

Voelcker Biomedical Research Academy Newsletter

Issue 2, Winter 2014

Dear Voelcker Scholars, Parents, Friends,

This issue of the VBRA newsletter is dedicated to the recent accomplishments of all of our VBRA Scholars. Established in 2009, The program now has 5 classes!

The class of 2013 completed their first year and selected their labs and mentors for the next two years. They presented their posters in the Science Symposium in July.

The class of 2012 successfully completed their first year of research and have compiled data for their projects. We are pleased to present the fruits of their labor in this collection of abstracts. In addition to their dedicated work in their labs, the class of 2012 also served as peer mentors to our incoming class of 2013. As part of their guidance, they held scientific presentations which included their data, as well as advice to our incoming students (pictured here).

The class of 2011 graduated from the VBRA this year!

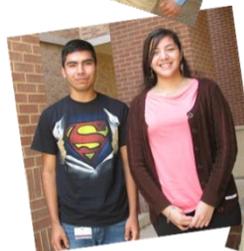
The class of 2010 graduated high school and are now enrolled as freshmen in college!

Finally, the class of 2009 successfully completed their first year of college and are now in their sophomore year. Four of them returned as peer advisors (pictured bottom right, in red).

For a complete listing of abstracts and pictures:
<http://voelckeracademy.uthscsa.edu/>

Congratulations to all of you on your wonderful accomplishments! We wish all of you a wonderful new year filled with great success and findings!

Your Voelcker Team



Class of 2012 Abstracts

Vanessa Alvarado

Mentor: Kathleen R. Stevens, RN, EdD, ANEF, FAAN

Academic Center for Evidence-Based Practice, School of Nursing Prevalence of communication failures in our healthcare system

Vanessa Alvarado, Darpan I. Patel, PhD, Frank Puga, PhD, Braulio Amezaga, BA, Kathleen R. Stevens, RN, EdD, ANEF, FAAN

Introduction: Organizational climate in hospitals, including organizational support for nursing in microsystem operations, are potentially modifiable to produce better patient outcomes. The purpose of the study is to conduct a secondary analysis of existing data collected as part of the Small Troubles, Adaptive Responses (STAR-2) study, specifically focused on communication failures.

Methods: A secondary analysis was conducted of existing data previously collected from the STAR-2 study. 14 hospitals from the Improvement Science Research Network participated in the STAR-2 study to report the type and frequency of operational failures that occur in medical surgical units. Three medical surgical units were recruited from each hospital. The STAR-2 Pocket Card[®] was used to report the type and frequency of operational failures. Descriptive analysis was conducted of existing data and is presented as percentages.

Results: A total of 24,014 operational failures were reported (6.15 operational failures per shift) by 716 nurses in the STAR-2 study. Information/communication failures (n=4,396; 18.6%) were the second highest reported category (1.14 failures per shift). The top Information/communication failures consisted of physician communication failures (illegible orders, unclear directions, difficulty paging/calling) and Staff/Peer communication failures (inaccurate information, unclear orders, no relay of messages, delayed requests) accounting for 22.6% and 20.8% failures, respectively.

Conclusion: Failure in communication can greatly impact patient outcomes, result in delays in medication administrations and effect other patient centered activities. Interventions to correct failures in communication may prove to be beneficial. TeamSTEPPS[®], an evidence-based system aimed at improving patient outcomes via fostering improvements in teamwork and communication among members of the healthcare team, is one potential solution to improve information and communication errors. Further research in on this topic is warranted.

Jessica Anthony

Mentor: Reto Asmis, PhD

Departments of Clinical Laboratory Sciences and Biochemistry Ursolic Acid and its Triterpenoid Analogs and Toxicity in THP-1 monocytes

Anthony, Jessica; Ullevig, Sarah; Nguyen, Huynh Nga; Piefer, Leigh Ann; Asmis, Reto

Introduction: Atherosclerosis begins with monocyte adhesion and migration into the arterial wall. Monocytes are recruited into the vessel wall by chemokines such as monocyte chemoattractant protein 1 (MCP-1) and then differentiate into macrophages, driving the progression of atherosclerotic lesions. Ursolic acid (UA), a phytonutrient found in fruits and herbs, has been shown to have cytoprotective effects *in vitro* and atheroprotective properties in a murine model of diabetic atherosclerosis. We sought to determine

in THP-1 cells, a human monocytic cell line, the toxicity of UA and its structural analogs (oleanolic acid, corosolic acid, hederagenin, asiatic acid, and madecassic acid). In addition, we investigated the effects of these phytochemicals at sub-lethal doses on normal monocyte migration in response to MCP-1.

