA decreased PGC-1 β and TFAM expression, increased PPAR α expression, and did not change PGC-1 α expression. This led to the hypothesis that CBA similarly alters mitochondrial protein expression in skeletal muscle of SIV-infected, ART-treated female rhesus macaques. Macaques (N=10) were administered a daily binge dose of alcohol (CBA, 13-15g/kg/week) or isovolumetric water (VEH) for three months prior to SIV infection and throughout the duration of the study. ART administration was initiated 2.5 months after SIV infection. At study endpoint (approximately 12 months post-SIV infection), skeletal muscle tissue was collected and homogenized. Cytosolic and nuclear-enriched protein fractions were extracted, and Western blotting was performed to determine expression of PGC-1 α , PGC-1 β , PPAR α , and TFAM. There was a trend ($p=0.056$) for CBA to increase PPAR α protein expression in the nuclear fraction, but no other CBA-mediated differences were observed. Decreased gene expression of PGC-1 β with no change in protein expression indicate the potential for post-translational modifications. The increase in nuclear expression of PPAR α could indicate increased reliance on fatty acid oxidation to meet skeletal muscle energetic demands. Future directions include analysis of CBA-mediated post-translational modifications to PGC-1 β (e.g., acetylation) that could alter its activity and measurement of expression of proteins downstream of PPAR α .

Tivon M. Eugene

Undergraduate

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Mentors: Lucas Albrechet-Souza, Ph.D & Nicholas W. Gilpin, Ph.D
Louisiana State University Health Sciences Center, Department of Physiology

“The Effects of Traumatic Stress on Reactivity to Acoustic Stimuli in Rats with a History of Alcohol Consumption”

Post-traumatic stress disorder (PTSD) is marked by symptoms of avoidance, re-experiencing, and hyperarousal that develop subsequent to one or more traumatic events. PTSD affects men and women differently. Not only are women twice as likely as men to develop PTSD, they experience different symptoms and comorbidities associated with PTSD. Alcohol Use Disorder (AUD) is commonly comorbid with PTSD. It has been estimated that approximately a third of people with PTSD also meet the criteria for AUD. PTSD-AUD comorbidity is also associated with a decreased response to treatment, as well as a poorer prognosis when compared to individuals with only one of either of the disorders. In our laboratory, we use the conditioned place aversion (CPA) model of PTSD, which involves exposure to bobcat urine. Male and female rats were initially exposed to alcohol consumption over a period of five weeks using an intermittent two-bottle choice model. During this period, alcohol was given on Mondays, Wednesdays, and Fridays for a period of 24 hours, and measured along with water consumption. Rats were then subjected to the CPA protocol and classified as Avoiders or Non-Avoiders based on their stress reactivity as measured by avoidance of the predator odor-paired context. Two days post-stress, rats were tested for responsivity to acoustic stimuli through the use of the acoustic startle response (ASR). With our research project, our lab's aim is to evaluate if a history of alcohol-drinking affects ASR in male and female rats exposed to traumatic stress. By increasing our understanding of comorbid PTSD-AUD and its effects on individuals, this information can then potentially be useful to improve the treatment options available to those individuals.

Acknowledgements: Funded by the Entergy Workforce Training Grant.

Emma R Freeman
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Angela Amedee, Phd.:
LSUHSC

“Characterizing Drug Resistant Virus in SIV-Infected Rhesus Macaques Treated with ART”

The Simian Immunodeficiency Virus (SIV) infected rhesus macaque exposed to chronic binge alcohol (CBA) has proven to be a highly useful model for elucidating the effects of alcohol misuse on Human Immunodeficiency Virus (HIV) disease. The use of antiretroviral therapy (ART) has significantly reduced the morbidity and mortality from HIV infections and now triple drug therapy is commonly used for treatment of people chronically infected with HIV. Characterizing and understanding drug resistance (DR) to ART in the SIV-infected CBA macaque is important for the refinement of our model. The objective of this study was to characterize the efficacy and development of DR in ART-treated macaques.

In our studies, rhesus macaques were given daily infusions via gastric catheter of ethanol or saline for three months prior to SIV-infection. Ten weeks post SIV infection, animals were treated with ART for the duration of the study. Viral loads were measured in plasma weekly. In our well-studied model design, 21 female and 22 male animals received a two ART regimen consisting of reverse transcriptase inhibitors and also received a typical macaque diet which is fairly lean. In a pilot study, 5 female animals received a three drug regimen that consists of two reverse transcriptase inhibitors and an integrase inhibitor, to better represent the clinical treatment currently prescribed for HIV. In this pilot study, animals also received a high fat diet, or a “western” diet, again to better represent our clinical HIV population. To evaluate DR, virus was purified from plasma, *pol* gene regions were then amplified by RT-PCR, cloned, sequenced and compared to DR database.

In the classical model study, viral loads decreased with the initiation of ART. However, this decrease did not reach undetectable levels in all of the animals, which is the ultimate goal of using ART. DR mutations were found in animals with detectable plasma virus. These mutations mapped to those observed in HIV. In the pilot study model, initial viral loads were higher than in the previous study animals, which may be due to their high fat diet. With initiation of the new ART regimen, viral loads have dropped lower in some animals, but the alcohol animals had a poorer response to ART. Viral isolates from these five animals are being analyzed to determine if DR is a factor contributing to persistent viremia. As the DR is thoroughly evaluated in this pilot study, it will provide valuable insight for developing a clinically-relevant model of HIV disease and alcohol misuse.

Mark Fisher

Undergraduate Degree
University of Colorado, Boulder, CO

Jennifer J. Lentz, Ph.D.
LSUHSC

“Pharmacokinetics of ASO therapy in a mouse model of Usher syndrome”

Usher syndrome (Usher) is the most common inherited cause of deaf-blindness. Type 1 Usher is the most severe form, characterized by hearing impairment at birth and retinitis pigmentosa (RP) beginning in early adolescence. 2.5% of Usher cases are caused by mutations in the USH1C gene, which encodes the protein harmonin. The function of harmonin in the retina and the cause of RP following its mutation are not known. The c.216G>A (216A) mutation in USH1C causes nearly all type I Usher in the Acadians of Louisiana and Canada. Previously, our lab created a knock-in mouse model that contains the human USH1C 216A mutation. The USH1C mice are deaf, and have vestibular and visual dysfunction similar to patients. We then developed antisense oligonucleotides (ASO) targeting the 216A mutation and showed that treatment with various deliveries and doses rescues hearing, balance and vision in the USH1C mice. To further develop this ASO therapy for the treatment of visual loss in patients, we sought to improve our understanding of the pharmacokinetics of ASOs after local injection in the eye. USH1C and control mice were treated with various doses of ASOs by intravitreal injection and ASO levels were quantitated in retinal tissues harvested 2 weeks post-treatment using HPLC and mass spectrometry. These results will inform future studies on the safety and biodistribution of ASOs in the retina after local treatment.

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Estrogenic Regulation of Lysyl Oxidase in Cardiac Fibroblasts

According to the American Heart Association's 2019 update, heart disease remains the number one cause of death in the United States, accounting for approximately 363,452 deaths in 2016 and costing 218.7 billion dollars annually. Although cardiac disease is the leading cause of death in both men and women, women develop heart disease ten years later than men. Yet, after menopause, the incidence of heart disease in women is similar to men, suggesting that estrogen may play a part in premenopausal cardioprotection. Previously, the Gardner lab found that ovariectomized rats lost the cardioprotective effects demonstrated by intact females. Further, adverse cardiac remodeling was associated with high lysyl oxidase (LOX) expression and activity in male rats, with mortality rates of 24.5% in male rats to 2.5% in females after 8 weeks of volume overload stress. It is not known if estrogen regulates cardiac LOX and if this regulation plays a role in premenopausal cardioprotection.

We hypothesize that estrogen regulates LOX activity and expression through the TGF β pathway. To assess how estrogen regulates LOX, cardiac fibroblasts were isolated from 10-week-old, adult, female rats and treated with estrogen (10 μ M), TGF β (10 μ M), estrogen + TGF β (10 μ M), estrogen +TGF β antagonist (SB-505124, 10 μ M), DPN (estrogen receptor β agonist, 10 μ M), PPT (estrogen receptor α agonist, 10 μ M) and vehicle (DMSO). After the cells were plated, and expanded, they were exposed to the above treatments for up to 24 hours. LOX activity in conditioned media was assessed via commercially available fluorescent assay (AAT Bioquest, Sunnyvale, CA). LOX expression was assessed using qRT-PCR of fibroblast mRNA. Findings in LOX activity assay showed an arbitrary fluoresce per minute of 17210 \pm 587.9 in DMSO, 23703 \pm 1044 in estrogen, 18460 \pm 769.5 in DPN (β agonist), and 22238 \pm 1068 In PPT (α agonist). Estrogen significantly increased LOX activity (38% versus vehicle DMSO; $p < 0.05$). The effects of TGF β and additional measures of LOX mRNA via qPCR are underway.

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“Norepinephrine causes a change in REST expression and subcellular localization in cerebellar interneurons”

Our lab has previously shown that stress induces a change in AMPA receptor (AMPA) subunit composition in molecular layer interneurons of the cerebellar cortex. This involves the upregulation of the AMPAR subunit GluA2 and induces a phenotypic switch in synaptic AMPAR subtype from GluA2-lacking to GluA2-containing. Such a switch alters neurotransmission within the cerebellar cortex and cerebellar output to other brain regions, with important implications for behavior and cognition. We therefore explored the mechanism by which stress may induce transcription, since the change in GluA2 is a transcriptional-dependent increase.

RE1-Silencing Transcription factor (REST) is a transcriptional repressor of many neuronal genes, including GluA2. Our results have shown that stress reduces the suppression of GluA2 transcription by REST. We explored the possibility that the expression of REST was downregulated after stress. To mimic stress on the cellular level, cerebellar neurons in culture were treated with norepinephrine (NE), a hormone that increases in the presence of stress. Previous in vitro experiments have shown that NE signaling is both required and sufficient to induce an upregulation of synaptic GluA2 and induces an overall decrease in REST expression in cerebellar interneurons. Here we examined a second mechanism underlying a stress-induced decrease in REST function, in which NE causes REST to translocate from the nucleus to the cytoplasm. This type of redistribution is seen in neurodevelopmental diseases, such as Huntington's, where REST gets trapped outside the nucleus and it cannot repress gene expression allowing for GluA2 to transcribe. The current study is focused on analyzing REST expression and subcellular localization in an in-vitro model of stress.

