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2011 Summer Research Internship Program

PATRICK F. TAYLOR
FOUNDATION

Poster Presentation Abstract Book

National Heart Lung and Blood Institute
People Science Health

2011 Summer Research Internship Program

This program was started to provide research experiences for medical students, undergraduate and high school students in Louisiana. The program directors Dr. Paula Gregory & Dr. Fern Tsien match students with mentors in laboratories or clinics at LSU Health Sciences Center, Tulane University Health Sciences Center or Children's Hospital of New Orleans. The 8-week summer research program allows students to cultivate their interest in pursuing research careers in either basic or clinical sciences. During the program students conduct their own small research project or work on part of an on-going research project.

Drs. Gregory and Tsien would like to extend their special appreciation to all mentors and poster session judges who helped make the Summer Research Internship a success! Their assistance with this project affords each student to be a part of a bigger, ongoing research project. The Directors would also like to thank supporters of this program: LSU Health Sciences Center, Louisiana Vaccine Center, Patrick F. Taylor Foundation, National Heart, Lung & Blood Institute and Tulane University School of Medicine.

Nicholas T. Alexander
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Xavier University of Louisiana, New Orleans, LA.

Mentor: Dr. Robert J. Reed, Dr. Wayne L. Backes
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Expression and Purification of the Full Length Human Cytochrome P450 Reductase (CYPR)

Cytochrome P450 is instrumental in the removal of environmental toxins, drugs and endogenous compounds that enter the body in a variety of ways. Cytochrome P450 accomplishes this by using the enzyme Cytocrhrome P450 Reductase (CYPR) as its electron donor, i.e. energy source. In this lab, the expression and purification of full length CYPR has been successfully implemented using a rabbit model.

The goal of our study was to purify and express full length Human CYPR using similar methodology as the rabbit. However, during the process of purifying and expressing full length Human CYPR, it spontaneously cleaved to a truncated, non-functional form with respect to Cytochrome P450.

Shilka S. Babu
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Mentor: Shyamal D. Desai, Ph.D.
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ISG15 Sensitizes Ataxia Telangiectasia Cells to Genotoxins by Disrupting Autophagy

Ataxia Telangiectasia (A-T) (Boder-Sedgwick/Louis-Bar syndrome) is an inherited immunodeficiency disorder. A-T patients are characterized by pronounced facial spider veins (telangiectasia), recurrent sinopulmonary infections, and an irregular gait (ataxia) that results from progressive neuronal dysfunction. Mutations in the *ATM* (*Ataxia Telangiectasia Mutated*) gene cause A-T. The *ATM* gene codes for 370 kDa ATM protein that harbors serine threonine kinase activity. ATM kinase is essential for repairing DNA damage. Consequently, A-T patients carrying mutant ATM kinase are defective in the repair of damaged DNA and are very sensitive to genotoxic agents. Hence, the central dogma has, thus far, been oriented towards the defective DNA repair as the major contributor to A-T patient's hypersensitivity to genotoxic agents. Here, we demonstrate that, in addition to the defective DNA repair, ISG15-mediated defects in protein turnover also in part, contributes to the genotoxic agent-mediated hypersensitivity of A-T cells. This conclusion is based on our two observations using anticancer genotoxic agent camptothecin: First, we demonstrate that camptothecin induces A-T cell death. Second, we show that the autophagy inhibitor bafilomycin protected A-T cell death induced by low-dose of camptothecin. In contrast, bafilomycin failed to protect A-T cells death induced by high-dose camptothecin. Combined with our previous findings that the protein turnover *via* autophagy (autophagic flux) is induced in genotoxically stressed A-T cells (Desai Lab, unpublished results), these results suggest that high-dose camptothecin induces autophagic flux in ISG15 overexpressing A-T cells. Increased autophagic flux in turn, leads to autophagic stress and A-T cell death. Supporting the model, we demonstrate that camptothecin induces protein turnover *via* autophagy in A-T but, not ISG15- silenced A-T cells. These results reveal an important role for ISG15 in determining sensitivity of A-T cells to genotoxic agents.

Nathan Backes

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Dehydroepiandrosterone and the Metabolism of Ethanol

Previous experiments performed in this laboratory with the neurosteroid dehydro-epiandosterone (DHEA) have found that this compound dose-dependently decreases ethanol self-administration in rats. However, blood-ethanol levels were disproportionately decreased at doses of DHEA that did not affect responding for ethanol. One explanation for these results is that DHEA may enhance the metabolism of ethanol. To further investigate this possibility, two groups of six male Long-Evans hooded rats were given intraperitoneal injections of 56 mg/kg of DHEA or an equal volume of cyclodextrin vehicle. After fifteen minutes both groups were administered 2.4 g/kg of ethanol intraperitoneally. At selected time intervals after the ethanol injection, blood was collected from two subjects in each group via saphenous venipuncture. After the blood was allowed to clot, the samples were centrifuged, and serum was extracted and frozen for analysis of blood-ethanol levels. The results indicated that DHEA had no effect on blood-ethanol levels compared to control. This demonstrates that DHEA does not decrease ethanol intake by enhancing its metabolism and that other pharmacological mechanisms are likely responsible for this effect of DHEA.

Glenn J Barras

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Production and HPLC purification of Fab–ricin A chain immune complexes by HPLC for crystallization and analysis of 3D conformation

Ricin is a potent toxin found in castor beans that can be used as an agent of biological warfare. Ricin is an AB holotoxin, and binding of the A or active chain by RAC18 antibody has been shown to inhibit the toxic effect. The three-dimensional binding conformation for this complex is not known. We aim to obtain a purified sample of complexed RAC18 and ricin A chain in sufficient quantity that would allow for crystallization followed by x-ray crystallography for conformational analysis. RAC18 Fab was used instead of whole Ab due to its smaller size and thus higher disposition to crystallize with minimum defects. RAC18 Fab fragments were made by papain digestion of RAC18 and subsequent separation from Fc fragments through protein A affinity chromatography. The Fab fragments were then complexed with isolated ricin A chain in a 1:1 molar ratio and the complex was purified through high performance size exclusion column chromatography on a Superose 12 column. Preliminary data has shown that RAC18 Fab forms crystals of sufficient size and purity for use in x-ray crystallographic analysis. The overall intent is to combine these results with data derived from computer molecular modeling to provide a basis for designing more effective antibodies for ricin by site directed mutagenesis of V-regions.

Indya Bruce

Undergraduate

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Mentor: Dr. Allan V. Kalueff

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ASSESSING STARTLE RESPONSES AND THEIR HABITUATION IN ADULT ZEBRAFISH (*Danio rerio*)

Zebrafish (*Danio rerio*) have become increasingly popular as a new model organism for neurobehavioral and psychopharmacological research. Here, the startle response, an organism's innate reaction to an adverse stimulus, was tested in adult zebrafish. Although startle is a well-established assay for anxiety-like behaviors in multiple species, little research has been conducted on the startle response or its habituation in adult zebrafish. Understanding startle response and habituation in organisms is crucial for assessing anxiety-related and cognitive phenotype, and may also have high translational value relevant to modeling human affective and cognitive disorders. To test startle response and its habituation, adult zebrafish were individually placed in a novel tank test, allowed to acclimate to the arena for 3 minutes, and then video-recorded (side view) for 10 minutes while receiving a startle-evoking stimulus (tapping on the surface of the tank) once every minute. In this experiment, the videos were recorded from the side view (as opposed to the top view) because startle-sensitive endpoints in the novel tank test such as distance traveled and average velocity are easier to measure in zebrafish from the side. The videos were recorded and analyzed using the Ethovision XT7 software (Noldus IT, Wageningen, The Netherlands). Our study showed that among the eighteen computer generated endpoints, the most sensitive to startle were average velocity and distance traveled within the sensitive 10-15 s time interval past startle. As the testing time elapses, zebrafish startle behaviors generally return to normal, therefore showing robust within-trial habituation. Also, with each consecutive trial, the startle-sensitive endpoints decreased, thereby showing robust between-trial habituation. Collectively, this supports the zebrafish as a viable model in neuroscience research, especially in the screening of stress-related behaviors and cognitive process.