Methods: Each phytochemical was tested for 24 h at several concentrations: 3 μ M, 10 μ M, 30 μ M, 50 μ M, and 100 μ M. To test the toxicity of the analogs, we performed propidium iodide (PI) viability assays. Normal cell migration in untreated cells was tested using a transwell chemotaxis assay to determine the optimal MCP-1 concentration for future studies.

Results: Corosolic acid and hederagenin decreased viability to 41% and 78% respectfully of THP-1 monocytes at concentrations greater than or equal to 30 μ M. Madecassic acid was not found to be toxic, maintaining viability (>90%) at concentrations up to 100 μ M. The transwell chemotaxis assay shows optimal results with an MCP-1 concentration of 1 nM.

Conclusions: Madecassic acid is not toxic to human monocytes *in vitro* up to 100 μ M and should be further investigated as a potential dietary supplement for the prevention of atherosclerosis. Despite the decreased cell viability at concentrations greater than 30 μ M, corosolic acid and hederagenin may have beneficial effects at lower, and therefore safer, concentrations. All three compounds will be further investigated for their effects on normal cell migration in response to 1 nM MCP-1. This work was supported by a fellowship (to J.A.) from the Max and Minnie Tomerlin Voelcker Foundation.

Sergio T. Arambula

Mentor: Dr. Charles W. Mathias Ph.D.

Department of Psychiatry Secure Continuous Remote Alcohol Monitor Study

Sergio T. Arambula

The Secure Continuous Remote Alcohol Monitor study or SCRAM study is a study focused Contingency Management of binge drinkers with a non clinical population (1). The SCRAM is an alcohol monitor commonly used by the judicial system but has not been utilized for research purposes. The monitor is secured to the participant's ankle and measures the amount of alcohol excreted from the participant's skin every thirty minutes thus, providing a continuous representation of the amount of alcohol in a person's system. There are several different phases of the study: Naturalistic phase – the participant wears the SCRAM for 4 weeks in order to capture their natural drinking behavior; Contingency Management – the participant wears the SCRAM for 12 weeks and is rewarded weekly for not binge drinking during the week; Monthly Follow-ups- the participant visits the lab once a month for 3 months to report what they have been drinking after the monitor has been removed. During the Naturalistic and Contingency Management phase participants report their alcohol use through the timeline follow-back method and Transdermal Alcohol Content (TAC) readings are downloaded from the SCRAM during weekly visits to the lab. The Timeline Follow-back Method (TLFB) is administered as a semi structured interview that is in a calendar format (2). Participants self report their alcohol use throughout the past week. TAC readings gathered by the monitors are used to confirm the participant's alcohol use during the past week. A fault of the TLFB is the reliance on self-report from participants. Participants often have trouble remembering events

further back from the date at the time. Participants may also have trouble recalling what they specifically had to drink after they have had a considerable amount to drink. Finally a major problem is participants not reporting all alcohol use due to various reasons such as fear of negative consequences. The data being collected is significant because it suggests that using objective measures like SCRAM might be more effective in research and clinical settings. Furthermost the information gathered serves as a foundation for future SCRAM studies.

References: 1. Leffingwell, T.R., et al. (2013). Continuous objective monitoring of alcohol use: twenty-first century measurement using transdermal sensors. *Alcoholism: Clinical and Experimental Research*, 37, 16-22. 2. Townshend, J.M., Duka, T. (2002). Patterns of alcohol drinking in a population of young social drinkers: a comparison of questionnaire and diary measures. *Alcohol & Alcoholism*, 37, 187-192.

Morganne Blaylock

Mentor: Brian Wickes, PhD

Department of Microbiology and Immunology Development of a Dominant Marker-Based Recyclable Transformation System in *Candida glabrata*