REST expression was analyzed by taking cerebellar cultures from 7 day old wildtype or GAD-GFP mice. Cultures were then maintained in vitro for 14-18 days. After incubation in the presence or absence of norepinephrine for 3 hours, cerebellar neurons were immunostained for REST and parvalbumin to label inhibitory interneurons. Immunofluorescence images were acquired using a confocal microscope and a 10-plane z-stack image was taken of each cell. The immunofluorescence intensity of REST (REST-ir) in the nucleus and cytoplasm were quantified in GFP- or PV-expressing interneurons. The nucleus to cytoplasmic ratio was calculated to evaluate the distribution of REST in the cell.

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Mentor: Dr. Li Shen M.D/PhD

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Construction of a short-lived fluorescent protein transcriptional reporter

Chlamydia trachomatis is an obligate intracellular Gram-negative bacterium that causes various acute and chronic diseases, including eye infections, trachoma, genital infections and the more invasive sexually transmitted infection; lymphogranuloma venereum (LGV). Despite the increased awareness and intensified testing of the *C. trachomatis* diseases, a rising number of new infections has been reported. Its high contagion and detrimental sequel have continued to be a global health burden. A better understanding of the *C. trachomatis* biology is critical for the development of new therapy against *C. trachomatis* diseases.

Studies of the unique chlamydial developmental cycle and gene expression have been greatly facilitated by recent success in the development of fluorescence protein reporter gene using transformation of *C. trachomatis* with a plasmid. This powerful tool allows to directly visualize dynamic growth of *Chlamydia in situ* in individual living cells. We have shown that a red-shift green fluorescence protein (rsGFP) driven by a chlamydial *ompA* promoter has a half live of ~3 hours in a transformed *C. trachomatis* LGV L2 strain under the condition that results in a productive infection. An ideal reporter gene of temporal transcription programs includes a short half-life that avoids extended accumulation when transcription is turning off. In an effort to meet this criterion, here, we adapted a GFP variant designated as GFP[LVA] which contained a short peptide sequence to the C-terminal end of intact GFP and rendered GFP's sensitivity to the action of endogenous tail-specific protease, *tsp*, in *E. coli*. *C. trachomatis* encodes a homologue of Tsp. To test the hypothesis that proteolytic degradation of GFP[LVA] is a conserved trait in *C. trachomatis*, we constructed a new reporter plasmid pCtGFP[LVA] for chlamydial transformation. Briefly, *E. coli* strain DH10 β cells (New England Biolabs) were used for the molecular cloning. A two-step cloning strategy was used to construct vector pCtGFP[LVA]. First, the pPeuo[LVA]E was constructed by ligation of *SpeI*/*BstBI* digested pBC-A1-009 (this vector contains gene encoding GFP[LVA]) and the *SpeI*/*BstBI*-elevated fragment from pPvGFP:SW2. The latter fragment carries a chlamydial promoter of *euo* flanked by a ribosomal binding site of *tuf* gene. Second, the *SpeI* and *SmaI*-digested DNA fragment of pPeuo[LVA]E was inserted into the *SpeI*-(*Sal*-blunted)-digested pBOMBeuo. Therefore, the resultant plasmid pCtGFP[LVA] contains gene coding for GFP[LVA] under the control of an early promoter from chlamydial *euo* gene as well as eight open reading frames (ORFs) and an origin of replication from conserved *C. trachomatis* plasmid. This plasmid also contains an *E. coli* origin of replication and a *bla* gene encoding resistance to beta-lactam antibiotics allowing for selection of recombinants with ampicillin. We are presently working on transformation of *C. trachomatis* with pCtGFP[LVA] and determining 1) what is the half-life in *C. trachomatis*, and 2) whether the short-lived GFP[LVA] reporter gene could accurately track and predict the transient mRNA profile of the early chlamydial gene, *euo* during infection.

Haley, L, Hill

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Mentor: Ping Wang, Ph. D

Louisiana State University Health Sciences Center, Department of Microbiology, Immunology,
and Parasitology**“Identification of Isoform-Specific Intersectin Mutants in Human Pathogenic Fungus
Cryptococcus neoformans”**

Cryptococcus neoformans is an encapsulated basidiomycetous fungus that is prevalently found in the environment including the fecal matter of avians, especially of pigeons. *C. neoformans* can infect immunocompromised and healthy individuals leading to the development of life-threatening meningoencephalitis and lung infections. In fact, cryptococcosis is the fourth most common opportunistic infection in HIV patients. Immunosuppressed individuals, such as those who have undergone organ transplants, are also at a leveled risk of infection. As a result of limited treatment options, there are high mortality and morbidity rates amongst infected individuals. Reaching a greater understanding of the underlying pathogenesis mechanisms of *C. neoformans* is crucial to the development of potential treatment options.

Virulence factors including melanin, a polysaccharide capsule, and extracellular urease are critical to the virulence of *C. neoformans*. It is understood that the secretion of these virulence factors to the cell surface is made possible by means of intracellular trafficking. During hematogenous dissemination, *C. neoformans* displays an inclination for the host brain through its ability to infiltrate the usually impermeable blood-brain barrier to enter the central nervous system. However, uncertainty exists on the mechanisms adopted by the fungi to thrive within the host CNS after entry. Previous research has identified cryptococcal intersectin 1 (Cin1) as a novel endocytic protein significant to *C. neoformans* growth, virulence, and intracellular trafficking. Cin1 contains multiple domains and is expressed as two isoforms, Cin1-S (short isoform) and Cin1-L (long isoform), as the result of alternate mRNA splicing. Interestingly, Cin1-L is highly homologous to human CNS-specific intersectin 1, ITSN1, whose expression of the long isoform ITSN1-L is CNS-specific.

Given that Cin1-L shares high sequence homology with ISNT1-1 and Cin1-S was previously shown to have a significant survival advantage in the CNS through a murine model of cryptococcosis, we hypothesize that Cin1 and its isoform formation may play a role in infection, especially in CNS. To test this hypothesis, we plan to create a Cin1-L mutant for infection in comparison to Cin1-S.

Cryptococcus neoformans wild type strain JEC21 was previously transformed with the *CIN1-L* allele linked to G418 drug resistance and 57 transformants were collected. The mutant strains were screened using colony-PCR amplification of the *CIN1-L* allele by DNA sequencing. The 30 initially screened transformants displayed either the wild type sequence or both the wild type and the *CIN1-L* allele, and none contained the *CIN1-L* allele alone. Screening of the remaining transformants are in progress. Once the Cin1-L mutants are obtained, they will be used to infect the mouse model. By comparing with infection of Cin1-S mutant, our study will help to examine the mechanisms of the neurotropic property and pathogenesis of *C. neoformans*.

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Mentor: Douglas Johnston, PhD
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“*T. cruzi*-induced Changes in Cardiac Endothelial Cells”

Chagas' Disease is a parasitic infection caused by the kinetoplastid parasite, *Trypanosoma cruzi* and is endemic throughout South & Central America, and Mexico. It is estimated that as many as 8 million individuals are infected worldwide, including more than 300,000 currently residing in the USA with associated costs of over \$9M. *T. cruzi* is most commonly transmitted by triatomine (aka “kissing”) bugs, but can also be transmitted congenitally, via blood transfusion, and through ingestion of food & drink. Acute Chagas' is an inflammatory condition resulting in mild to moderate signs and symptoms lasting several weeks or months, but often goes undiagnosed. Untreated disease may advance to include serious cardiovascular and/or digestive pathology. Up to 30% of those infected with *T. cruzi* will develop Chronic Chagas' Disease (CCD) involving life-threatening cardiomyopathy. Treatments options are currently limited to only 2 drugs, both associated with limited efficacy and side-effects, and no vaccine is currently available. Symptoms of CCD include cardiac hypertrophy and dysfunction caused by excessive inflammation and fibrosis. While several cell types have been shown to contribute to this pathology, any potential involvement by cardiac endothelial cells is largely unknown. In this study, we aim to identify changes in cardiac endothelial phenotypes that may result in increased pro-fibrotic functions. As endothelial cells are known to undergo endothelial-to-mesenchymal transition (EndMT), we hypothesize that *T. cruzi* infection induces EndMT in cardiac endothelium, leading to increased cardiac fibrosis.

We have chosen to develop a multicellular, 3D model of chronic *T. cruzi* infection. In order to establish this model, we must first identify a baseline of gene expression profiles in both control and infected cell types, as well as validate our reagents- in 2D culture. For this report we have utilized Quantitative Reverse Transcriptase Polymerase Chain Reaction (QRT-PCR) to measure changes in gene expression, and immunocytochemistry (ICC) to characterize changes in protein expression in both cardiac microvascular endothelial cells (cMVEC) and cardiac fibroblasts (cFb) in 2D mono- and co-cultures. We find that cMVEC infection with *T. cruzi* tissue-culture-derived trypomastigotes (TcTs) results in both early and late changes in gene expression. The pro-inflammatory genes TGF β and IL-1 β are rapidly increased at early timepoints, as are the EndMT-associated transcription factors SNAIL and SLUG. By 24 hrs post-infection, expression of key endothelial markers had dropped, although no upregulation of fibrosis-associated genes was observed. We also stained fixed cell cultures for characterization of both endothelial and fibroblast cellular markers. We found that our current reagents (Abs, etc) successfully identified key markers on both cell types as evidenced by immunofluorescent microscopy. These findings in a 2D model set baseline parameters for the identification of potential EndMT-associated changes in cellular phenotypes as we move forward into our 3D model.

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Jennifer Klein and Dr. Arthur L. Haas
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“Arginine-170 is Important in Stabilizing the Active Parkin Oligomer”

Parkinson's disease (PD) is the second most common neurodegenerative disease, with more than 10 million people affected worldwide and 60,000 new cases reported in the United States annually. It is estimated that 5-10% of these cases are linked to underlying genetic causes. The predominant autosomal recessive forms of PD are associated with mutations in the gene PARK2, which encodes the enzyme Parkin. Parkin is a ubiquitin ligase required for the degradation of mitochondrial proteins damaged by oxidative stress. With its loss of function, the neural cell death associated with PD occurs. This targeted degradation of damaged mitochondrial proteins occurs through the ubiquitin proteasome system in which ubiquitin ligases recruit a charged E2~ubiquitin carrier protein and catalyze the transfer of activated ubiquitin from the E2 to the protein substrate. Polyubiquitin chains are subsequently formed on the protein substrate that are recognized by the proteasome and result in the degradation of the targeted protein. Despite information available on Parkin and its relation to PD pathogenesis, Parkin's mechanism of action is not well understood. Parkin is an RBG ligase similar in catalytic function to HECT domain ligases. The Haas lab has recently extended earlier work on HECT domain ligases to RBG ligases with current experiments aimed to explore the mechanism of human Parkin conjugation. Previous kinetic studies show that Parkin exhibits cooperativity with a Hill value of 2, suggesting that its active form is a dimer.