Nick Carbajal

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Mentor: Dr. John D. Clements
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Purification of Heat Stable Enterotoxin

The Heat Stable Enterotoxin (ST) is one of the two plasmid encoded toxins produced by Enterotoxigenic *Escherichia coli* (ETEC). ETEC, the most common of the diarrheagenic *E. coli* virotypes, causes secretory diarrhea in children and travelers to underdeveloped countries. There are no current vaccines or consistently available treatment methods for ETEC and roughly 400,000 people die from ETEC infection annually, mostly children in underdeveloped countries. Dr. Clements and his colleagues have set out to formulate a vaccine against ETEC that produce ST. In order for the vaccine to be effective, the lab must determine a way to immunize individuals against this small, 2,000 dalton peptide/toxin.

The lab proposed to construct a series of mutated ST toxins that would lack toxicity but still be capable of triggering a protective immune response. Purification of ST was the initial step and was one of the main projects that I was involved in during my time in the lab. The purification process, which took an estimated fifteen months to establish, integrated an array of steps in order to successfully purify ST. In short, ST-producing ETEC strains: B41 (producing porcine STp) and 64111 (producing human STh) are cultured in minimal medium to a desired density. Once the cultures have grown stationary phase, they are clarified by centrifugation and the supernatant is filtered through a 0.2 micron filter to remove remaining bacteria. The supernatant is then passed through a hydrophobic matrix (amberlite XAD-2) column and ST eluted with methanol. The eluate is roto-evaporated to remove excess methanol and the remaining material is separated by gel-filtration chromatography on a P6 Bio gel column. The ST-containing fractions from the P6 column are pooled and concentrated by roto-evaporation. This product is then chromatographed on a second hydrophobic matrix (C-18) column and the ST separated with a methanol gradient. The ST-containing fractions are pooled, roto-evaporated for the last time to remove the methanol, and resuspended in physiologic saline. Both STp and STh were purified and used to establish immunologic (ELISA) and biologic (T-84, suckling mouse) assays for ST. In addition, an intermediate experiment was also conducted in order to make the ST purification process more efficient. The human strain 64111 was transformed with a plasmid (P_{BAD}-ST) to increase expression of ST in the wild-type strain. P_{BAD}-ST contains the gene for STh under control of the arabinose promoter (AraC) to facilitate increased ST production when presented with a specific monosaccharide (L-Arabinose).

The suckling mouse assay is the primary means of demonstrating biologic activity of ST. This assay consists of orally administering small doses of toxin to 1-3 day old Swiss Webster mice and then measuring the gut/carcass ratio after a 3-hour incubation. In the future, the suckling mouse assay will be used to provide evidence that mutants of ST are not toxic and may be suitable as toxoids for vaccination. These studies are important because they may lead to development of a vaccine against one of the leading causes of childhood morbidity and death.

Victoria A. Carter

High School Student

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Dr. Gagliardi

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Expression of DCAMKL-1 in colorectal adenoma-carcinoma sequence

Background: Stem cells are found in all adult tissue and have been shown to have self-renewal capacity. This self-renewal ability has also been demonstrated in some neoplastic cells that are now referred to as cancer stem cells. Cancer stem cells are the only cancer cells capable of metastasis and may originate from normal adult stem cells. DCAMKL-1 is an intestinal stem cell marker that has been shown to have a functional role in colorectal cancer and metastasis; therefore it may be a new marker for colorectal cancer stem cells as well as a therapeutic target for anti-cancer stem cell therapy. Expression of DCAMKL-1 in colorectal adenoma to carcinoma sequence is not known therefore our aim was to study the immunoreactivity for DCAMKL-1 in colorectal adenomas, primary colorectal cancer and liver metastasis.

Methods: Immunohistochemical staining, using a rabbit polyclonal anti-DCAMKL-1 antibody, was performed on formalin-fixed, paraffin-embedded samples from patients with adenomatous polyps, primary colorectal adenocarcinoma and colorectal liver metastasis. As a secondary antibody we used a horse radish peroxidase conjugated goat anti-rabbit antibody for 30 minutes. The staining intensity and pattern of DCAMKL-1 immunohistochemistry was done by two independent pathologists who were blinded to the clinicopathological variables. The Institutional Review Board at Tulane University approved the study.

Results: Normal colonic mucosa showed staining of 1 of 2 epithelial cells per crypt towards the crypt base. Staining was localized to the basal part of the cytoplasm. Of 18 polyps 13 (72%) expressed DCAMKL-1. Staining was localized to the apical part of the cytoplasm. Percentage of stained cells varied between 5% and 50%. Of 41 colorectal cancers 31 (76%) showed immunoreactivity for DCAMKL-1. Staining was localized to the apical part of the cytoplasm. Percentage of stained cells varied between 5% and 100%. In normal mucosa adjacent to the tumor DCAMKL-1 staining was found in the apical part of the cytoplasms and diffused the whole crypt. Of the liver metastasis evaluated 6 out of 15 (40%) were positive for DCAMKL-1. All positive liver metastasis showed cytoplasmic staining while 2 also showed strong membrane staining.

Conclusions: We found that DCAMKL-1 immunoreactivity in normal colonic mucosa is present in single cells at the crypt base but it is over expressed in a high percentage of colorectal adenomas and carcinomas. DCAMKL-1 is also over expressed in the mucosa adjacent to the tumor which is suggestive of a colonel expansion causing a "field defect". Further characterization of DCAMKL-1 positive colorectal cancer cells both in vitro and in vivo is needed to prove their role as cancer stem cells.

Kenia Carvajal

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Expressing ALS Associated Mutant Human Genes in *Drosophila*

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease that affects specifically the motor neurons impairing mobility, speech, swallowing and even breathing. ALS is a late onset disease that starts affecting adults 40-60 years of age. The majority of patients diagnosed with ALS have a prognosis of dying within a year of having the symptoms of this genetic neurodegenerative disease. Only 10% of ALS cases are familial (FALS) and about 90% of them are sporadic (SALS) but it is hoped that studying the familial forms will shed light on the mechanisms involved in both types.

Recently, it has been reported that mutations in Fused in Sarcoma/ translated in liposarcoma (FUS/ TLS) lead to both sporadic and familial forms of ALS. The protein FUS is predominantly found in the nucleus in normal patients, however in ALS patients FUS is found in both the nucleus and cytoplasm suggesting that this mislocalization is correlated with neurotoxicity. Recently, the Pandey lab demonstrated that ectopic expression of ALS associated mutant human FUS in *Drosophila melanogaster* (fruit fly) causes neurodegenerative phenotypes such as external eye degeneration, reduced life span, impairment in larval crawling ability and pupal lethality. The Pandey lab has previously identified that the RNA binding protein, Drosha affects FUS toxicity in fly eyes. Drosha is a conserved RNA binding protein that is involved in the RNA interference system. The RNA interference system is a highly conserved pathway that degrades specific RNAs in a cell.