Morganne Blaylock, Brian Wickes

Candida glabrata is a pathogenic yeast that is common in immunosuppressed patients. *C. glabrata* infections are problematic because this yeast is innately more resistant to antifungals than the more commonly encountered *C. albicans*. The manipulation of genes in *C. glabrata* can be cumbersome due to a lack of well defined genetic and transformation system. Therefore, the development of a recyclable, dominant marker-based system would greatly further the study of this organism. Multiple species of *Candida* were tested for their ability to grow on acetamide, resulting in four species, including *C. glabrata*, showing no growth. In other fungi, growth on acetamide can be complemented by the *Aspergillus nidulans amdS* gene, which is an acetamide transporter, and has recently been shown to work in *Kulcyveromyces lactis*, a relative of *C. glabrata*. In order to further develop a *C. glabrata* transformation system, we created a number of mutants of *C. glabrata* using various methods. We first used UV irradiation to create adenine mutants. A dose response curve was used to select a dose for the irradiation of *C. glabrata*. The irradiated plates of *C. glabrata* were screened for pink colonies, suggesting adenine auxotrophy. Pink colonies were tested for adenine auxotrophs as well as reversion frequency. Out of 21 mutants, 9 reverted at $<2 \times 10^7$ and were selected for further study to determine if they were *ade1* or *ade2*. Next we used an analog of a uracil biosynthetic pathway intermediate (5-fluoro-orotic acid) to select for spontaneous uracil auxotrophs. Twenty were selected and tested for reversion frequency and mutation type (*ura3* or *ura5*). Amino acid analogs (alpha-amino adipate and 5-anthranilate) were used to screen for lysine and tryptophan auxotrophs, respectively. Each of these mutants will be complemented with the native wild type gene to confirm the exact mutation. Finally, each wild type gene will be disrupted with the gene *amdS*, counterselected with fluoroacetamide to evict the *amdS* from the locus, and then complemented with the wild type copy, to confirm that this system works.

Jeffrey Childers

Lance McMahon, PhD

Department of Pharmacology Tolerance Observation through Hypothermic Effects of Nicotine and RTI-102 in a Mouse Model

Jeffrey Childers, Lance McMahon

Lung cancer is the leading cause of cancer death in the United States, which highlights a need for more effective smoking cessation pharmacotherapies. Nicotine and other smoking cessation pharmacotherapies are usually agonists that bind to and activates nicotinic acetylcholine receptors (nAChRs). However, these pharmacotherapies can differ along two pharmacological dimensions from nicotine, that is affinity and efficacy. Affinity describes the likeliness of a drug to bind to a particular receptor, while efficacy is themagnitude of receptor stimulation that a drug produces. This study will focus on possible differences in efficacy between nicotine and a new potential pharmacotherapy for smoking cessation RTI-102. To examine differences in efficacy between these two drugs an approach examining the hypothermic effects of the compounds before and after chronic treatment was used. One unique aspect of chronic treatment is often a phenomenon called tolerance. Tolerance is defined as a state of progressively decreased responsiveness to a drug as a result of which a larger dose of the drug is needed to achieve the effect originally obtained by a smaller dose. We can use this approach to examine possible differences in efficacy between nicotine and RTI-102, because if RTI-102 has lower efficacy than nicotine, the magnitude of tolerance should be greater.

The effects of nicotine and RTI-102 will be assessed in 40 total mice (20 for nicotine and 20 for RTI-102). Each mouse will be given only one dose of either nicotine or RTI-102 and rectal temperature will be taken 30 min post-injection. These results will make up our control dose-response curve. On the next day mice will receive 1.78 mg/kg dose of nicotine base three times a day with an hour and a half in-between each injection. Temperature will be taken before each injection and thirty minutes after each injection. After 7 days of nicotine treatment the effects of nicotine and RTI-102 will be reassessed. I expect the results from this study to show that a development of tolerance can affect physiological effects in mice, like hypothermia. Furthermore, I expect greater cross-tolerance to RTI-102 compared to nicotine. This would be indicative of RTI-102 having lower efficacy at the particular nAChRs mediating the hypothermic response.

Rebecca Dawes

Mentor: Reto Asmis, Ph. D.

Department of Biochemistry Altered DNA Methylation of Nox4 Promoter in Response to Metabolic Stress

Dawes, Rebecca; Kim, Hong Seok; Asmis, Reto

Introduction: Atherosclerosis, a major cause of myocardial infarction and stroke, is accelerated in diabetic patients. An essential step in atherosclerotic plaque development, monocyte recruitment into the vasculature, is accelerated by increased levels of NADPH Oxidase 4 (Nox4) in monocytes. Metabolic stress is a known inducer of Nox4 protein expression in monocytes; however, the mechanism(s) by which Nox4 is induced by metabolic stress are poorly understood. A hypothetical mechanism involves the demethylation of the Nox4

promoter (removal of methyl groups from CpG islands on the promoter region of the gene), which would allow transcription factors Sp1 and Sp3 to bind to CpG islands and promote the transcriptional activation of Nox4. This study tested this hypothesis and investigates the potential correlation between demethylation of the Nox4 promoter region and Nox4 protein expression in a human monocytic cell line (THP-1). This study also investigates the earliest time at which Nox4 protein levels are increased in response to metabolic stress and the duration that they remain elevated.