Prior to these experiments, a synthetic gene for human Parkin (variant 1; accession number BAA25751) was optimized for bacterial codon usage and the resulting synthetic Parkin coding region was subcloned into the pGEX-4T3-6A expression plasmid. Recombinant GST-Parkin was expressed in Escherichia coli BL21 (DE3) cells at 16°C for 14 hours after induction with 0.40 mM IPTG. The resulting protein was then purified by affinity chromatography using glutathione Sepharose. The GST-Parkin was processed using thrombin and was resolved from processed GST using glutathione Sepharose. The resulting free, unbound Parkin was used in subsequent experiments. A similar approach was used for the generation of GST-PINK1, with the thrombin processing step omitted. Parkin was then activated by incubation with GST-PINK1 under optimized conditions to phosphorylate the Ser-65 residue of the former. GST-PINK1 was removed by passing the mixture through glutathione Sepharose. Final experiments were designed to test the hypothesis that the active form of Parkin is a dimer stabilized by the Arg-170 residue using kinetic assays. Guanidine HCl was used in the assays to determine if inhibition of Arg-170 would affect polyubiquitin chain formation. Results of the experiments indicated Parkin functions as a dimer stabilized by side chain interactions between Arg-170 and Lys-220. Thus, Guanidine HCl represents a potential lead compound for the development of drugs targeting Parkin activity.

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“The Role of HPV & EBV in the Detection of Biopsy-Proven Cervical Dysplasia in HIV+ Patients”

Human Papillomavirus (HPV) is a virus contracted from skin-to-skin contact. Though HPV can find a host throughout the body, the most concerning form of HPV is found in genitalia. As the most commonly found sexually-transmitted infection, HPV plays a defining role in the development of several cancers, including but not limited to: anal, oral, penile, vulvar and, most frequently, cervical cancers. Additionally, the Epstein Barr Virus (EBV) is transmitted through saliva and previous data has been proven to increase the risk of abnormal cervical pap smears by 30%. The combination of these two viruses in patients can lead to early detection of cervical dysplasia. As cervical cancer is the second highest cancer found in women worldwide, early detection is a necessity and can be done through biopsies of possible pre-cancerous lesions in individuals with HPV and/or EBV. Through this process, the lab aims to detect cervical dysplasia (CIN), which is an abnormal mass of cells in the cervix that has a high chance of developing into cervical cancer as soon as a patient is diagnosed with HPV in hopes of detecting and treating cervical cancers early.

In a joint clinical and laboratory research cohort, the Hagensee lab sampled 125 HIV+ women in the Infectious Disease Clinic at University Medical Center over the course of 10 years. The participants ranged in age from 22 to 65 and the majority of participants (88%) were African-American, followed by Caucasians (11%) and Hispanics (0.8%). The lab collected CD4 counts and HIV viral loads as a marker of the severity of the HIV disease. Additionally, patients would receive a cervical pap smear, cervical swabs to test for HPV and EBV and biopsies of infected areas were taken.

The results from these procedures were analyzed to determine any patterns between the presence of the HPV and/or EBV viruses and the development of cervical dysplasia. Of 110 patients with documented HPV/EBV lab results: 46% tested positive for both EBV and HPV, 38% tested positive for HPV but negative for EBV, 8% tested positive for EBV but negative for HPV, and 7% tested negative for both. Patients were also grouped by the severity of their cervical pap smears on a scale of 1 to 4, where 1=negative results and 4=high-grade lesions. Of 118 patients with documented cervical pap smear lab results, a significant number of patients (52) progressed to low or high-grade lesions at some point in the study. Lastly, patients were grouped on the severity of their cervical biopsies on a scale of 1 to 3, where 1=little to no dysplasia and 3=severe dysplasia. Out of 125 patients, 61 had biopsies taken over the course of their involvement in the study. Of those 61, a significant number had moderate to severe results, demonstrating the importance of early detection to possibly pre-diagnose cervical cancer.

Though the results show an upward trend in HIV+ patients who also have HPV and/or EBV developing cervical dysplasia, there is insufficient data to present definitive results. Only 54 of the 125 study participants were not missing any of the lab results analyzed in this study (HPV, EBV, biopsies and pap smears). Obtaining all of these results in future studies is essential to confirm the role of HPV and EBV in detecting dysplasia. The Hagensee lab also plans to evaluate biopsies taken before study dates to determine any patterns in the development of dysplasia.

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“Human Papilloma Virus, Herpes Simplex Virus, Syphilis, Gonorrhea and Chlamydia infections among HIV-positive Patients at the Emergency Department [ED] of University Medical Center New Orleans.

Background: Although progress has been made for the health and wellness for HIV+ patients, the sexual health of these patients falls behind the general population’s (Shapiro et al). HPV and HIV are often co-infected, and HIV+ patients are disproportionately affected by HPV-related cancers (Konopnicki et al, 2013). Cervical cancer, anal cancer, genital warts, vaginal cancer, vulvar cancer, penile cancer are the most common HPV-related health conditions and cancers (CDC). Despite being more at risk for HPV-related cancers, almost one in four HIV+ women do not receive an annual cervical cancer screening. (Norwood et al, 2018). Moreover, HIV+ patients are less likely to receive an HPV vaccination (Wigfall et al). Being HIV+ puts patients at a higher risk for STDs, as HSV, syphilis, gonorrhea, and chlamydia are associated with HIV co-infection. Because STD and HIV cases are rising in New Orleans, it is imperative to study the correlation between the two. This study aims to assess the prevalence of HPV-related cancers, other STDs, demographic variables, and clinical referral rates.

Methods: This was a retrospective chart review of 792 HIV+ patients at the UMC ED during a five-year period (March 2013 to December 2018). In this study, medical charts were reviewed for demographic variables and clinical variables related to the disease conditions of interest (HPV, HSV, gonorrhea, chlamydia, and syphilis).

Results: Of patients studied, 75.88% were Black, 17.38% were White, and 6.74% were Other. The mean age is 38.7 years. 20% of the population had IV drug use, 55.53% had current alcohol use, and 64.94% had current tobacco use. 20% of the population was homeless, and 20% was uninsured. 76.25% of the population is male, 22.09% is female, and 1.66% is transgender. Over 10% of the study population had an HPV diagnosis, but only 3.58% had received an HPV vaccination. Patients who are younger than 40 are 11.9 times more likely to get an HPV vaccination. Patients who use alcohol are 4.2 times more likely to get a vaccination. Patients who use tobacco are .4 times less likely to receive an HPV vaccination. With regards to lifetime diagnosis, 11.28% had gonorrhea, 9.36% had chlamydia, 26.15% had syphilis, 11.04% had HPV, and 11.04% had HSV. Of patients not referred for STD treatment, 25.71% had gonorrhea, 24.67% had syphilis, 19.61% had chlamydia, 16.39% had HPV, and 21.21% had HSV. There is no correlation between syphilis, gonorrhea, chlamydia and HPV cancer; however, there is a relationship between HSV, HPV, and HPV cancer. 19 out of 126 females and 26 out of 595 males have HPV cancer. Blacks are more likely to have two or more STDs when compared to whites and other.

Conclusion: Our results show that Blacks are over-represented. Over 95% of the population have not been vaccinated against HPV as the average age is 38.7 and the recommended vaccination cut-off age is 26. Moreover, the vaccine is relatively new and marketed towards pre-teens. Literature has shown that tobacco users are more likely to get HPV, and in this study, were less likely to be vaccinated against it. HSV, HPV, and HPV-related cancer are related, which is consistent with current literature. Low referral rates were noted for STD patients across all the clinics, highlighting the need for specific interventions for both patients and ED providers. Over 25% of the population had syphilis, thus emphasizing the need for sexual health education in the HIV population. Past studies have proven a relationship among tobacco, alcohol, and HPV, respectively, in the general population. Therefore, it is recommended for further research to investigate these correlations with HPV-related cancer in the HIV population.

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Martin Ronis, James Watt:
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“Ethanol dysregulates chondrocyte differentiation via different sources of reactive oxygen species in chondrocyte ATDC5 cells”

Chondrocytes are cells that produce and maintain the cartilage matrix and form the growth plate of long bones. Understanding chondrocyte differentiation under unique circumstances is the key to unlocking the mechanism by which alcohol causes osteoporosis. During differentiation, chondrocytes undergo phases of proliferation, hypertrophy and apoptosis. NADPH oxidases (NOX) enzymes, in particular NOX2 and NOX4-generated reactive oxygen species (ROS) have been found to be vital in chondrocyte differentiation. In contrast, ethanol (EtOH) has been shown to dysregulate bone turnover via NOX2 and NOX4 in osteoblasts and suppress osteoblast differentiation *in vitro* via mitochondrial ROS. Additionally, it has been found that alcohol dysregulates the growth plate leading to an observed shortness of long bones *in vivo*. This effect is reversed through co-administration of antioxidant n-acetyl cysteine (NAC). The hypothesis of these studies is that alcohol will dysregulate chondrocyte differentiation but the correct dose and type of antioxidant will mitigate that effect. To test this hypothesis, we administered ethanol (0, 25mM, or 50mM) to chondrogenic ATDC5 cells in the presence of either mitochondrial inhibitors (mitoquinone (MitoQ) and mitoTempo), a NOX-specific inhibitor (GKT), or NAC for 16 days. Starting at day 5, RNA was extracted every two days to determine expression of genes related to chondrocyte differentiation (*Col2a1*, *Col10a1*, *Acan*, *Runx2*) via rt-qPCR. At 14 days, cells were fixed and stained with alcian blue to determine cartilage formation; at day 16, cells were stained with alizarin red to determine mineral deposition. Preliminary analysis of time-dependent gene expression patterns suggest a suppression of *Col2a1* and *Acan* by EtOH and an overall suppression of *Col2a1*, *Col10a1*, and *Acan* by MitoQ and NAC, where MitoQ and NAC abolished the effect of ethanol on *Acan* expression. Consistent with these initial results, EtOH suppressed alcian blue staining in control cultures. NAC and MitoQ independently suppressed alcian blue staining and protected against ethanol's effect. In contrast, EtOH appears to increase chondrocyte mineralization. These initial studies will help identify important sources of ROS in chondrocytes and determine the mechanism of EtOH's effect to dysregulate growth plate dynamics in bone.