The lab is also interested in expressing mutant human forms of FUS/TLS and in the role that the fly homologue of FUS/TLS, which is called Cabeza, plays in neurodegeneration. Cabeza is a homologue of human FUS and, consistent with the role of FUS in ALS, Cabeza mutations cause neurodegeneration in fruit flies.

The primary goal of my project was to observe six fly strains and validate their expected genotype. The particular genes of interest were FUS (human), Cabeza (*Drosophila*) and Drosha (RNAi knockdown line in *Drosophila*). Each fly strain contained two components – a "driver" which activates expression of exogenous genes, and a gene of interest. I validated these fly strains using genetic crosses and a western blot. I then used these strains to investigate whether reducing the amount of Drosha had any protective effect on ALS associated mutant human FUS toxicity in fly motor neurons.

Claire Fitzgerald

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The Effect of Mismatch Repair on Transcription-coupled GAA·TTC Repeat Expansion

Freidreich's Ataxia (FRDA) is a recessive neurodegenerative disease caused by an expansion of GAA·TTC repeats in the first intron of the *frataxin* (*FXN*) gene. The GAA·TTC repeat region is unstable and prone to both somatic expansion and contraction, though disease relevant tissues of FRDA, such as the dorsal root ganglia, show a bias towards somatic expansion. Expansion of the GAA·TTC repeat in disease relevant tissues of FRDA is thought to contribute to the progression of the severity of the disease as patients age, but the mechanism of expansion in affected cells is still poorly understood.

Long GAA·TTC repeat sequences have been shown to form unusual DNA structures during transcription. A link between transcription levels and repeat expansion rates observed in cell lines with an unstable GAA·TTC repeat suggests that the expansion of the repeat sequence in *FXN* gene is caused by some mechanism involved with repair of the unusual DNA structure. We are looking at the proteins of the mismatch repair (MMR) pathway to determine if this system may be involved with the repair of these unusual structures and subsequently the expansion of the GAA·TTC repeat. We have already found a link between the MutS branch of the MMR pathway and GAA·TTC expansion. Our study is now focused on determining whether proteins involved in the next step in the MMR pathway, the MutL proteins, are also involved in the expansion process.

Our preliminary findings show that one of the MutL proteins, MLH1, does affect the rate of GAA·TTC repeat expansion. Our study will continue to look at the other MutL proteins, MLH3 and PMS2, to determine if they also have a role in GAA·TTC expansion.

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Hippocampal Interneuronal-Parvalbumin Modification as a Consequence of Status Epilepticus

Epilepsy is a disease characterized by repeated spontaneous seizures in patients preceded by little to no warning. The mechanisms of the disease are poorly understood. The majority of seizures that are not directly attributed to mass legions or CNS trauma arise from structures in the brain such as the hippocampus and the amygdala. Epileptogenesis, the fundamental transformation from healthy brain tissue into epileptic damaged brain tissue, seems to produce a phenomenon called hippocampal hyperexcitability in which normally tolerated electrical impulses spiral out of control because of a loss of inhibitory mechanisms. Interneurons high in parvalbumin, which have previously been shown to be highly susceptible to damage, seem to be responsible for this inhibitory failure.

In this experiment, the parvalbumin interneurons were studied as an attempt to indentify clear morphological differences in the brains of naïve and status epilepticus mice, particularly in the dentate gyrus- CA3 and CA1 regions of the brain. This experiment aimed to quantify the dendrite density, along with average dendrite area, from status epilepticus mice in parvalbuminal interneuron subpopulations using the computer imaging software, Image J. These test subjects were originally injected with pilocarpine to induce status epilepticus, and some were treated with the neuroprotectin D1 (NPD1) before the brains were harvested and sectioned.

Based off initial data collected, it was observed that the naive rat brain's dendrite density was generally less than that of the brain of NC-SE (Non Convulsive) and SC-SE (Convulsive) mice, supporting the hypothesis that because of the brain damage caused by epileptogenesis, the plasticity of the brain will cause an interneuronal rewiring, causing an increase in the number of dendrites present. It is also interesting to note that in the naïve rat brain, the dendrites that were counted were, on average, larger than those in epileptic brains.

Future studies may focus on hippocampal interneuronal modification as potential compensatory mechanisms for hippocampal brain damage caused by status epilepticus, both convulsive and non-convulsive.

Zachary M. Harris

Medical Student

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Mentor: Jacob Sloane, MD, PhD

Mentor's Affiliation: Beth Israel Deaconess Medical Center, Boston, MA

Microarray analysis of oligodendrocytes in multiple sclerosis

Multiple sclerosis is a disease characterized by immune-mediated bouts of inflammatory demyelination and neurodegeneration throughout the entire CNS. Inflammatory foci in the brains of multiple sclerosis patients often undergo the reparative process of remyelination. In this process, lost myelin is replaced by oligodendrocytes, resident CNS glial cells derived from neuroepithelial stem cell progenitors. Certain chronic multiple sclerosis lesions, called shadow plaques, show significant remyelination, whereas most chronic lesions exhibit poor remyelination. The mechanisms behind why some lesions remyelinate and others do not are completely unknown. For this project we have focused on why remyelination is incomplete in progressive MS. We propose that an in-depth microarray analysis of olidogendrocytes from MS lesion subtypes will elucidate the process of remyelination in shadow plaques and its impairment in chronic inactive lesions. Also, we have performed cell-counts of oligodendrocytes in the cores and margins of different lesion subtypes, elaborating our understanding of the role of this cell population in multiple sclerosis.

Using Luxol fast blue staining and immunohistochemistry, we classified lesions from MS brains into five major subtypes: early acute plaques (EAP), late acute (LAP), chronic active (CAP), chronic inactive (CIP), and shadow (SP) plaques. We confirmed pathological features of lesion subtypes by characterizing various degrees of microgliosis, astrocytosis, and myelin vacuolation or loss. Oligodendrocytes were identified by immunofluorescence and laser capture microdissected from the cores and margins of MS lesions and control tissue. Total RNA was extracted, amplified, and tested to ensure integrity. Now that we have collected sufficient mRNA from multiple lesions, we plan to microarray analyze 10μg samples of labeled cRNA using an Affymetrix chip in order to yield expression profiles of oligodendrocytes from the different lesion subtypes.

In addition to this work, we have begun a review of the literature and current research indicating axonal loss as a distinct pathological process in multiple sclerosis. Our review has centered around why axonal loss is considered a major factor to permanent disability in multiple sclerosis, and as such why elucidating the etiology and pathogenesis of this phenomenon lies at the heart of understanding the progressive disease process in MS. We describe why axonal loss in MS is thought to be a distinct pathological process independent of inflammatory demyelination. In terms of progressive MS, we examine the relationship between axonal dystrophy and neurodegenerative progression. We review the mechanisms behind axonal injury, including those intrinsically tied to oligodendrocyte pathology. Finally, we consider how remyelination may counteract neurodegeneration, and dissect mechanisms by which the cellular milieu in MS works against or facilitates myelin repair and axonal preservation.