Methods: THP-1 monocytes were treated with high glucose (HG; 25 mM glucose) or high glucose and LDL (HG+LDL; 25 mM glucose and 100 ug/mL LDL) and were harvested at 5 h, 24 h, 48 h, and 72 h following treatment. Unstimulated monocytes were treated with 5-Aza-2'-deoxycytidine, a demethylating agent, for 72 hours to completely demethylate the Nox4 promoter. Protein levels were determined by Western blot analysis, and the methylation status of the Nox4 promoter sequence was determined by bisulfite sequencing and PCR.

Results: Preliminary data show that Nox4 protein expression is elevated after 24 h in response to HG and HG+LDL, and remains elevated through 48 h. Nox 4 protein expression was no longer elevated after 72 h in HG and HG+LDL treated samples. Further studies will validate these results. **Conclusions:** Induction of Nox4 protein levels in response to *in vitro* metabolic stress is sustained for at least 2 days, suggesting epigenetic mechanisms might be involved. Further studies will investigate the directionality and strength of association between demethylation of the Nox4 promoter and expression of Nox4 protein levels. This work was supported by a fellowship (to R.D.) from the Max and Minnie Tomerlin Voelcker Foundation.

Angelica Fernandez

Mentor: Dr. Lora Watts Ph.D

Department of Cellular and Structural Biology

The effects of Methylene Blue following oxidative stress in astrocytes.

Angelica Fernandez, Dr. Lora Watts Ph.D, Dr. Timothy Duong Ph.D

Neuroprotection is a therapeutic intervention that aims to prevent or attenuate neuronal degeneration and loss of function in neurological diseases. However, despite decades of research, none of the neuroprotective approaches have proven to be effective against TBI in the clinical setting. Mitochondrial targeting strategies following injury have been increasingly studied as their maintenance will potentially preserve brain function. Methylene Blue (MB) is one such mitochondrial targeted strategy. MB is FDA approved agent currently used in the treat of malaria, methemoglobinemia, and cyanide poisoning. MB acts as an electron cyler that allows MB to redirect electrons to the mitochondrial ETC, thereby enhancing ATP production and promoting neuronal survival. In bypassing complex I-III activity, MB reduces ROS production from the mitochondrial ETC, which has the potential to minimize ischemic and reperfusion injury. The majority of *in vitro* studies have focused on the effects of MB on neuronal functioning, however there are no studies on MB treatment in astrocytes. Astrocytes are the most numerous cell type within the brain, outnumbering neurons 10 to 1. Astrocytes are thought to play a fundamental role in signaling, maintenance and protection of the brain. Therefore we have explored whether MB provides primary cultured astrocytes protection from an oxidant stressor (t-BuOOH) using a cell viability assay (MTT assay) and measuring mitochondrial membrane potential. We have seen an increase in the membrane

potential following MB treatment in primary cultures of astrocytes. We are currently determining the effect of MB on astrocyte viability. The studies presented here may demonstrate a novel protective mechanism in astrocytes that could improve protection following an injury that could be translated clinically.

Maria Free

Mentor: Yidong Bai, Ph.D

Department of Cellular and Structural Biology

The Effects of Stress on Mitochondrial Heteroplasmy

Maria Free, Amanda Milstein, Yidong Bai Ph.D

Heteroplasmy normally stays stable within a cell line; however, in a previous study by Dr. Bai a shift due to ethidium bromide was shown. The current study will test to see if a change happens because of oxidative and mitochondrial stress. Appropriate drug dosages and time of exposure to paraquat and rotenone dictated by viability trials will be used to apply stress to mouse cell cultures. The cells exposed to stress will be compared to those of a control to see if a shift in heteroplasmy occurred. Understanding how the mitochondria function under normal and stressful conditions will lead to future research related to the quality control mechanisms of the organelle.