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Martin Ronis

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“Exploration of the Role of Indian Hedgehog Signaling in PCB Toxicity on Skeletal Bone”

Polychlorinated Biphenyl (PCB) is a toxic chemical commonly used in electrical equipment. Consumption of PCB through bioaccumulation leads to adverse health risks like carcinogenesis in humans. In recent experimental studies performed on rats in our lab, showed that bone health in PCB-126 exposed animals were compromised. Compared to rats in the control group, rats treated with PCB had an overall smaller tibia bone size. In a related experiment, our lab was able to show that there is some protection from PCBs toxic effects on the bone in Aryl Hydrocarbon Receptor (AHR) knockout rats. This data provided more evidence that PCB acts through this receptor. However, the signaling pathway leading to bone toxicity is still unclear. To further investigate how exactly PCB may be affecting bone growth, RNA sequencing was done. Interestingly, Indian Hedgehog (IHH) is one of the genes that is drastically upregulated in wild type rats treated with PCB, but not in AHR knock out rats. Given that IHH plays an important role in bone growth and elongation, we hypothesize that IHH signaling is perturbed by PCB effects on bone growth. By performing real time PCR experiments on rat bone samples, we expect to see amplification of IHH verifying the upregulation of the gene seen in the RNA sequencing data results. We will further investigate IHH signaling in the marrow and shaft. With this information, more can be done to understand the role of IHH on the bone.

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Voluntary Ethanol Consumption and Alterations in Reward Circuitry in Ethanol Exposed Adolescent Male Mice

Adolescent drinking has been shown to contribute to the development of alcohol use disorders (AUDs) later in life. Our lab utilizes a mouse model of adolescent intermittent alcohol vapor exposure (AIE) to study neuroadaptations and behavioral correlates of this early alcohol use. One brain region of particular interest is the bed nucleus of the stria terminalis (BNST), which is a key brain structure in mediating both reward and stress/negative affect related behaviors. Thus, it is critical to understand how adolescent alcohol affects the BNST. Previous work in the lab has demonstrated that withdrawal from AIE enhances glutamate release and subsequent excitatory plasticity in the BNST, yet the specific inputs and cell populations affected are still unknown. The current work set out to test two hypotheses: 1) withdrawal from AIE increases the activity of BNST inputs from areas involved in reward and 2) AIE enhances subsequent voluntary alcohol intake. To test this, male C57BL/6J mice underwent adolescent intermittent ethanol vapor exposure (AIE) consisting of two four-day cycles (16hrs in, 8hrs out) separated by three non-vapor chamber days. One cohort first received bilateral microinjections of fluorescent green retrobead tracer into the BNST and then underwent AIE treatment. Green retrobeads are used to identify those brain regions that send projections to the BNST. These mice were perfused during withdrawal from AIE (4-6hrs after final vapor exposure), had their brains removed and were subsequently processed using immunohistochemistry for the immediate early gene, c-fos. This strategy allows us to determine if acute withdrawal alters the activation from reward brain structures projecting to the BNST (nucleus accumbens shell, ventral tegmental area, prefrontal cortex, peri-aquiductal gray, and hippocampus). A second group of mice received a two-bottle choice (2BC) voluntary alcohol consumption protocol following AIE. In 2BC, subjects were allowed intermittent, 24hr access to 20% alcohol solution or water, separated by 24 hr withdrawal periods, where they were provided only water. Voluntary ethanol consumption in 2BC was monitored for four-weeks following AIE or air vapor exposure. There was no significant interaction between history of vapor treatment and alcohol consumption or alcohol preference (p 's > .05). However, a main effect of time was significant for alcohol consumption, alcohol preference, and water consumption (p 's < .002). These results do not replicate previous findings that alcohol vapor exposure increases voluntary alcohol consumption in rats and suggest potential differences across rodent models. Future work will continue to quantify changes in reward circuitry inputs into the BNST from adolescent alcohol exposure.

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“Evaluation of the Efficacy of Various types of Tourniquets Utilizing an Exsanguinating Limb Simulator Model”

Tourniquets are devices that encircle a wounded limb or tissue and prevent the blood flow by tightening around major arteries and veins. Tourniquets have been in use for centuries and have proven effective at hemorrhage control in numerous settings, from battlefields to modern intraoperative applications. There are many different producers of commercially-available tourniquets such as the Military Emergency Tourniquet (MET), Combat Application Tourniquet (CAT), Israeli Silicon Tourniquet (IST), SWAT Tourniquet, Special Operations Forces Tourniquet (SOF-T), SOF-T Wide and Mechanical Advantage Tourniquet (MAT), all of which take slightly different design approaches to achieve effective circumferential compression of a limb to achieve cessation of distal blood flow. Despite the widespread use of tourniquets, new national efforts to educate the lay public in their use are relatively new. Recent studies have shown that tourniquets work differently. Recently, there has been a “Stop the Bleed Campaign” that has promoted awareness and provides a training opportunity to learn the proper usage of this technique. However, testing the effective usage of different tourniquets in the lay population has not yet been conducted.

The purpose of this study was to evaluate the efficacy of two types of tourniquets including the CAT (older and the newer version) and IST tourniquet on an exsanguinating limb simulator (ELS) to determine which one is most effective. An ELS was constructed from commercially available materials to simulate a human limb. Physicians and nurses were timed and data were collected on (1) time it takes to tighten the tourniquet until cessation of flow, (2) speed of application of tourniquets, and (3) subjective ease of use.

Due to the novelty of our study, results are pending and will be presented at a later date. It is a process that we started fairly recent and data is still being collected. Our research team plans to use not only this model but to incorporate patients and lay people into the study by teaching them how to use tourniquets in emergencies. We plan to compare patients' ability to apply tourniquets to those of physicians and nurses. Eventually, we will compare the efficacy of the tourniquets on the ELS model against the tourniquets on actual people to determine efficiency of the model.

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“Sex Differences in Fat Signaling in Obesity Prone and Obesity Resistant Rats”

Obesity affects one in every four Americans. The development of obesity is due to many factors including sex, susceptibility, and diet. Dysregulation or variations in orosensory perception can lead to overconsumption of highly palatable, calorically dense foods, which increases the risk for developing obesity. CD36 is a fat receptor found in the oral cavity and is proposed to be the fat taste receptor. Multiple studies support a role for lingual CD36 in fat intake and preference and differences in lingual CD36 expression have been shown in rats that differ in their susceptibility to develop obesity. Fat signals from the tongue are predicted to regulate homeostatic and hedonic signals in the brain. The hypothalamic neuropeptide, QRFP-26, increases fat intake and is increased by the consumption of dietary fat. GPR103a, the receptor for QRFP, is located in the nucleus accumbens (NAc), a key region for hedonic eating. Therefore, expression levels of lingual CD36 may regulate homeostatic and hedonic eating via the QRFP system.

We hypothesize that when fed a high fat diet (HFD), obesity-prone Osborne-Mendel (OM) rats will express higher levels of lingual CD36 mRNA, hypothalamic prepro-QRFP mRNA, and GPR130a mRNA in the NAc compared to obesity-resistant S5B/PI (S5B) rats. We further hypothesize that male rats will have higher expression levels than female rats and will exhibit higher rates of weight gain. To test this, male and female OM and S5B rats were fed a HFD (60% kcal from fat) for seven weeks. Gene expression in the circumvallate papillae of the tongue (CV), the ventromedial hypothalamus/arcuate nucleus (VMH/ARC), and the NAc were assessed by Real Time PCR.

Obesity-prone OM males gain more weight and consume more HFD than obesity-resistant S5B male rat. Additionally, males gain more weight and consume more HFD than females. When fed a HFD, lingual CD36 mRNA expression and GPR103a mRNA levels in the NAc were higher in female rats, compared to male rats. Prepro-QRFP mRNA levels were higher in S5B rats compared to OM rats. These data suggest that females may have an increased sensitivity to dietary fat, which decreases their preference for highly fatty foods and leads to decreased consumption of fat. Future directions include assessing fat taste sensitivity using food choice tests and determining the role of estrogen on fat taste preference.

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“Sulindac Sulfide’s Apoptotic and Anti-proliferative Effects on Human Colon Cancer Cells”

Non-steroidal anti-inflammatory drugs (NSAIDs) have been proven to decrease the incidence and mortality of colon cancer (11). One specific NSAID that has garnered much intrigue for its exceptional ability to prevent precancerous lesions and to act as a chemotherapeutic is sulindac. The purpose of our study was to evaluate a novel anti-proliferative mechanism of sulindac sulfide (SS) in colon cancer cells. We hypothesized that sulindac mediated its apoptotic effect through the inhibition of the p65 subunit of NF- κ B resulting in increased p53 stability and ultimately cell cycle arrest/cell apoptosis. In order to simulate sulindac’s effect on colon cancer cells, we treated three colon cancer cell lines (HCT-116 wild-type (WT), HCT-116 p53^{-/-}, and HT-29) with SS to observe the effects on proliferation, apoptosis, and the proteins that influence cell cycle. First, we evaluated the effect of SS on colon cell proliferation using HCT116 p53 WT cells; we observed a significant decrease in cell growth at the 48 and 72 hour time points. Next, we evaluated the protein expression of transcription factors known to regulate cell cycle progression and cell growth. HCT-116 WT cells were treated with 50 μ M of SS for 24, 36, and 48 hours, lysed and used for western blotting. The transcription factor E2F1 was downregulated in the presence of SS starting at 24 hours; but its downstream target, dihydrofolate reductase (DHFR), is not inhibited until 48 hours of treatment. Based on these results we decided to evaluate if the observed decrease in proliferation was due to cell cycle arrest. Next, we stained the three colon cancer cells with Propidium iodide, a commonly used dye to quantify DNA content in cells when using flow cytometry, we evaluated the effect of SS (50uM) on cell cycle progression of HCT-116 WT, HCT-116 p53^{-/-} and HT-29 colon cancer cell lines. With our results, we concluded that there was no significant difference in cell cycle observed between the control and treated tests of each respective cell line. To confirm these results we evaluated the protein expression of the cyclin kinase inhibitor, p21, which is known to function as a regulator of cell cycle progression at G₁ and S phase. Based on our western blot results we determined SS does not regulate the expression of p21. Previous literature has stated that NSAIDs use reactive oxygen species (ROS) in mediating their cytotoxic effects. To evaluate if this was the potential mechanism of SS in colon cancer, we treated cells with SS followed by dichlorodihydrofluorescein diacetate (H₂DCFDA) and used flow cytometry to evaluate the reduction of this compound. In the SS treated cells there was an increased presence of reactive oxygen species (ROS) present. Based on the known interaction of ROS and NF- κ B we decided to evaluate if SS mediated its function through the regulation of NF- κ B. We determined that SS treatment decreases the nuclear translocation of the p65 subunit of NF- κ B but has no effect on the presence of the phosphorylated/activated p65 subunit. We evaluated the effect of SS treatment of cell apoptosis induction using flow cytometry analysis of Annexin V and PI staining and we found that the HCT-116 WT cells had an increase in apoptosis while the other two cell lines experienced no significant difference in apoptosis. In conclusion, SS is most effective in inducing apoptosis of cells with a normal expression of the p53 protein due to an increase in ROS and may be an effective method for the treatment of cancers with a normal p53 protein due to its induction of apoptosis.