Matthew E. Haskins

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Histologic Types and Risk Factors in Familial Lung Cancer Cases from Southern Louisiana

Lung cancer is the second most common cancer and the leading cause of cancer-related deaths among both men and women in the U.S. The association between lung cancer and smoking is well known. However, only 15% of smokers are diagnosed with lung cancer. In addition, about 10% of lung cancer cases (22,000 cases per year in the U.S.) have at least one relative affected with lung cancer. Evidence from epidemiologic studies reveals a complex interaction between environmental risk factors and genetic predisposition in the development of lung cancer. Some studies also have shown an association between familial lung cancer and histologic subtypes. Persons with non-small cell lung cancer, especially squamous cell, are more likely to have a family history of lung cancer than those with small cell lung cancer. Similarly, females, non-smokers, and those with early age of diagnosis are more likely to have a family history. The objective of this present study was to analyze histologic subtypes and their association with smoking behaviors and other risk factors of interest among familial lung cancer cases from southern Louisiana. Eligible subjects (N=148) with two or more relatives affected with primary lung cancer were recruited from 34 hospitals within 24 parishes in southern Louisiana. Diagnosis of primary lung cancer was confirmed through medical records, and histologic subtype (N=114) was abstracted from pathology reports. Preliminary results indicated no significant difference in the age of diagnosis between cases with non-small cell lung cancer and those with small cell lung cancer; mean number of pack years was about twice as high in cases with non-small cell lung cancer; and about 81% of cases had non-small cell lung cancer, with adenocarcinoma being the most common histologic subtype. These results suggest the need for population-specific evaluation of risk factors in familial lung cancer cases.

Byron Bryce Hills
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Mentors: Erich O. Richter, MD
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Sensorimotor Representation in Human Subthalamic Nucleus: Single Spike Analysis

The possibility in which the human and particularly the Parkinsonian human STN possesses a topographically-organized map of contralateral musculature has been addressed in the literature. The degree to which there is a fully coherent map is still debatable. Although there is some evidence of this localization, we propose that an adequate study of quantitative rigor has not been used to clarify the correlation between limb movement and cellular responsiveness. We question if receptive fields lose their specificity during the progression of the disease. Therefore, we aim to test the hypothesis that the human Parkinsonian STN receptive fields respond in an isolated manner, and that a fully coherent map exists.

To test the hypothesis, neuronal activity is amplified, filtered, and fed to an A/D converter which collects samples across the 5 channels at 25 KHz at 16 bit resolution. These signals, including a time-stamp, are collected into a file for offline analysis. Spikes are then sorted using a custom algorithm written in MATLAB that initially sorts either local minima or maxima in the frequency range of action potentials (~2 kHz). The waveforms are extracted, smoothed using a spline interpolation, and then characterized for rising and falling amplitudes and slopes. These parameters are then used to group the waveforms as belonging to a single neuron. Usually amplitude provides a good criterion in determining whether an action potential belongs to a single neuron. Due to pulsation and oscillations in the brain, amplitude alone does not suffice. Thus, to assess the goodness of the estimates generated by the program we used the refractoriness of the group. None of the spikes should occur within 1 msec, and relatively few below 2 msec. Once the spikes are isolated, their relation to movement is assessed by aligning them with goniometer traces and constructing peri-movement epochs. These peri-movement epochs are then used to analyze intergrated activity using statistical measures (i.e. Chi-square distribution, etc).

The specific aims of this project are to verify the method of multi-unit analysis by determining the degree to which individual cells exhibit peri-movement activity. Through single spike analysis, we also aim to characterize the frequency of spectra of isolated cells as it might relate to tremor-related activity as well as oscillatory activity associated with Parkinson's disease and to determine the degree to which there is synchronized activity across channels.

Jasmyne S. Hudson

Undergraduate

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Mentor: Dr. Renee Gardner

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A Study of Parental Attitudes and Knowledge about Penicillin Prophylaxis in Sickle Cell Disease

Children with sickle cell disease (SCD) usually become functionally asplenic during the first 5 years of life. Pneumococcal infection occurs at an increased frequency that is up to 400 times that is seen in normal children. Penicillin prophylaxis has been used to prevent pneumococcal infection and its complications, such as meningitis, pneumonia, and septicemia. Recently a debate has risen over how long penicillin prophylaxis should be employed and there is concern over possible emergence of penicillin resistance.

We hypothesize that parents of children with SCD have been adequately educated on the risks of pneumococcal disease and the benefits of penicillin prophylaxis and will readily concur with the recommendations made regarding discontinuation of the antibiotics.

To gather information regarding parents, knowledge of the risks of infection, role of antibiotic prophylaxis, and parental reaction or attitude towards current recommendations regarding penicillin use. Methods; We surveyed the parents of patients with SCD (0-21 years), questioning them about their knowledge of the importance of infection in SCD; their utilization of antibiotic; their practices and awareness of symptoms when confronted with signs of infection; and their opinion regarding current recommendations for the discontinuation of the antibiotic. We assess the importance of factors influencing their opinion, such as race or ethnicity, gender, educational attainment, and socioeconomic status. Patients' charts were reviewed also to compile data on hospitalization for febrile episodes and/ or proven bacteremia. Parental decisions regarding continuation or discontinuation of antibiotic was correlated with this data.

The study is ongoing and data is still being collected. So, the results and data will be presented and discussed in the poster.

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Effects of Human Cytomegalovirus on Viral Replication, HIF-1α Protein Expression, and MMP 2/9 Gene Expression Under Hypoxic Conditions

Human cytomegalovirus (HCMV) is a betaherpesvirus that is the leading cause of congenital birth defects for which there is no cure or vaccine. HCMV has the ability to cross the placenta, and infect the fetus resulting in long-term sequelae. It is known that HCMV can infect extravillous trophoblast (EVT) cells and impede their invasion of the myometrium, which results in poor placentation. EVT invasion is an essential event prior to spiral artery remodeling, providing a constant supply of maternal blood to the placenta.

Prior to spiral artery remodeling, an oxygen gradient exists at the fetal-maternal interface. The oxygen gradient exists during the first trimester of pregnancy and is an essential regulator and main promoter of EVT proliferation and differentiation. Expression of the transcription factor, Hypoxia-Inducible Factor 1-Alpha (HIF-1 α), adjusts to changes in oxygen levels and is regulated through its ubiquitin-proteasomal degradation by the Von Hippel Lindau protein (pVHL) pathway in an oxygen-dependent manner. Cells that induce a hypoxic response aid in cell respiration, metabolism, and survival. HCMV is known to upregulate HIF-1 α expression after infection in human foreskin fibroblasts (HFF) under hypoxic conditions. We would like to determine if growth of EVTs in a hypoxic environment provides optimal conditions for infection by HCMV because it is known that EVTs provide an entry path for HCMV to the fetus. We hypothesize that EVTs are more favorable to infection when grown under conditions of hypoxia. Previous studies from our laboratory demonstrate that HCMV inhibits EVT invasion that correlates with decreased activity of matrix metalloproteases-2 and -9 (MMP-2 and -9), which functions to break down particles in the extracellular matrix to navigate EVT invasion.

To determine the effect of hypoxia on the kinetics of productive HCMV replication, four cell lines: HFF, first trimester EVT cell lines, SGHPL-4 and -5 cells, and retinal pigment epithelial cells, ARPE-19, were grown under normoxic or hypoxic and mock-infected or infected with HCMV (strain TRpMIA; MOI of 5) for 24, 48, 72, and 96 hours. At each time point, supernatants were collected for plaque titration analyses and RNA was isolated from cell lysates for real-time RT-PCR to determine gene expression of *MMP-2, -9*. In addition we also isolated protein from cell lysates to look at HIF-1 α expression by Western blot analysis. Collectively, these studies should establish whether hypoxia facilitates HCMV replication, upregulates HIF-1 α gene expression, and enhances the inhibition of invasion-inducing matrix metalloproteases during placentation.