Jocelyn E. Hernandez

Mentor: Ratna Vadlamudi, PhD

Department of Obstetrics and Gynecology

Natural Estrogen Receptor Agonist in the Treatment of Therapy Resistant Breast Cancer Cells

Jocelyn Hernandez, Ratna Vadlamudi

The inability to efficiently combat metastasis makes breast cancer preclude homeostasis. Current cancer treatments such as Tamoxifen, a selective estrogen receptor modulator (SERM), lead to cancerous cell resistance and human system weakening. A novel therapy for breast cancer includes natural compounds that have not induced cell resistance such as S-Equol. This agonist has the capability of triggering tumor suppression and may reduce or prevent tumor growth. Therefore, it was hypothesized that upon different levels of S-Equol treatment the appearance of therapy resistant breast cancer cells would be inhibited and substantially decreased. The experiments involved MCF7, MCF7 PELP1, and MCF7 Tamoxifen resistant cell lines. The control cell lines did not receive S-Equol treatment and were cultured in the absence of estrogen/tamoxifen (negative control) as well as with estrogen/tamoxifen (positive control). The independent variable for this study was the S-Equol treatment, while the dependent variables centered around the percentage of surviving cells, apoptotic markers, potential inhibition of cell growth and proliferation rate. Each experiment had various trials of cell lines with different doses of S-Equol (100um, 200um, and 400um). Data was gathered by an MTT Assay and a Colony Formation Assay. Proliferation rates, as shown through the MTT Assay, were substantially decreased with the second highest dose treatments of S-Equol in all cell lines. Results from the Colony Formation Assay highlighted a decrease in survival rate of the tested cell lines. The data supports that upon S-Equol treatment, the proliferation of cells was in fact inhibited and the metastatic potential was mitigated.

Christopher A. Herrera

Mentor: Nathaniel A. Jeske, Ph.D

Departments of Oral and Maxillofacial Surgery, Pharmacology, and Physiology Inflammatory Response of Neprilysin

Christopher A. Herrera, Nathaniel A. Jeske

Neprilysin (NEP) is a zinc metalloendopeptidase that plays an important role in turning off peptide signaling at the cell surface. One such peptide substrate, enkephalin, serves to reduce nociceptor activation, thereby reducing pain. However, during inflammatory injury, multiple mechanisms cooperate to increase peripheral pain. One such mechanism involves an increase in bradykinin (BK) which may effect NEP expression at the cell surface. We hypothesize that BK stimulates an increase in NEP expression and activity in nociceptors that innervate peripheral tissues. We hypothesize using caveolin which serves as scaffolding proteins for the integration of signal transduction will also stimulate NEP activity. We will monitor NEP expression by Western blot analysis and immunocytochemistry, and will measure NEP activity by using a commercially available enzymatic fluorescent assay. Research from this work will identify a new and potentially significant target for the development of peripheral inflammatory analgesics. Furthermore, results from this work will support studies that focus on the pharmacological significance of opiorphan, and endogenous NEP expression produced in the human saliva.

Christine Lamaie

Mentor: Molly Bergman, PhD

Department of Microbiology and Immunology Center for Airway Inflammation and Research (cAIR) Identification of Factors Required for *Serratia marcescens* Flow Biofilm Formation

Christine Lamaie, Norberto Gonzalez-Juarbe, Molly Bergman

Serratia marcescens is a Gram-negative rod-shaped bacterium that is frequently associated with nosocomial infections and causes a diversity of diseases including acute pneumonia, cystitis, pyelonephritis, prostatitis, endocarditis and sepsis. *S. marcescens*, like many opportunistic pathogens, can form biofilms on hospital equipment and tubing, a process likely required for subsequent seeding of patient tissues. There is a lack of knowledge regarding the molecular mechanisms driving *S. marcescens* biofilm formation. Some factors have been identified using static biofilms, but the importance of these or other factors for continuous flow biofilms, which more closely approximate natural *S. marcescens* biofilms in hospital equipment, is unknown. **The objective of this project is to identify *S. marcescens* factors critical for flow biofilm formation and maintenance.** To date, we have been developing important tools that will be used as a negative controls in our flow biofilm assays. Using standard molecular biology techniques (polymerase chain reaction, restriction digest, DNA gel electrophoresis), we have constructed mutant alleles for two genes, *oxyR* and *bsmA*. We chose to mutate these genes because of their importance in static biofilm growth and survival. Both play a critical role in static biofilm formation - *oxyR* in transcriptional regulation and *bsmA* in the production of exopolysaccharides. We have successfully constructed the suicide vectors containing the deletion mutant alleles, and will next use a selection/counter-selection strategy to replace the wildtype alleles on the *S. marcescens* genome with the mutated ones.

We are also evaluating possible chemical inhibitors of biofilms formed by other bacteria for their effects on *S. marcescens* biofilms. Results from this project will enable future studies aimed at preventing and disrupting *S. marcescens* flow biofilms in clinically-relevant settings.