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“Workplace Wellness and Cancer Screening”

Everyday, over 1,500 people die of preventative cancers. In Louisiana, the burden of cancer is especially high. These cancers include breast cancer, cervical cancer, and colorectal cancer. Barriers to care include getting time off of work, not being able to make an appointment, or even fear. One of the most powerful tools to preventing cancer are screenings. In 2016, breast cancer screening rates in Louisiana were 78.1-81.8% while colorectal cancer screening was 64.1% and cervical cancer was 84%. The *CA: A Cancer Journal for Clinicians*, states that 40% of all cancer related illnesses/death can be prevented with proper screening.

Per the World Health Organization, employee wellness is a policy which assists in the making and implementing of a healthy environment and lifestyle for its workers. Through analyzing employee wellness initiatives at other institutions, this project provides recommendations to Louisiana State University Health Sciences Center (LSUHSC).

Best practices from The University of Alabama at Birmingham (UAB), University of Maryland, and the Louisiana Department of Health were utilized during this project. UAB focuses on the promotion of colorectal cancer screening and prevention while the University of Maryland requires completion of health activities by all employees throughout the year. UAB took initiatives such as sending the message through flyers, incentive systems, and creating pledges. A list of recommendations from these establishments was then compiled for administration at LSUHSC. Finally, visual aids and tools were created to support the project.

UAB was able to increase their screening rates for colorectal cancer from 67.06% to 68.43% in just one year. LSUHSC is the employer of approximately 4,000 people. Based on data from other institutions, we hope to see an increase anywhere from 1-3% percentage points in data here at LSUHSC as well.

LSUHSC must implement the following strategies to produce a successful employee wellness program. First and foremost, champions must be appointed. These champions will be able support and initiate the main goal: stop deaths from preventable cancers. Although LSUHSC has helpful programs which allow one to track their health by fitness tracking and meal planning, they can accomplish the specific goal of targeting preventable cancers through exercising awareness, advocating for using sick days to take care of screenings, and implementing a point by point incentive system which would then encourage participants.

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“Electrophysiological Techniques to Study Activity in Rat Hippocampus”

Electrophysiological techniques are adept at investigating neuroanatomical functions and mapping brain activity to behavior. In this study, we use electrophysiology to study how the hippocampus contributes to associative learning, which is the ability to learn and remember relationships between arbitrary stimuli. Previous research has shown that the hippocampus is involved in interpreting context and environmental cues, which is a key component of associative learning. Rats were trained to perform a visuomotor conditional association task in which they were presented with a visual stimulus and required to nose-poke a left or right port depending on the pattern of the stimulus. Correct choices were reinforced with chocolate milk, and incorrect trials were repeated until the correct action was made. Once the behavior was learned to 70% accuracy, the rats underwent a craniotomy where handmade electrodes were inserted into the CA1 area of the hippocampus. Postmortem histology confirmed placement in desired structures. After recovery, the rats performed the same task while the electrodes recorded their neural activity for analysis. Spikes were detected by an algorithm which isolated the downward deflections greater than three times the standard deviation using custom MATLAB programming. Inspection of spiking activity one second before and after the nose poke showed evident correlation between the task and the neural activity. Results demonstrate that electrophysiological techniques are effective at evaluating neurological activity in vivo and further suggest that there is a correlation between hippocampal activity and associative learning.

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“Of Mice and Many Repeats: Tissue Specific Expansion in a Friedreich Ataxia Mouse Model”

Friedreich ataxia (FRDA) is a life-shortening and progressive neurodegenerative disorder with no available treatment or cure. FRDA is caused by continuous expansion of the GAA·TTC trinucleotide repeats within the first intron of the Frataxin (FXN) gene. Early symptoms of FRDA, such as ataxia of the limbs, typically occur between the ages of five and fifteen years old, leaving most patients wheelchair bound by their late teenage years. As a direct result of this expanded repeat, FXN expression decreases and energy-hungry cells in the brain and heart are affected. Clinically, patients exhibit neurodegeneration and cardiomyopathy, with an average life expectancy of only 36.5 years. Research shows that the larger the repeats are, the more severe the disorder. Our goal is to find a way to slow down the expansion of these repeats to slow disease progression and improve patient outcomes.

We have found a way to slow repeats in patient cells (primary fibroblasts) and our next step is to test our treatment in a FRDA mouse model. Research shows that repeats in the heart and brain expand in FRDA patients, therefore the purpose of our experiment is to examine the expansion rate in these same tissues in an animal model with a single human FXN (hFXN) transgene.

Throughout our research, we have found that in 23 days, when the mice are first weaned, there are very few differences between the tissues within each mouse. However, after 8 weeks we begin to see differences: the brain and heart tissues show the most expansion, the ears show some expansion, and the kidneys show no expansion. Over time, repeats in these tissues continue to grow larger, as seen in mice at 6 months old. With the ability to detect changes of GAA repeats over time in our target tissues, this mouse model is a good model to test our therapy to slow progression.

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“Expression of Nicotinic Acetylcholine Receptors in Pulmonary Artery Endothelial and Smooth Muscle Cells”

Nicotinic acetylcholine receptors (nAChRs) mediate the body's response to nicotine; expression of these receptors defines the tissue and organ specific response to nicotine. Previous studies from our lab show that in mice chronic nicotine inhalation leads to pulmonary vascular remodeling and increased right ventricular systolic pressure. The aim of the current study is to identify the nAChRs that are expressed by the pulmonary vasculature, including pulmonary artery endothelial cells (PAEC) and smooth muscle cells (PASMC). Using quantitative real time polymerase chain reaction (qRT-PCR), we looked at relative mRNA expression of $\alpha 3$ -, $\alpha 4$ -, $\alpha 7$ -, and $\alpha 9$ -nAChR in human PAEC and PASMC. These receptors were chosen because $\alpha 7$ and $\alpha 9$ have been shown to be involved in many signaling pathways, $\alpha 3$ is involved in nicotine-induced carcinogenesis, and $\alpha 4$ is involved in nicotine-induced addiction. In addition, we used immunohistochemistry (IHC) on mouse lung tissue sections to detect $\alpha 7$. QRT-PCR results showed that HPAEC predominantly express $\alpha 7$, and HPASMC express $\alpha 3$, $\alpha 7$, and $\alpha 9$ with $\alpha 3$ being expressed at the highest level followed by $\alpha 7$. Further, IHC on lung tissue sections showed that $\alpha 7$ is prevalent in the smooth muscle cells and epithelial cells, however expression in the endothelial cells is unclear. Our future direction is to examine the functional role of $\alpha 7$ -nAChR in PAEC, and the role of both $\alpha 7$ and $\alpha 3$ in PASMC in nicotine induced vascular remodeling *in vitro* and *in vivo*.

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“Application of Machine Learning to Biomarker Discovery and Outcome Prediction in Colon Cancer using Genomic Data”

Background: Despite extensive screening campaigns, colon cancer remains the second-most common cause of cancer-related death in the United States. The recent surge of next generation sequencing of cancer genomes has led to an expanded molecular classification of colon cancer and increased our understanding of the molecular taxonomy of the disease, as genetics play a major role in its pathogenesis. However, despite the remarkable progress in mapping the genomic landscape of colon cancer, challenges remain. One of the more significant challenges is the identification of patients at high risk of developing aggressive disease that should be prioritized for treatment to improve clinical outcomes. Current prognostic and risk prediction markers include lymph invasion and tumor size, but these markers lack specificity and sensitivity. Thus, there is an urgent need for the development of more robust method for stratifying colon cancer patients according to risk to improve clinical outcomes. The application of machine learning (ML) to analysis of genomics data provides new opportunities for the development of such algorithms for patient stratification to guide treatment decisions. The objective of this investigation was to develop and apply ML to the discovery of molecular markers for stratifying patients and the prediction of patient outcome in colon cancer.

Methods: To address this unmet need, we used gene expression data generated using RNA-sequencing of 509 patients from The Cancer Genome Atlas (TCGA), classified as either tumor (n = 468) or normal (n = 41). We processed and screened the data before normalization to account for differing library sizes and gene lengths. We performed supervised analyses comparing gene expressions levels in tumors and controls to identify genes associated with the disease. Using clinical information, we then sorted the patients according to the disease status at follow-up as either tumor presenting (TP, n = 77) or tumor free (TF, n = 266). We performed supervised analysis comparing gene expression levels of genes significant associated with colon cancer between the two patient groups. Significantly differentially expressed genes between TF and TP were used in four algorithms to classify patients based on disease status. The algorithms included: Naïve Bayes (NB), Very Fast Decision Tree (VFDT), Support Vector Machine (SVM), and Logistic Regression (LR). Because of the unbalanced design of the project, we applied both class-balancing and boosting meta-algorithms to all four methods to improve performance of each classifier. Accuracy was chosen as the primary evaluation metric.

Results: Comparison of gene expression levels between tumors and controls revealed 13,108 ($p < 0.05$) differentially expressed genes; only these genes were considered in the following analyses regarding disease status. Comparison of gene expression levels with respect to disease status produced 537 significantly differentially expressed genes. Application of ML to multiple subsets of these genes was executed in WEKA 3.8.2. Among the four methods used, VFDT performed the best, achieving an accuracy of 82% on 98 genes (LFC > 0.75).

Conclusion: Despite limitations, including time to follow-up and disease heterogeneity, our results validate the use of genomic data to stratify patients based on risk of developing aggressive cancer, in addition to discovering clinically relevant biomarkers. Further studies are recommended to integrate transcriptome data with somatic and epigenomics data to develop more robust classifiers for potential clinical use.