Thomas V. John
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Creation of Pax3 Phosphorylation Compound Mutants

Pax3 is a transcription factor important for the early development of skeletal muscle that also plays a critical role in the development of the childhood solid muscle tumor alveolar rhabdomyosarcoma (ARMS). ARMS is characterized by a t(2;13) chromosomal translocation, which creates an oncogenic-protein Pax3-FOXO1through the fusion of the 5' Pax3 sequences to the 3' FOXO1 sequences. Phosphorylation is a means to regulate transcriptional factors. The Hollenbach lab has previously determined that Pax3 and Pax3-FOXO1 are phosphorylated at serine 205 by the protein kinase CK2. Serine 201 and serine 209 have also been determined to be phosphorylation sites in Pax3, with serine 201 being phosphorylated by the protein kinase GSK3β. Phosphorylation on Pax3 can occur during two different stages of muscle development: proliferation and differentiation. The Hollenbach lab has previously demonstrated that phosphorylation on 205 only occurs during proliferation, while phosphorylation on 209 only occurs in differentiation. Further, phosphorylation at Ser201 is dependent on the prior phosphorylation of Ser205. It is not known, however, whether there exists a relationship between phosphorylation at Ser205 and Ser 209. The question posed by this project is to determine phosphorylation at 205 inhibits phosphorylation at Ser209 and vice versa. In order to answer this question, two different types of mutant Pax3 were created through overlap extension PCR: S201A205S09D and S201A205D09S.The three phosphorylation sites can be mutated to two forms by adding aspartic acid (D), which results in a permanently phosphorylated state, alanine (A), which disallows the site to be phosphorylated, or leaving the site as serine(S). These mutants were created and cloned into pGEX-5X-1, which fuses Pax3 to glutathione-S-transferase, to facilitate purification, and underwent sequence analysis to confirm the presence of the desired mutations. Future experiments will place these mutants in bacteria cell to express the proteins and use the purified proteins in *in vitro* kinase assays, standard in the lab, to determine their ability to be phosphorylated.

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Mentors:

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Baseline Activity and Behavior Levels in a Primate Model of Depression

The prefrontal cortex (PFC) is a significant evolutionary landmark in humans. Its roles include mediation of the integration of cognition with both emotional and motivational behaviors through connections with limbic and sensorimotor structures. Abnormal PFC activity has been observed in depressive disorders, such as major depression or bipolar disorder, and in schizophrenia. Previous imaging studies of patients experiencing depressive disorders have demonstrated a hypoactive dorsolateral prefrontal cortex (DLPFC). Other observations have suggested that there is a deficit or inefficiency present in the cognitive processes controlled by the DLPFC of patients suffering with these disorders.

The role of the DLPFC in behavior and affect can be studied by inactivating it in a primate model using muscimol, a γ-Aminobutyric acid (GABA) agonist. GABA is a major inhibitory neurotransmitter and regulates neuronal excitation. Within brain tissue, muscimol binds and activates the GABA-a receptor, effectively silencing all activity of cell bodies. Previously done in this lab, muscimol was injected into the right superior colliculus of a rat resulting in unilateral turning.

In preparation to examine the behavioral effects of muscimol inactivation of the DLPFC in a primate model, we performed assessment of baseline behavior. The primate was housed in a single cage and videotaped for 24 hours. Only 22 of the 24 hours were analyzed. Behavior was scored based on one second intervals, and divided into three categories: normal, anxiety, and depressive. Behaviors classified as depressive were observed during the night but were considered normal because these were the hours the monkey slept. Also, increased anxiety-type behaviors were seen during the hours between 6am-5pm, but correlated with periods of increased worker-related activity. This baseline forms a valid diurnal pattern for comparison in future experiments with muscimol inactivation. By discretely localizing function of the DLPFC, we can further our understanding of the role of the DLPFC in behavior and affect

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Social Factors Which Predict Penetrating Trauma: Has Anything Changed in the Past Twenty Years?

BACKGROUND: Penetrating trauma and the incidence of morbidity and mortality that are associated with it occur at alarmingly high rates in the United States and subsequently place a great strain on healthcare resources. Conditions such as poverty and social isolation have been documented in the literature as strong correlates for higher rates of penetrating trauma, and inner city areas are prime breeding grounds for these particular conditions. We examine several social and epidemiologic factors to determine whether they correlate with a greater predisposition to becoming a victim of penetrating trauma. The study also looks at the severity of injury, using procedures performed in the Emergency Department (ED) and the operating room (OR) as well as type of discharge as surrogates for severity.

HYPOTHESIS: We hypothesize that male gender, younger adult age, Black or Latino ethnicity, lower levels of education, and illicit drug use increase the risk of being a victim of penetrating trauma.

METHODS: Our study is a retrospective chart review of patients who have suffered penetrating traumatic injuries. We included all patients enrolled in the trauma registry of University Hospital, New Orleans for a six month period. We documented the presence or absence of our hypothesized risk factors in the medical records of each of the penetrating trauma patients. Our study was IRB approved, and all patient data has been de-identified.

DATA ANALYSIS: Once the data collection is completed, the variables will be entered into an excel spreadsheet and analyzed for relative risk and odds ratio with a multivariate regression model. Once the data from the New Orleans area is processed, it will be compared to data obtained from an identical study performed in the South Bronx in New York to form a multi-site study of penetrating trauma in the inner-city. The risk factors in the studies that are demonstrated as increasing the risk of penetrating trauma will form the basis for the initiation of future studies in how to address this epidemic.

FUTURE IMPLICATIONS: The findings of the study will be used as incentives to provide methods of intervention to areas where penetrating trauma has been shown to occur at particularly high rates. The goal is to create preventive efforts aimed at educating the inner city population about the risks of penetrating trauma. This new wave of education would focus on factors whose correlation with higher rates of penetrating trauma is the most significant. With time we hope to eventually see a decrease in the strain on healthcare and a decrease in the deaths that are associated with penetrating trauma

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Chronic Δ-9-tetrahydrocannabinol modulates SIV disease progression in the duodenum: potential protection of barrier function via upregulation of anti-inflammatory cytokines and epithelial tight junction genes

Δ-9-tetrahydrocannabinol (THC), the primary psychoactive component in marijuana, is FDA-approved to ameliorate AIDS-associated wasting. Our studies have demonstrated that chronic THC administration results in generalized attenuation of the classic markers of simian immunodeficiency virus (SIV) disease progression in rhesus macaques. Gut-associated lymphoid tissue is considered an important site for HIV replication and inflammation; thus impacting disease progression and is a possible site for THC immunomodulation. SIV/HIV disease progression in the gut results in a pro-inflammatory and pro-oxidative milieu, which exacerbates gut barrier dysfunction, leading to enteropathy and increased viral load. We hypothesized that the anti-inflammatory modulation by THC on the immune system would maintain gut barrier function and decrease viral replication.