Johanna Lawrence

Mentor: Murat Digicaylioglu MD, PhD

Department of Neurosurgery Autophagy and repetitive mild concussive brain injury

Lawrence J, Fletcher L, Sprague S, Jimenez DF, Digicaylioglu M

Traumatic brain injury (TBI) is a type of brain injury which can cause damage ranging from mild to life-threatening. Symptoms can range from nausea/headache to death. Children from 0 to 4, 15 to 19, and adults over 75 are most likely to suffer from a traumatic brain injury. Seventy five percent of the 1.4 million traumatic brain injuries each year are milder injuries, such as concussions. Autophagy is the main mechanism for self-degradation of cytoplasmic components of the cell. Autophagy is involved in traumatic brain injury induced cell death. The purpose of this study is to observe the short term effects of repetitive concussive brain injury on autophagy. A series of four closed-skull impacts were made in 30 minute intervals on young adult (3 month) C57 Black/6 mice with controlled repose time and impact speed. The samples were harvested at time marks 0 hour, 1 hour, 3 hours, 24 hours, 48 hours, and 72 hours from the left and right cortices, the left and right hippocampi, and the cerebellum. The samples were lysed. The lysates were processed using the Western blot analytical technique to observe the effects of repetitive traumatic brain injury on the autophagic proteins beclin-1, LC3, and Atg.

Natalia Legarreta

Mentor: Vivek Singh, MD

Department of Psychiatry Sequential Multiple Assignment Randomized Treatment (SMART) for BD

Natalia Legarreta, C Bowden, V Singh, P Thompson, C Martinez, J Calabrese, K Gao, M Quiñones, J Mintz, M Tohen.

This open methods advancement study will randomize BD patients with clinically significant symptoms to treatment with one of two mood stabilizers (MS), lithium [LI] or divalproex [DV]. Those who develop protocol defined depression will then be randomized to a MS alone, MS + quetiapine [QT] or MS + lamotrigine [LM]. The SMART strategy employs a rule for adding new treatments based on each patient's current illness state and response during the trial, mimicking the adaptive nature of treatment selection which occurs in clinical settings, but in a controlled way which allows application of causal inference. SMART eliminates unmeasured confounders associated with treatment decisions that are not randomized. The first specific aim will be to assess the feasibility of a SMART design in the conduct of an effectiveness study over 26 weeks in patients with BD. The second specific aim will be to compare the effectiveness of LI to DV as a primary component of treatment for BD over 26 weeks. The third specific aim will be to assess the effectiveness of MS + QT and MS + LM vs MS in subjects who develop depression. The final specific aim will be to determine the effects of ethnicity, language facility, education and stress as moderators of treatment outcomes,

as well as explore the use of novel statistical methodologies to more informatively characterize illness trajectories in response to the interventions. LI/DV will be dosed to attain LI or DV levels of $\geq 0.5\text{mEq/L}$ or $\geq 45\text{mg/L}$. LM will be incrementally dosed up to 400 mg/day, or, in combination with DV, 200 mg/day. Dosage may be reduced for adverse effects to one half of the target dose. QT will be started at 50 mg/day and titrated up to 300 mg as tolerated. QT will be discontinued if not tolerated at 100mg/day and the patient will be treated according to guidelines. The majority of patients at all sites will access medications through prescription benefit programs. DV, LI, LM and are available generically for subjects lacking prescription benefit. This sequential adaptive design represents a methodological innovation in bipolar trial history which will have particular implications for effectiveness studies.

Paige Livingston Lopez

Mentor: Murat Digicaylioglu MD, PhD

Department of Neurosurgery

The correlation between multiple concussive traumatic brain injuries and the activation of phosphorylated mTOR

Livingston Lopez P, Fletcher L, Shane S, Jimenez DF, Digicaylioglu M

Traumatic brain injury (TBI) occurs from head injuries that causes brain damage, and can range in severity from mild cognitive deficits to death. Military personnel, athletes at all levels, and victims of motor vehicle accidents are commonly subject to such injuries [facts obtained from NIH]. TBI affects upwards of 1.4 million people every year in the United States, killing 50,000, and leaving many survivors with impairment of the ability to learn, form new memories, and access memories from before the TBI [Shaoyi, 2007]. As the primary regulator of the cellular response to nutrient availability, which decreases during TBI, the mTOR pathway plays a critical role in neuroplastic responses to TBI. However, the underlying mechanisms for these responses are insufficiently understood. The aim of this study is to observe the effects of multiple concussive injuries on the activation of mTOR and its downstream target, S6 Kinase. After a series of 4 impacts, with controlled impact speed and repose time, made in 30 min intervals on closed skull subjects, the right and left cortex and hippocampus and the cerebellum from young adult (3 month) C57 Black/6 mice were harvested at 0h, 1h, 3h, 24h, 48h, 72h time points to observe the phosphorylation of mTOR (ser 2448) and S6 Kinase.