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Undergraduate

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Mentor: Lisa Moreno-Walton MD, MS, MSCR, FAAEM
LSUHSC School of Medicine, UMCNO Emergency Department**“Low Clinic Attendance Rates for Hepatitis C Appointments at UMCNO”**

Background: Hepatitis C virus (HCV) is a single-stranded RNA virus and often contracted through the use of unsterile needles and syringes during the course of IVDU and blood transfusion. The infection is also less commonly contracted during sexual intercourse (Modi and Liang, 2008). Chronic Hepatitis C is typically asymptomatic. When left untreated, the infection can lead to cirrhosis, hepatocellular carcinoma, liver failure, and death (Poll et al., 2017). This highlights the importance of early clinic appointments and treatment. At the Emergency Department (ED) of UMCNO, 178 patients of the 297 patients who tested positive for HCV and were referred for a clinic appointment in 2017 failed to keep that clinic appointment. This study aims to identify patient specific and clinical variables that correlate to low and high attendance rates.

Methods: We conducted a retrospective chart review of 632 patients who were 18 years or older diagnosed with HCV at the UMCNO ED between January 1, 2017 and May 31, 2019. Medical charts were reviewed to obtain demographic and clinical variables pertaining to various patient groups with Hepatitis C infection to assess correlations with low and high clinic attendance rates.

Results: Our results showed that 75% of subjects were males. 48.4% were black, 45.4% were white, and 6.17% were other. There is a significant correlation between age (p-value= 0.0002), race (black vs white: p-value= 0.0521; other vs white: p-value= 0.0129), and drug usage (p-value= 0.0083) with attendance to the first infectious disease (ID) clinic appointment. There was no significant correlation between counseling by testing nurses, follow-up phone calls, and social work intervention and first ID clinic appointment. Average time to linkage was 115 days. The average time to linkage in 2017 was 188 days, and the average time to linkage in 2018 was 81 days, demonstrating a 57% reduction in time from diagnosis to first appointment. There were 69 fewer appointments scheduled in 2018 than in 2017. Analyzing data across these years, we saw linkage within one-month increase by a factor of 7 (with 3 out of 67 in 2017 and 15 out of 48 in 2018 linked to care in a month).

Conclusion: We set out to identify correlations with attendance rate and compare the attendance rate between 2017 and 2018. As age increases by one year, patients are 1.042 times more likely to attend their first appointments. Patients who use one or more drugs are 0.5 times less likely to attend their first appointments. While an older age and no drug usage were correlated to a high attendance rate, modifiable variables such as counseling, phone calls, and social work intervention were found to have no significant correlation with attendance rate. Even though fewer appointments were scheduled in 2018 compared to 2017, there was a higher first ID clinic attendance recorded in 2018. This may be impacted by the ID nurse clinic visits and the Opioid Survival Program initiated in 2018 by the ID clinic and the ED. These programs may be improving attendance rates and may need to be expanded.

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“Neighborhood Concentrated Disadvantage and Smoking among Young Adults in Greater New Orleans”

Concentrated disadvantage is a robust concept of neighborhood environment in public health and behavioral research. Neighborhood concentrated disadvantage has been shown to be an environmental risk for a number of public health outcomes, from crime to cardiovascular disease, as well as health behaviors like diet and physical activity. While rates of smoking have decreased dramatically in the US over the past few decades, the smoking epidemic is still responsible for a large burden of preventable disease. The objective of this study is to develop an index of neighborhood concentrated disadvantage for the Greater New Orleans area and assess the relationship between neighborhood concentrated disadvantage and current smoking rates in young adults.

We derived a composite index of neighborhood concentrated disadvantage for the Greater New Orleans area using US Census American Community Survey (2012-2016) 5-year estimates of ten census-tract level socioeconomic measures. We evaluated the association between concentrated disadvantage and smoking in a retrospective study of young adults. Data was collected from electronic medical health records for young adults (18-30 years) presenting to the emergency department of a major health system in the Greater New Orleans area between January 2013 and December 2016. Smoking status was modeled using multilevel logistic regression models of individuals nested within census tracts. Regression models were stratified by race/ethnicity and adjusted for age, gender, insurance, marital status, religion and primary care provider. Statistical analyses were completed in SAS version 9.4.

The study area encompassed 387 census tracts. An exploratory factor analysis identified a dimension of concentrated disadvantage for which measures of census tract poverty, education, public assistance, female-headed households, unemployment, abandoned houses and lack of vehicle loaded highly. The study dataset included 51,733 young adults in the study area. The majority (55%) were non-Hispanic black, 35.6% were non-Hispanic white, 7.8% were Hispanic and 1.6% were of other race/ethnicity. Rates of smoking were highest in non-Hispanic whites (38.6%). Neighborhood concentrated disadvantage index was significantly associated with smoking status for non-Hispanic whites ($p < .0001$), non-Hispanic blacks (0.0188) and other race/ethnicities ($p = 0.0306$) but not for Hispanics. The association between concentrated disadvantage and smoking status among young adults was strongest in non-Hispanic whites, where the odds of smoking were 30% greater high disadvantaged tracts compared to low disadvantaged tracts [OR 1.30 (1.11, 1.50)].

Smoking is a risk behavior that is most often established before the age of 25. The results of this study indicate there are subgroups of young adults in the Greater New Orleans area for which significant rates of smoking persist. While rates differ by race/ethnicity, we found that after controlling for individual characteristics, greater neighborhood concentrated disadvantage was associated with higher rates of smoking.

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Dr. Calandria:
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“PLA2G6 and α -synuclein interaction in human RPE cells”

Abstract:

Phospholipase A2 group VI activity is critical for the survival of dopaminergic cells. Mutations in the Calmodulin binding site are proposed to induce a dysregulation of PLA2G6 activity and a progressive degeneration of dopaminergic neurons located in Substantia Nigra pars compacta (SNpc) thus causing Parkinson's- like symptoms at early age (Paisan-Ruiz et al., 2009; Karkheiran et al., 2015; Zhou et al., 2016). Despite the importance of PLA2G6 in the abnormalities observed in idiopathic and familial PD, the specific function that determines its pathogenicity remains unclear and only a few of its products have been investigated, for example, DHA and Cardiolipin. To produce a model to study dysfunction of PLA2G6, we designed a morpholino oligo that protects the initial ATG codon in exon 2 (MO-ATG1) and forces the translation to start in a cryptic site at exon 4 (MO-ATG2). The result is a truncated PLA2G6 (tPLA2G6) that does not reside in the membrane as the normal long isoform of the protein. We tested the idea in retinal pigment epithelial (RPE) cells as a first step. Under the hypothesis that PLA2G6 activity dysregulation interferes with the survival and function of retinal pigment epithelial (RPE) cells, our goal was to test species of PLA2G6 synthesized in the presence of MO-ATG1 by the means of Real-time PCR, Western blot assay, and the changes induced by the toxic isoform tPLA2G6 using immunocytochemistry. We found that MO-ATG1 induced and increased in the PLA2G6 transcript, however, the levels of the protein were noticeably reduced. MO-ATG2 alone did not affect the levels of transcript or protein as predicted, but in combination with MO-ATG1, the reduction of both species was potentiated as well as the colocalization with α -synuclein suggesting a silencing of all the possible isoforms may induce accumulation of α -synuclein. The addition of BEL, a suicidal substrate that inhibits PLA2G6 activity, exacerbates the appearance of colocalization vesicles and signal of both PLA2G6 and α -synuclein in the perinuclear zone, suggesting an increased expression of both proteins. Together, these results show for the first time that 1) morpholino strategy used to silence PLA2G6 is a useful tool to model L-PLA2G6 dysregulation and 2) deficits in the activity of the enzyme induce colocalization of this protein with α -synuclein suggesting a possible mechanism of interaction. In future studies, these results will serve as bases to test PLA2G6 dysregulation and interaction with α -synuclein in dopaminergic neurons and astroglial cells.

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“Purification and Functional Analysis of Monoclonal Antibodies Protection Against *C. auris* Invasive Infection”

Disseminated candidiasis is the leading cause of life-threatening fungal infections in humans. This is especially the case in immunocompromised individuals, hospitalized patients that include intensive care unit patients. Despite the availability of antifungal therapy, crude mortality in the last decade has remained unacceptably high. The infection is caused by multiple species of the fungal genus *Candida* with *C. albicans* being the most common. Altogether, *C. albicans*, *C. tropicalis* and *C. glabrata*, cause >90% disseminated candidiasis in humans. Of particular concern, the “superbug” *C. auris* is a multi-drug resistant, health care-associated fungal pathogen, and has recently emerged as the first fungal pathogen to cause a global public health threat.

Xin lab has identified and isolated a panel of monoclonal antibodies (mAbs) specific for *Candida* cell surface peptides, and the mAbs provide protection in mice against disseminated candidiasis caused by medically important *Candida* species, including *C. auris*. The goal of this study was to produce these mAbs by cell culturing hybridoma cells, adapt cells to CELLline flasks, and purify mAbs by using a Centricon device. Cell culturing is a useful technique in producing monoclonal antibodies in large quantities and in controlled conditions. The hybridoma cells were cultured in a nutrient rich media and were gradually adapted to serum free media. Once adapted, the cells were transferred to CELLline culture flasks where the cells were able to grow at high concentrations. Cells were harvested periodically to obtain each cell line’s supernatant containing mAb. The supernatants of hybridomas were then purified using centrifugal filtration and dialysis. Next, each mAb supernatant was isotyped to determine antibody subclass identity. Finally, the purified mAb endpoint titers and concentrations were determined with ELISA assays. ELISA, fluorescence staining and Flow cytometric analysis were performed to validate the binding of each mAb to specific *Candida* cell wall peptides.

We conclude that the Immunoassay results show evidence of high functional titers of each peptide specific mAb, which provides protection against *C. auris* infections in mice. Antifungal antibodies could provide long-awaited novel therapies for use alone or in combination with antifungal agents.

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Diet Analysis Apps: What's Under the Hood?