We examined the impact of chronic THC administration (0.18-0.32 mg/kg i.m., 2 X daily), starting one year prior to inoculation with SIV_{mac251} (100 $TCID_{50}$/ml, i.v.) on the duodenum, an important immunological site. Viral load, immunological parameters, and transcriptome was determined in gut mucosa from chronic THC- (N=4) and vehicle (VEH)-treated (N=3) SIV-infected 4-6 yr old macaques at necropsy (~5 mo post-inoculation). Gut viral load was lower in THC/SIV than in VEH/SIV (2.04±0.41 vs 3.05±0.68 RNA Log copies/ng tissue mRNA) macaques. Using Illumina Custom algorithm to compare gene expression, 115 genes were identified to be differentially expressed in gut mucosa samples of THC/SIV when compared to VEH/SIV macaques. Using GeneGo software, a total of 12 pathway maps were identified to include 3 or more differentially expressed genes ($p<0.05$). Of these maps, several were related to cytoskeleton remodeling and cell adhesion. Cytokine expression was determined using a 23-plex MilliPlex panel. Chronic THC administration resulted in differential cytokine expression. G-CSF and IL-17, both important for production of antimicrobial defensins, modulation of enterocyte homeostasis, and maintenance of barrier function were increased in the THC/SIV animals. Gut mucosa of THC/SIV macaques had significantly more $CD8^{+}$ central memory cells and $CD4^{+}$ central memory cells that were integrin β7 positive vs. the VEH/SIV animals. These findings indicate that chronic THC administration modulates SIV disease progression through suppressed gut viral replication, maintenance of gut barrier function, and preservation of protective immune cell populations.

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Validation of MRS water referencing derived from conventional proton density images versus unsuppressed water spectroscopy

Multiple Sclerosis (MS) is an autoimmune disease that affects 400,000 Americans every year. Half of these people will not be able to walk without assistance within 15 years of their diagnosis without treatment. Magnetic resonance imaging (MRI) and spectroscopy (MRS) have provided new insights into the pathogenesis of MS by providing a means by which to assess tissue health. By determining the relative concentrations of the tissue's metabolites, MRS provides volumetric data that discloses the tissue's anatomical and physiological aspects as well as metabolite concentrations, *in vivo*. One of the most highly concentrated metabolites in the human brain, N-Acetyl-Aspartate (NAA), is found primarily in the mitochondria of healthy neural cell bodies. Histopathological studies have shown that NAA is a selective marker of neurons and axons and therefore the NAA signal provides an indirect means by which to use MRS to quantify neuronal health. N-Acetyl-Aspartate is our primary outcome measure in evaluating the neuroprotective effects of epigallocatechin gallate (EGCG), the major antioxidant in the green tea.

Different methods may be used to adjust the NAA signal to compensate for differences in the magnetic fields that are induced by the patient's head interacting with the receiving coil. The easiest approach seems to be referencing the NAA to creatine, which is assumed to be constant; however, under certain circumstances, creatine levels may change. More precise methods entail referencing NAA to the water content. In our pilot data, we found that EGCG affected both levels of creatine and NAA. MRS spectra with water suppression were acquired along the anterior-posterior commissural line and were referenced to an axial MRI proton density (PD) image. The PD image provides an indirect reference for water content.

For this project, we want to compare the method of referencing NAA to the water signal using the PD image versus a second spectroscopic acquisition of the same brain region without water suppression to determine the absolute metabolite concentrations. to be determined by referencing the spectral peak for water. We have standardized a protocol to use the proton density data to determine the water content. Our hypothesis is that the water suppression spectra, referenced to the proton density image, will provide an accurate water content reference.

Specific objective 1: To implement the absolute metabolite concentration measurements.

Specific objectives 2: To compare the measurements of NAA levels using PD reference versus the unsuppressed water acquisition.

We have scanned 4 people without water suppression and will compare these images to those acquired with water suppression. By comparing MRS metabolite peaks to MRS water peaks we get a direct measurement, instead of having to use a non-MRS acquired water content value like PD, which gives us an indirect measurement.

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Effect of a Novel Polymer on the Lipid Biofouling of Silicone Hydrogel Contact Lenses

Purpose: To determine the extent of lipid deposition on four types of silicone hydrogel (SiH) contact lenses and the effect if any of use of a novel block copolymer multipurpose cleaning solution (MPS) on that deposition.

Methods: Four types of SiH lenses, comfilcon A (Bioinfinity®, CooperVision, Inc., Pleasanton, CA), senofilcon A (Acuvue Oasys™, Johnson & Johnson Vision Care, Inc, Jacksonville, FL), lotrafilcon B, (AirOptixAqua™,CIBA Vision, Inc, Duluth, GA), and balafilcon A, (PureVision™, Bausch & Lomb, Inc., Rochester, NY), were used in this study. Fluorescently-tagged (FT) cholesterol (NBD Chol, Invitrogen, Carlsbad, CA) and phosphatidylcholine (BODIPY PC, Invitrogen, Carlsbad, CA) were doped individually in artificial tear film (ATF) solution to image the lipid deposition. Lenses were exposed to the FT-lipid doped ATF under simulated wear conditions for 24 hours either prior to or after soaking overnight in MPS. Then, using confocal microscopy, the total amount of lipid present on the lenses was quantified.

Results: There was a significant difference between lens and lipid types in the amount of lipid deposited. In general, more cholesterol was found on all lenses than PC. The novel block copolymer MPS significantly decreased the amount of lipid on the lens when used after ATF exposure. The decrease in total lipid deposition was not as significantly affected with pre-use of MPS.

Conclusion: The novel block copolymer MPS had a significant cleaning effect on the lipid deposited on all of the SiHy lenses tested and therefore a viable method for increasing MPS effectiveness.

Brittany McCain

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Western Blot Analysis of Protein Expression You

Western blot analysis (also known as the protein immunoblot) is a procedure used for protein detection. Western blots can reveal a protein's size and expression level. The steps for performing a Western blot are: protein preparation, gel electrophoresis, transferring, blocking, detection with primary antibodies and secondary antibodies, and visualization of the samples. In protein preparation a cell culture is needed. The purpose of culturing cells is to get the cells to multiply/grow. The cells are treated with certain agents and then are collected for protein isolation. The protein samples are separated by gel electrophoresis. Gel electrophoresis is the process of making and running the proteins on the Western blot set-up. The gel is made by combining six liquids: water, 40% Acrylamide Mix, 1Mtris pH 6.8, 10% SDS (sodium dodecyl sulfate), 10% APS (ammonium persulfate), and TEMED (tetramethylethylenediamine). After gel electrophoresis, the samples get transferred to a membrane. The membrane is blocked by using dry milk to block the other proteins from the one that needs to be detected. Doing this, results in a more-clear outcome. The overall aim of Western blot is to find the protein size and expression level.

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Aberrant synaptic plasticity in the hippocampus during epileptogenesis

Seizures are periods of abnormal brain activity associated with convulsions, loss of consciousness, and lapse of attention, or other neurological symptoms. Epilepsy is a brain disorder characterized by recurrent seizures. In order to be diagnosed with epilepsy, one must have more than one spontaneous seizure. The period between the initial precipitate factor and the first clinical seizure is known as the "latent" or "silent" period. During this time, there are anatomical and morphological changes occurring in the interneurons of the brain.

While other studies take a more longitudinal approach in understanding epileptogenesis and the latent period, this experiment is focused on observing the morphological changes of the dentate gyrus, DG (a hippocampal structure) – CA3 and CA1 areas – during the early development of epilepsy. We studied the neuronal projections in the pilocarpine model of epileptogenesis in mice and rats using Growth Associated Protein-43 (GAP-43) and a modified Golgi staining. GAP-43 is a pre-synaptic marker that is highly expressed during growth and development of neurons. In our experiment, GAP-43 helped to evidence the plasticity and development of dendrites during epileptogenesis, while the Golgi stain technique stains dendritic spines.