Joshua Singer

Mentor: David Weiss, PhD

Department of Physiology

Incorporation of the π Subunit in CNS α_1 and β_2 into the GABA_A Receptor

Joshua Singer, David Weiss

γ -Aminobutyric acid (GABA) functions primarily as an inhibitory neurotransmitter in the central nervous system (CNS). The GABA_A receptor is a multisubunit chloride channel that mediates the fastest inhibitory synaptic transmission in the CNS. It consists mainly of α , β , and γ units; six α subunits, three β subunits, and three γ subunits have thus far been reported. Atypical GABRP (π) can assemble with these known GABA_A receptor subunits, and the presence of this

subunit may alter the sensitivity of GABA_A receptors to GABA or modulator compounds. GABA/GABAR receptors are also present in non-neural tissues, including cancer, but their precise function in non-neuronal or cancerous cells has thus far been poorly defined. The π subunit (GABRP), a peripheral type γ -aminobutyric acid (GABA) receptor subunit, was identified as a novel molecular target for this disease. The main goal of this research is to identify how the π subunit co-assembles with most abundant in CNS α_1 and β_2 GABA receptor subunits affects pharmacological and biophysical properties of receptor and to see if π could be potentially involved in promoting cancer cell growth through affecting proliferation, migration, and differentiation of non-neuronal cells. π is suspected to alter GABA or/and allosteric modulators affinity (EC-50 value) or/and efficacy (maximum current or I_{max}) when co-assembled with α_1 and β_2 GABA receptor subunits producing recombinant GABARs with distinct pharmacological and biophysical properties (the EC-50 value different from $\alpha_1\beta_2$ receptors for GABA is 5 micromolar). The project will require *in vitro* RNA synthesis, Xenopus oocytes injections, electrophysiological recordings, and mammalian cell transfections.

Abhinav Suri

Mentor: Yidong Bai, Ph.D

Cellular and Structural Biology

Identifying Factors Regulating Super-complex Assembly in Mitochondria Respiratory Chain

Abhinav Suri, Christina Parros, Rasika Vartak, and Yidong Bai Ph.D

The respiratory chain consists of 5 complexes responsible for ATP synthesis and is located in the mitochondrion, the powerhouse of the cell. The traditional textbook model of respiratory complexes as discrete enzymes floating in the inner mitochondrial membrane has been replaced by a solid state structure. In this model individual respiratory complexes associate with each other to form super-complexes, which are now thought to be the units of respiration. Defects in the formation of these super-complexes have been associated with many genetic and neurodegenerative disorders; however, very little is known about how these super-complexes form. To identify which proteins regulate the assembly of respiratory super-complexes, a criterion was developed to narrow down possible protein assembly factors using homology database and protein interaction software which was applied to known and putative assembly factors in the mitochondria. Using this *in silico* approach, within the next year, systematic knockdowns on genes coding for potential proteins will be conducted and their effects on super-complex assembly at various time points will be studied in order to determine whether potential proteins affect assembly of super-complex or not.

Blanca Hernandez Uribe

Mentor: Borries Demeler, PhD

Department of Biochemistry

A Biophysical study of DNA anisotropy in the solution phase: Can Salt Change DNA?

Blanca Hernandez Uribe, Borries Demeler

The purpose of this experiment was to determine how different sodium concentrations affected DNA anisotropy. Because DNA has a negatively charged phosphate backbone which repels itself and other

DNA molecules, the DNA cannot coil/bend freely causing it to be stiff and rod-like in any aqueous solution. Two differently sized DNA fragments were placed into 14 different sodium chloride concentrations in order to neutralize the negative charge of the DNA's phosphate backbone with the positively charged sodium ions, and in turn increasing the flexibility of the DNA. By using an analytical ultracentrifuge, the sedimentation coefficients (or *s* value) of these DNA samples were measured by sedimentation velocity. The sedimentation coefficient, which is inversely proportional to the DNA's anisotropy, was found to increase with increasing salt concentration. The change in DNA anisotropy was found to be independent of the length of the DNA molecule; however the amount of change is dependent on DNA fragment length. For example, the small strand of DNA (the 208 bp fragment) only changed in *s* value from 5.2 to 5.7, whereas the larger plasmid fragment changed from 10.4 to 12 in *s* value. The greatest change observed occurred in both samples between 0 to 20 mM sodium concentration. It was also found that for sodium concentrations larger than 30 mM the *s* value of the DNA fragments no longer increased. Based on the data obtained, it can be concluded that different sodium concentrations do affect DNA anisotropy.