With the technological advances made in the past couple decades, it is not surprising that 81% of Americans own a smartphone. In a time of such advances, failing to utilize these pocket computers to the fullest extent in the pursuit of a healthier public would be a grave mistake. In the burgeoning mHealth field, food-tracking apps have become very popular. Whether designed to help the individual lose weight or just maintain a generally healthier diet, the market is booming. In a 2015 survey, 58.23% of smartphone users said that they had downloaded a diet app; over half of those positive responses reported continued use and high trust in the accuracy of these apps. Several studies have shown deviations between these apps and databases recognized as the standard in the diet analysis field. These deviations could have a significant impact on an individual's health if they choose to use this nutrient data for health guidance. The interaction between the user and the app's interface is also critical in the reliability and accuracy of the data output; features that are more prone to miss-selection of a food entry or incorrect portion sizes will lead to inaccurate and misleading data. The focus of our study is to ultimately determine the accuracy of these apps; we begin that evaluation with a look at various features that could result in inaccuracy. In this study, apps were selected from the Google Play store using their search algorithm and two other independent methods. The following criteria were used for the selection in the Google Play Store: 1) the app had to be free; 2) a customer star rating of at least 4 out of 5, and 3) have a diet evaluation function that included more nutrients than just calories. All apps/programs not found in the Google Play store also had to adhere to criteria 1 and 3. Seventeen apps and two web-based computer programs met the criteria and were included in our evaluation. Then, how each app obtained its food database—and from what sources—was investigated, as well as characteristics related to monetization and food search techniques were determined. This information was obtained from several sources: 1) information contained within the app or computer program; 2) blogs, frequently asked question (FAQ) lists, or chatboxes on app-associated websites included with the app and/or computer program; 3) email communication with the app developers. Fifty-nine percent of apps/programs utilized the USDA food composition database. A yes-or-no qualitative assessment of the app was completed for the following features: 1) monetization (account required, in-app purchase offers, premium version, self-advertising of other apps and/or physical products, advertisements), 2) search methods (search bar, common/popular foods, bar code, photo, voice, food recall prompts, custom foods, others), and 3) other (verification labeling, track daily value of non-kcal nutrients, socializability with other users, retroactively view other entries, graphing of nutrient data). A premium subscription for several apps was needed to obtain a more extensive list of nutrients. At least eight different methods of entering foods were found, but all the selected apps and web-based programs had a search bar to find foods. Only 50% allowed quick entry of common or popular foods, while two-thirds recognize bar codes and let the user enter that food's data. Only 40% visually display a verification label that assures the user of the accuracy of an entry. Most apps allow the user to enter a food from their diet, but not in the database, which, in some instances, can be shared with other users. This data may or may not be verified. In conclusion, some features have the potential to introduce new errors compounding inaccuracies that may exist in the nutrient databases.

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Disparities in Motor Vehicle Collision (MVC) among the Pediatric Patients

Background: A motor vehicle collision is a collision between a vehicle and a pedestrian, animal, road debris, or another vehicle. Each year 1.25 million people lose their lives in motor vehicle collisions (MVC). An estimated 4.5 million people were severely injured in a motor vehicle collision in 2018. Motor vehicle collisions are one of the three leading causes of death in pediatric patients in the United States. The focus of this study is to discover trends in demographics, the use of protective devices, severity of injuries, surgery rates, and length of stay among pediatric patients presenting to the University Medical Center New Orleans (UMCNO) following an MVC. This study can potentially reveal disparities in MVC rates, length of stay, and imaging (X-rays, CT scans, and MRIs) practices in the care of pediatric patient sub-groups.

Method: The Louisiana Trauma registry was queried for patients between birth and 18 years of age involved in an MVC meeting level 1 criteria for trauma activation at UMCNO. The charts for all patients meeting criteria were reviewed in EPIC. Variables collected included race, age, dates of admission and discharge, gender, injury severity score (ISS) and protective devices used. All data was de-identified, and then analyzed using Statistical Analysis System (SAS) 9.4. Relationships between categorical variables were assessed using Pearson's chi-square or Fisher's exact tests. We also performed Wilcoxon rank sum tests to compare independent groups.

Result: Of the cohort of 668 pediatric patients, 435 were males and 233 were females. 403 identified as black, 218 identified as white, and the remaining 47 patients identified as neither. When analyzing by age, the two largest groups were the 13-18 year olds (302 patients) and the 4-8 year olds (155 patients). Only 20.75% of all patients studied underwent surgery. The most common surgeries were Orthopedic (61), General (47) and Neurosurgery (25). Length of stay and injury severity scores were similar for both males and females. Of the surgical patients, 11.13% were black and 9.17% were white. White patients had the highest injury severity scores (ISS) with a mean score of 11.65. All races had similar rates of x-ray imaging on day 1. There was no correlation between race and age and the number of MRIs performed. Patients in the 13-18 year age group had a higher percentage of surgeries (13.98%) ($p < 0.0001$), longer length of stays (5 days) ($p < 0.0001$), and the highest injury severity scores ($p < 0.0001$). Of the 397 male patients, only 121 used seatbelts. Of the 212 female patients, only 84 used seatbelts.

Conclusion: This study reveals a disparity in gender, race, and age regarding motor vehicle collisions. Black teenage males have the highest rates of level 1 trauma activations from MVCs. Although blacks are a minority in the United States, they represented more than 50% of all patients in this study. There is a wide disparity between teenage patients and patients in all other age groups regarding ISS, incidence of MVC and surgery rates. The use of a seatbelt was noted to be low across all groups, highlighting a need for more awareness and better enforcement of existing safety laws.

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Retinal Sensitivity of Hormonally Modulated *Hyla cinerea* Using Electrophysiological Techniques

Hormones modulate the nervous system to regulate reproductive behavior and the processing of visual signals. However, little is known of how endocrine mechanisms modulate the retina, the peripheral sensory layer in the eye that is responsible for the transduction of light to electrophysiological events in the central nervous system. The goal of this study was to determine whether reproductive state is correlated with increased retinal sensitivity in vertebrates. The retinal physiological sensitivity of non-reproductive and gravid green tree frog, *Hyla cinerea*, females were measured using scotopic electroretinograms (ERGs). This nocturnal frog is used as an experimental model due to its ability to respond to wavelengths spanning the human visible light spectrum with similar ocular and cellular anatomy. The electrical response to flashes of light were collected using two different experimental protocols. The first approach examined relative spectral sensitivity by comparing ERG b-wave amplitudes for isointensity light flashes at different wavelengths. The second approach measures b-wave amplitude across a series of light intensities, enabling calculation of retinal threshold at each wavelength. Our preliminary data show increased electrophysiological response size and decreased thresholds in reproductive females. The results are significant, as the elucidation of peripheral sensory function under different endocrine states has potential clinical applications for treating degenerative retinal diseases such as retinitis pigmentosa. These data provide the first step toward such therapeutic approaches.

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Glioblastoma multiforme (GBM): Clinical presentation, experimental animal models and novel treatments

Glioblastoma multiforme (GBM) is a highly aggressive brain tumor with low expected survival rates (12-15 months). Many reasons of the low survival rate include high system toxicity, acquired chemoresistance, and the low penetrance of the blood brain barrier by many anti-cancer drugs. Female nude athymic mice aged 6-8 weeks were injected U87 GBM cells that express a luciferase reporter into the left striatum of the brain to induce GBM. The mice were anesthetized with ketamine and xylazine, buprenorphine was administered for pain relief. Physiological monitoring by rectal temperature and body weight was used as well as the observation of seizures and abnormal gait or posture. Treatment groups are as follows: LAU – 0901, Avastin, LAU – 0901 + Avastin, Elovanooids, LAU – 0901 + Elovanooids, Elovanooids + Avastin, and a Vehicle (Saline) group. Treatments were administered starting on Day 13 after GBM implantation. In-vivo imaging was performed on days 13, 20, and 30 post-implantations to show how the cells divided and grew over time by use of bioluminescence and quantitative analysis. All treatment groups exhibit decreased tumor growth and increased overall survival compared to the vehicle. One mouse from the saline group died, while all mice of the treatment groups survived. The best combination of treatment was Elovanooids + Avastin, showing the highest rate of decreased tumor growth on Day 30. Future studies explore combining and comparing more treatments to target the cocktail that exhibits the best and most aggressive tumor fighting capabilities.

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“The Protein S LG1+2 domain Contributes Significantly to Inhibition of Factor IX_a”

Protein S (PS) is a key vitamin K-dependent, anticoagulant protein. Homozygous PS deficiency causes fatal purpura fulminans in neonates, a dramatic phenotype that emphasizes the critical function of PS in the regulation of human blood coagulation. Similarly, heterozygous PS deficiency is associated with increased risk of venous thromboembolism. Consistent with these clinical manifestations, gene ablation of PS in mice results in an embryonic lethal phenotype related to consumptive coagulopathy and intracranial hemorrhage.

Recently, the Majumder lab identified an important function of PS, i.e., PS inhibits Factor IX_a(FIX_a) in the coagulation pathway. Coagulation occurs by a cascade of reactions, culminating in the formation of a fibrin clot. A key event in the coagulation cascade is the activation of Factor X(FX). The activation of FX relies on FIX_a binding to its cofactor, Factor VIII_a(FVIII_a), forming a complex that activates FX to FX_a. FX_a, along with its cofactor, Factor V_a(FV_a), activates prothrombin to thrombin, which in turn generates fibrin. The binding of PS to FIX_a inhibits formation of the FIX_a/FVIII_a complex, thereby inhibiting FX generation. This newly recognized inhibitory function of PS presents a novel possibility of using PS as a therapeutic agent to treat hypercoagulation disorders.

PS is composed of a GLA domain, a thrombin sensitive region, four EGF domains, and the LamninG-1/G-2 domains (LG1+LG2). LG1 and LG2 were cloned and overexpressed in E.coli. The LG1+2 protein reduced the rate of FX activation by 69% in the presence of the FIX_a/FVIII_a complex; this reduced rate agreed with previous measurements that used intact PS. However, the separate LG1 and LG2 domains reduced the rate of FX activation by FIX_a by only 41% and 28%.

In this project, we used circular dichroism spectroscopy and isothermal titration calorimetry to determine structural changes that accompanied binding of the PS LG1 and LG2 domains to FIX_a. In addition, we used ex vivo plasma assays such as activated partial thromboplastin times (aPTT) and thrombin generation assays (TGA) to identify the interactions between the LG domains and FIX_a in physiological conditions.

We observed that the binding of the LG1+2 and LG1 domains with FIX_a were exothermic with a 1:1 stoichiometry. The binding affinities LG1+2 and LG1 to FIX_a were $1.2 \times 10^7 \text{ M}^{-1}$ and $5.16 \times 10^6 \text{ M}^{-1}$, respectively. The calculated ΔG values of LG1+2 and LG1 binding to FIX_a were -9.46 Kcal/mol and -8.90 Kcal/mol, respectively. These parameters agree well with the parameters for intact PS binding to FIX_a. The circular dichroism data revealed significant conformational changes that accompanied binding of LG1+2 domain to FIX_a. We also observed conformational changes with binding of LG1 domain to FIX_a; however, the change was greater with LG1+2 domain.