The hippocampal images were analyzed images using the ImageJ software. We quantified the thickness of two different bands when observing GAP-43 immunoreactivity. In addition, we determined the dendritic spines from neurons and quantified the data using ImageJ.

It is hypothesized that there are observable changes that take place early during the development of epilepsy that are evidenced by the plasticity of neurons and its dendrites. We observed a difference in the expression of GAP43 – there was a banding pattern. In the control sections, the DG had a dark banding pattern. The epileptic brains, even those treated with NPD-1 and CSF, showed a lighter banding pattern. Preliminary studies show that dendritic spines were determined in control animals using modified Golgi staining. We observed the normal hippocampus as a U-shaped structure with somas typically located within the banded areas, and the dendrites mostly occupying the regions around the banded area.

Caitlin E. Mitchell

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Segregation of a Translocation Involving Chromosomes 12 and 22 in a Family Ascertained Through a Child with Phelan-McDermid Syndrome

Phelan-McDermid Syndrome, or 22q13 deletion syndrome, occurs when the distal segment of the long arm of chromosome 22 is deleted or lost. This deletion includes the SHANK3/ProSAP2 gene. This gene is known to help with development of the nervous system. Symptoms of this syndrome include: floppy muscle tone, delayed speech, delayed sitting up, walking, and crawling, normal to tall growth, large fleshy hands, thin underdeveloped toenails, and the inability to perspire. This syndrome is currently widely under diagnosed because of similarities to Angelman syndrome and the large parallel to autism. Currently, studies are showing that 1% of individuals with autism have 22q13 deletions or mutations of the SHANK3 gene.

Typically, Phelan-McDermid Syndrome results from a de novo that occurs in the egg or sperm, leading to the birth of an affected child. Some cases do show, however, an inherited unbalanced translocation that causes the deletion. This translocation can be inherited from either the mother or the father who has a balanced translocation. In this study, TJE has the 22q13 deletion causing Phelan-McDermid Syndrome. She, her mother, (TAE), and her father,(JE), were tested previously. It was found that TJE was missing 22q13, TAE had a balanced translocation of t(12; 22)(p13.31;q13.2), and JE was chromosomally normal. The maternal family history shows at least three intellectually impaired members of the family. This study hopes to locate the origin of the translocation on the maternal side of the family, as well as diagnose the intellectually impaired family member being tested.

For this study, chromosomal analysis was performed on peripheral blood to identify the suspected translocation or deletion. The white blood cells were grown in culture media at 37°C for 72 hours, the cells were harvested, dropped onto slides, and banded for the chromosomal analysis. These slides were then studied under the microscope at 100 times magnification and the chromosomes were cut and analyzed in a focused study on chromosomes 12 and 22.

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Dr. Jason Gardner:
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Fibroblast Isolation from Rat Cardiac Tissues

Fibroblasts are the most prevalent cell type within the connective tissue of animals. They function to secrete collagen proteins via extracellular matrix, in order to maintain a structural framework for tissues. Cardiac fibroblast cells were isolated from the tissues of adult female rats via tissue digestion with the enzyme liberase 3(a proprietary collagenase). Series' of five successive digestions were conducted using the enzyme and the amount of fibroblast cells were determined after each digestion by performing a cell count. This allowed for an indication of which digestion is most significant during fibroblast isolation. In order to digest the tissues and retrieve cells, the hearts were first removed from the female rats and immediately stored in a solution of cold KHB (Potassium Hybroboride). After removal from solution shortly afterwards, the left ventricles of hearts were minced in a solution of warm KHB. The minced heart fragments were then placed into conincal tube in which 10ml of the Liberase 3 enzyme was added as well. The conincal tube was subsequently shaken in a water bath for 15 minutes. Once the bath was completed, the tube was vortexed for 30 seconds and the supernatant was drawn Next, the supernatant was centrifuged at ~800 RPM for 8 minutes. The resulting pellet was resuspended in 11 ml of media (DMEM/10%NBS/5%FBS/PSF/10ug/ml Gent.) and placed into aT75 flask containing 9 ml of media. The process, beginning with the addition of Liberase 3, was repeated 4 times to generate a total of 5 digestions. Media was changed two hours after digestion, then every two days for (time length). After (time length) the amount of cells were counted for each digestion. It was hypothesized that the fourth and fifth digestions with liberase 3 would yield a greater number fibroblast cells than digestions one through three.

Adeem Nachabe

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Dr. Erik Flemington
Tulane University Health Sciences Center, Department of Pathology

An Introduction to Molecular Biology Techniques

During my internship in Dr. Flemington's Pathology Lab, I learned multiple molecular biology lab techniques including molecular cloning, cell culture, transfection, and real-time RT-PCR.

The first project involved the successful cloning of two new plasmids through the steps of DNA digests, ligations, transformations, minipreps, and sequencing. Later, these two new plasmids, as well as two additional plasmids, were transfected into 293 suspension cells using the calcium phosphate transfection method.

A second project involved running real-time RT-PCR tests as part of an ongoing project within the lab. After designing primers for several genes and making cDNA from RNA, several real-time RT-PCR assays were run in an attempt to verify if certain genes were induced by anti-Ig treatment in Akata cells.

Outside of the lab there was a final project involving computer analysis of next generation sequencing results to determine if there is a correlation between lymphoma cells and *Acinetobacter*.

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Cyto-toxicity in Human Lung Cells Exposed to Oil and oil-Dispersants mixture

The BP oil spill disaster caused not only large amount of crude oil to spill, but also large amount of oil dispersants. The dispersants used in response to the disaster were mainly Corexit including 9500 and 9527. Nearly two million gallons of those oil dispersants were applied to the waters of the Gulf in an attempt to break up the spill. However, the safety of these spilled oil and oil-dispersants mixture, especially their human toxicity, is still a topic for debate.

The objective of this study was to assess the cyto-toxicity of PBS-soluble fraction (PSF) of oil and oil-dispersants mixture (Corexit 9500, 9527 and 9580). To address these questions, Human lung epithelial cells A549 were cultured and then exposed to the selected dispersants for 2 hours and 24 hours. Cell viability was measured by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. The morphologic change of A549 exposed to PSF was also investigated.

The results showed that the viabilities of A549 cells were significantly decreased in response to 2% of PSF from oil-Corexit 9527 and 5% of PSF from oil-Corexit 9500 whereas no decrease was found in cells treated for 2 hours by PSF from PBS, crude oil and oil-Corexit 9580 at this experiment. For 24 hours, the viabilities of A549 cells were significantly decreased in response to 2% of PSF from oil-Corexit 9527, 5% of PSF from oil-Corexit 9500 and 20% of PSF from oil-Corexit 9580 whereas no decrease was found in cells treated by PSF from PBS and crude oil. The morphologic changes of A549 treated by 5% of PSF from oil-Corexit 9527 and 5% of PSF from oil-Corexit 9500 for 2 hours. The results suggest that the cyto-toxicity of tested PSF from crude oil and oil-dispersants mixture is different. Dispersants may increase the PBS soluble of crude oil. However, further studies are required to understand the mechanism of cell death in response to oil and oil-dispersants mixture.