Samantha Van Koughnet

Mentor: Lora Watts, Ph.D.

Cellular and Structural Biology

Methylene Blue neuroprotection in a cortical impact model of Traumatic Brain Injury

Samantha Van Koughnet, Lora Watts, Ph.D., Timothy Duong, Ph.D.

Traumatic brain injury (TBI) is a multi-faceted injury and is considered the leading cause of death in both children and young adults. During injury, a surge in ROS facilitates a vicious cycle that accelerates mitochondrial damage, excitotoxicity, lipid peroxidation, and inflammation. Further, mitochondrial targeting strategies in TBI have been increasingly studied as their maintenance will potentially preserve brain function. Methylene Blue (MB) is one such mitochondrial targeted strategy. MB was synthesized in 1876 and is FDA approved to be used in the treat of malaria, methemoglobinemia, and cyanide poisoning. MB acts as an electron cyler that allows it to redirect electrons to the mitochondrial ETC, thereby enhancing ATP production and promoting cell survival. In bypassing complex I-III activity, MB reduces ROS production from the mitochondrial ETC, which has the potential to minimize ischemic and reperfusion injury. TBI was induced over the S1 region of the cortex in male Sprague Dawley rats to mimic a moderate injury. Subsequently, MRI scans were performed on the day of injury and on 1, 2, 7 and 14 days post injury to determine lesion volume in TBI versus TBI + MB treated animals. On day 14, the animals were sacrificed and immunohistochemistry was performed using Nissl and Fluro Jade to further characterize the lesion. We determined that MB treatment significantly reduced lesion volume and the number of Fluro Jade positive cells compared to TBI. Additionally, behavioral improvements were seen with MB treatment using the asymmetry test and the foot fault test. This novel mechanism has established a new neuroprotective strategy for future studies that may lead to the discovery of effective TBI treatments as stroke and TBI share common pathologies.

Rachel Weems

Mentor: Maria Danet Lapiz-Bluhm, PhD, RN.

**Family and Community Health Systems, School of Nursing
 Promoting Health Literacy using the Ask Me 3 Program**

Rachel Weems, M. Danet Lapiz-Bluhm, PhD, RN.

An estimated 90 million Americans have low health literacy. Health literacy is defined as “the degree to which individuals have the capacity to obtain, process, and understand basic health information and services needed to make appropriate health decisions” (Johnson, et al. 2013, Health Literacy: A primer for pharmacists). Patients with low health literacy will generally not understand their diagnosis and/or treatment. Patients need to be taught a quick and simple method of communication with their healthcare provider so that they can better understand and manage their disease. An approach promoted by the National Patient Safety Foundation (NPSF) is the “Ask Me 3™” campaign. The Ask Me 3™ Program encourages patients to ask their healthcare providers three questions : 1) *What is my main problem?*; 2) *What do I need to do?*; 3) *Why is it important for me to do this?*

The aim of this study was to assess the level of knowledge of the Ask Me 3™ program by community members attending a health screening. Further, it assessed whether surveyed health screening participants would be comfortable in asking these three questions at their next health care visit.

Health screening participants (N=150; mean age = 49) at the Texas Folklife Festival held in June 2013 in San Antonio were asked to fill out a survey on their knowledge of the Ask Me 3™ Campaign and their level of comfort in using the tool at their next healthcare visit. They were also given a wallet size of the tool to use. Among the 74% (N= 111) who responded to the survey, 92% had no prior knowledge of the Ask Me 3™ program. 96.4% of those surveyed reported that they would be comfortable in asking their healthcare providers the three questions at their next visit. A follow-up phone call is on-going to assess if these participants actually used the Ask Me 3™ tool and if it helped the communication with their healthcare provider.

The data suggested the Ask Me 3™ questions are not widely known in the community. However, community participants were very receptive to the tool. They expressed comfort in using the tool at their next healthcare visit. Results from the follow-up call will help determine the actual use of the tool and its usefulness for the patients.



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