The LG1+2 domain decreased thrombin generation in PS-deficient plasma by ~50%, whereas the LG1 domain reduced thrombin generation by only ~25%. The clotting time of PS-deficient plasma (~30 s) was prolonged by 10 s by addition of 300 nM LG1+2 domain.

On the basis of these results, we conclude that the LG1+2 domain is needed to bind and inhibit FIX_a. However, LG1 or LG2 alone is not sufficient to recapitulate the inhibitory effects of PS on FIX_a.

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**“Neuroadaptations in Estrogen Signaling in an
Animal Model of Complex Regional Pain Syndrome & Alcoholic Neuropathy”**

Complex Regional Pain Syndrome (CRPS) is a chronic pain condition that occurs following injury or immobilization to a limb and shares many symptoms with alcoholic neuropathy, including an increased sensitivity to painful stimuli (allodynia). Both CRPS and alcoholic neuropathy disproportionately affect women, leading us to hypothesize that estrogen and progesterone may play a role in the pathophysiology of these two conditions. Based on estrogen's neuroprotective properties, we predicted hyperalgesia would correlate with reduced estrogen receptor phosphorylation and G protein-coupled estrogen receptor (GPER) levels in pain-associated brain areas. CRPS was modeled in female rats by using unilateral hind limb immobilization for seven days, allowing the other limb to serve as a within-subjects control. To model alcoholic neuropathy, half the rats were subjected to a ten-week Liber-DeCarli alcohol liquid diet prior to immobilization. To investigate the role of ovarian hormones, half of the animals underwent ovariectomy (OVX) and the other half a sham operation. Allodynia was quantified as mechanical paw withdrawal thresholds using the von Frey method. The behavioral findings from this experiment showed that rats that had been subjected to cast immobilization ($p=0.014$) and a liquid alcohol diet ($p=0.005$) had lower paw withdrawal thresholds the days following cast removal, and these effects were seen both separately and additively. This demonstrated that chronic alcohol intake increased pain sensitivity in our model of CRPS. However, OVX did not interact with these effects. Through the use of Western blot analysis, several estrogen and progesterone receptors were targeted and their concentrations were analyzed to determine the relationship between the hormone levels and pain sensitivity. In this study, we have focused on the cingulate cortex, a main cortical area that brings pain into conscious awareness.

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“Mechanisms of Nicotine-dependent Activation of Cardiac Fibroblasts”

The rising popularity of electronic nicotine delivery systems (ENDS) has created concern over the physiological effects of inhaled e-liquid components, particularly nicotine. The increasing presence of tobacco companies in this market and access to open-system devices, which allow for individual customization of the e-liquid's composition, add to the worry that ENDS use could be more dangerous than is advertised. Cigarette smoke has a multitude of detrimental effects, and inhaled nicotine alone may trigger similar responses. Of particular consideration is the effect of nicotine use on fibroblasts because of their role as producers of the extracellular matrix (ECM) proteins that provide stability and aid in the function of the surrounding tissue. Pulmonary fibroblasts undergo accelerated proliferation and increased ECM collagen production following exposure to nicotine. Likewise, cardiac fibroblasts show nicotine-dependent transdifferentiation into myocardioblasts. Myocardioblast activation is typically an acute process providing cardioprotection in response to cardiac stress or injury. Chronic activation, however, may accelerate progression of heart disease via an over-accumulation of ECM.

We hypothesize that nicotine activates cardiac fibroblasts by binding to the alpha-7 nicotinic acetylcholine receptors (nAChR), leading to the disruption of compensatory mechanisms in favor of a more deleterious cellular response, which will result in excess ECM production, fibrosis, and increased susceptibility to cardiovascular disease. Fibroblasts extracted from cardiac tissue of naïve male rats are grown in vitro and exposed to nicotine for 12, 24, and 48 hours in the presence and absence of inhibitors of the renin-angiotensin system (RAS) – the signaling pathway believed to be responsible for increased ECM production. Following nicotine treatment, quantitative reverse transcription polymerase chain reaction (qPCR) measurements are utilized to monitor changes in the mRNA transcription of markers of myocardioblast activation, including lysyl oxidase (LOX), alpha-smooth muscle actin (α -SMA), and collagen I and III. Wound healing and collagen gel contraction assays are used to obtain functional information about potential nicotine-dependent alterations in the cells. Ultimately, we plan to expand these experiments to include a mouse model of nicotine vapor exposure to closely mimic inhaled nicotine use. This research could provide new insight into physiological responses to nicotine consumption and a basis for tighter regulation on ENDS devices.

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Differences in patient-provider communication between smokers and nonsmokers

Cigarette smoke causes cancer and increases one's risk of cancer-related mortality. People under the age of forty who quit smoking reduce their chances of dying from smoking-related diseases by 90%, and effective patient-provider communication aids in smoking cessation efforts. Patients that experience effective patient-provider communication have better recovery habits, mindsets, and health outcomes. This study examined differences in quality measures of patient-provider communication among smokers and nonsmokers.

Using a cross-sectional study design, we examined nationally represented data from the 2017 and 2018 Health Information National Trends Survey (HINTS). Measures of patient-provider communication included how often respondents reported providers 1) always listened carefully, 2) explained things, 3) showed respect, 4) spent enough time, and 5) involved them in joint decision-making. Descriptive statistics included age, gender, race, income, education, and whether the patient ever had cancer. Chi-square analysis determined differences between respondents who reported current tobacco use and respondents who reported no tobacco use. Logistic regression determined the relationship between smoking status and patient-provider communication variables after adjusting for covariates.

The sample included 6,789 participants, of which 38% were current smokers. Chi-square analysis revealed a significant difference ($p < 0.05$) between smokers and non-smokers for each measure of patient-provider communication except for "*explain[ing] things in a way [they] could understand.*" After controlling for all variables, compared to non-smokers, smokers were more likely to report that a health care provider did not "give [them] the chance to ask all the health-related questions [they] had" (OR 0.744, 95% CI 0.562-0.933, $p = 0.041$), "involve [them] in decisions about [their] health care as much as [they] wanted" (OR 0.666, 95% CI 0.523-0.853, $p = 0.001$) nor "help [them] deal with feelings of uncertainty about [their] health or health care" (OR 0.777, 95% CI 0.630-0.963, $p = 0.019$).

Our analysis revealed room for improvement in patient-provider communication for smokers. Future interventions that improve patient-provider communication among smokers may aid tobacco cessation efforts.

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“Higher SIV Levels are observed in Blood and Tissue Reservoirs of ART-Treated Female Macaques Exposed to Chronic Binge Alcohol”

Anti-retroviral therapy (ART) has greatly increased the life span of people living with HIV (PLWHIV), however ART does not eliminate viral reservoirs. PLWHIV experience disease co-morbidities that affect their quality of life, and they are more likely to misuse alcohol. We hypothesize that despite the use of ART, alcohol misuse leads to increased levels of virus in tissue reservoirs contributing to co-morbidities. To explore this hypothesis, female macaques infected with Simian Immunodeficiency Virus (SIV) were exposed to chronic-binge alcohol (CBA) or saline. Blood and tissue samples from 19 animals were available for quantification of proviral DNA and viral RNA. At 10 weeks, SIV RNA levels in blood and lymph nodes were higher in CBA animals when compared to the controls. After 1 month of ART, viral levels decreased in both groups, however CBA animals had significantly higher SIV RNA levels. After 8 months ART, proviral DNA levels in tissue showed little change. In contrast SIV RNA levels decreased from pre-ART time points but remained detectable and were higher in the CBA animals. These observations support our hypothesis and suggest that ART may be less effective in controlling virus in tissue reservoirs of CBA animals. Analyses of additional tissue site will identify variations in discrete tissue reservoirs to determine their relationship to co-morbidities and disease progression.

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“Effect of fasting on the distribution of immune cells in the mouse adrenal”

The adrenal gland is a (neuro) endocrine gland located above the kidney that releases hormones that maintain homeostasis including the control of blood glucose levels. The adrenal gland has two regions, the outer adrenal cortex and the inner adrenal medulla. The cortex produces steroid hormones including corticosterone, and the medulla produces the catecholamine hormones, epinephrine and norepinephrine. These hormones are released during the fight-or-flight response to stress, particularly when the levels of blood glucose fall (such as during fasting).

The adrenal gland also contains a diverse population of immune cells but whether these are involved in the function of the adrenal during the fight-or-flight response is not known. The aim of this project was to test the hypothesis that activation of the adrenal gland during fasting would lead to a local change in the number or distribution of adrenal immune cells.

To test this idea we compared the location of CD45-immunoreactive cells in the adrenals of mice that were fed or fasted for 24 hrs. At the end of the experimental protocol the mice were euthanized, the adrenals were removed and fixed in paraformaldehyde. Then cryosections were made and processed for immunohistochemistry. Immune cells were stained with a rat anti-CD45 antibody (that labels all hematopoietic cells except erythrocytes and platelets).

Preliminary results indicate that there was no difference in the number of immune cells between the fed and fasted mice.

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“Suppression of Dendritic Cell Maturation by Triple-Negative Breast Cancer Exosomes”

Exosomes are spherical membrane-enclosed nanoparticles released by cells that play a significant role in intercellular communication. The content of exosomes depends on the cell of origin and can include RNA and protein. After being released from their parental cell, exosomes are taken up by target cells and thus transfer their biological content causing a series of molecular changes. Cancer cells release significantly more exosomes and these exosomes have a unique composition relative to their normal counterparts. In addition, tumor-derived exosomes can rewire neighboring cells in order to create a tumor promoting microenvironment.

Dendritic cells (DCs) are immune cells important in the host response to many diseases including infections and cancer. DCs function by presenting antigens on their outer cell membrane to T-cells which then activate and initiate a cytotoxic killing response. Immune cells including DCs are often suppressed during malignancy and are unable to induce tumor cell killing. The role of exosomes in the suppression of immune cells remains unclear. We hypothesize tumor-derived exosomes are responsible for the suppression of dendritic cell activation. In addition, we explore the effect of sulindac, a non-steroidal anti-inflammatory drug known to have anti-cancerous effects, on attenuating the ability of exosomes to suppress DC maturation. In this study we collected exosomes from the aggressive mouse murine triple-negative breast cancer cell line 4T1 and administered the exosomes into DC culture media. DC maturation was assessed using flow cytometry. Our results support our hypothesis and demonstrate tumor cell-derived exosomes can suppress the maturation of DCs. This study is significant because it provides insight into the involvement of exosomes in systemic and local malignancy-associated immune suppression and will aid future studies in developing treatment strategies to inhibit this process.