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Constructing an *ede1* allele for gene knockout to study the Cin1-mediated endocytic pathway in the pathogenesis of *Cryptococcus neoformans*

Cryptococcus neoformans is a pathogenic yeast-like fungus that causes meningoencephalitis especially in immunocompromised patients. The ability of *C. neoformans* to grow and infect patients heavily relies on the expression of several virulence factors, including a polysaccharide capsule, a melanin pigment, and extracellular enzymes such as ureases and phospholipases. Of particular importance is the polysaccharide capsule, the synthesis of which depends on the intracellular transport of membrane vesicles containing capsular components and extracellular enzymes. Previous studies have shown that the endocytic adaptor protein Cin1 (cryptococcal intersectin 1) links vesicle trafficking events to signaling processes and regulation of the actin cytoskeleton. Despite the demonstrated significance of Cin1 and other protein mediators of transport pathways in the pathogenesis of *C. neoformans*, the precise interactions between these proteins and their individual roles in transport pathways have yet to be elucidated.

Interestingly, Cin1 is homologous to human intersectin ITSN1, which is similarly involved in membrane trafficking, actin cytoskeleton organization, and signal transduction. However, homologs of Cin1/ITSN1 have not been found in ascomycetous fungi such as *Candida albicans* and *Saccharomyces cerevisiae*. Instead, *C. albicans* and *S. cerevisiae* rely on the adaptor protein Pan1 to coordinate the endocytic cycle and actin cytoskeleton dynamics. Although Pan1 and Cin1 appear to have emerged through divergent evolution, *C. neoformans* retains a Pan1/Ede1 endocytic protein homolog. Knowledge of the Pan1/Ede1 function will help us to determine whether Cin1 contributes a unique function to the intracellular transport mechanisms of *C. neoformans*, or whether Cin1 is a redundant protein that provides the fungus with 'Pan1' function.

In order to characterize Pan1/Ede1 function, a method was developed for constructing a Pan1/ede1 gene knockout allele. The *PAN1/EDE1* gene was first amplified with PCR and cloned into the vector TOPO® TA. The *PAN1/EDE1* gene was then re-cloned into the vector pUC18, and an internal partial fragment was released via a double digest using Apa1 and Xho1 restriction enzymes. Ongoing experiments will involve the insertion of a selectable marker gene cassette encoding resistance to the antibiotic nourseothricin (*NAT*) into the location from which the partial sequence was deleted in order to generate a *pan1/ede1::NAT* allele. This mutant allele will then be transformed into the fungal strain through biolistic genetic transformation, and mutant strains will be selected based on growth in the presence of *NAT*. Characterization of the resulting *pan1/ede1* mutants will lead to a better understanding of Cin1-mediated endocytic machinery and its function in intracellular trafficking and fungal virulence.

Daniel B. Noel

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UTILITY OF ULTRASONOGRAPHY IN EVALUATING VOCAL CORD MOBILITY AFTER THYROID AND PARATHYROID SURGERY

Objective: To determine the utility of ultrasonography (USG) in determining vocal cord function (VCF) following thyroid and parathyroid surgery. .

Study Design: Prospective, Observational Study.

Methods: Patients undergoing thyroid and parathyroid surgery by a single surgeon (RRW) at a tertiary care facility from January 2011 to date were included. Recurrent laryngeal nerve monitoring was used in all procedures. VCF was documented with preoperative fiberoptic laryngoscopy (FL). An immediate perioperative ultrasound examination was performed after extubation. VCF was documented as "Normal" or "Abnormal". The ultrasonography performed by the attending surgeon (RRW) or the ENT resident. Postoperative voice and FL in follow up examination were documented.

Results: Nine female patients with a mean age of 52.7 years (range, 34 – 75) underwent a total of 10 procedures. The procedures included parathyroidectomy (4/10), hemithyroidectomy (5/10), and total thyroidectomy (1/10). 30% were endoscopic video-assisted and 70% were open procedures. The preoperative VCF was normal in all 10 patients. In 9 procedures, nerve was localized and stimulated intra-operatively. In one patient undergoing a total thyroidectomy, recurrent laryngeal nerves were identified but had erratic nerve stimulation. Bilateral VCF was normal and visualized without patient discomfort in all patients. The postoperative USG finding correlated with good voice quality and normal bilateral VCF on follow up FL.

Conclusions: USG provides a non-invasive and accurate estimate of VCF immediately after thyroid and parathyroid surgery. USG for VCF assessment is technically easy and can be easily adapted into the practice by physicians in various levels of training and experience.

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Toxicity Testing of Novel Antibacterial Compounds with *Caenorhabditis elegans*

Approximately 1 in 20 hospital patients will contract a nosocomial infection caused by a multi-antibiotic resistant microorganism. Chlorhexidine (CHX) is an antiseptic effective against many of these pathogens and resistance is uncommon. However, CHX is poorly absorbed and relatively toxic compared to antibiotics. GUMBOS (**G**roup of **U**niform **M**aterial **B**ased on **O**rganic **S**alts) are compounds created by binding CHX to various β-lactam antibiotics. These ionic liquids show great promise as new therapeutic agents to treat antibiotic-resistant bacteria. Toxicity testing of GUMBOS with mammalian cell lines is in progress. The purpose of this study was to determine GUMBOS toxicity to a whole organism other than a vertebrate animal. *Caenorhabditis elegans* is a nematode extensively studied as a model organism for a number of research fields. We used this worm to determine the concentration of two GUMBOS (CHX di-ampicillin [CHX $(Amp)_2$] and CHX carbenicillin [CHX Carb]) that resulted in a lethal dose to 90% of a population (LD_{90}) relative to CHX alone. L1 stage *C. elegans* were exposed to serial dilutions of GUMBOS or CHX for 24 hours at 20°C. Worms were then transferred to agar plates and scored for motility (live) or non-motility (dead). The LD_{90} values for CHX, CHX $(Amp)_2$, and CHX Carb were 25, 50, and 25 μM, respectively. These results indicate that the GUMBOS CHX $(Amp)_2$ is two-fold less toxic than CHX alone. Further toxicity testing using mice is warranted.

Jesús Pérez

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Heat shock protein beta-8 rescues eye neurodegeneration in a *Drosophila* model of Amyotrophic Lateral Sclerosis

Neurodegenerative diseases are age-related conditions caused by the gradual loss of neurons and the accumulation of ubiquitinated protein aggregates. Amyotrophic lateral sclerosis (ALS), commonly known as Lou Gehrig's disease, is a late onset disease affecting motor neurons in the brain and spinal cord, with most patients suffering from fatal paralysis and respiratory failure 1–5 years from diagnosis. Mutations in TDP-43 and FUS have been identified in both sporadic and familial forms of ALS. TDP-43 and FUS are DNA/RNA-binding proteins that are structurally and functionally similar and are involved in several RNA processes including microRNA processing, transcription, splicing, transport and translation.

Recently, our lab developed a *Drosophila melanogaster* (fruit fly) model of FUS-related neurodegeneration that imitates mutation-dependent toxicity. Overexpression of mutant FUS, but not WT FUS, caused an accumulation of ubiquitinated proteins, neurodegeneration, larval-crawling defects and early lethality in the flies. Here, we examined the role of 22 kDa heat shock protein beta-8 (HSPB8) in suppressing the neurodegenerative effects of mutant FUS. Heat shock proteins (HSPs) often act as molecular chaperones to help stabilize misfolded proteins and protect the cell from protein-induced toxicity such as the accumulation of ubiquitinated proteins in ALS. Therefore, we hypothesized that overexpression of HSPB8 would result in a decrease in the neurodegenerative effects of ALS-associated FUS. Our preliminary results show that co-expression of the mutated form of FUS with both human and *Drosophila* forms of HSPB8 causes a suppression of FUS-related neurodegeneration. Our data indicates that heat shock proteins may play a protective role in proteinopathies. Next, we will determine if overexpression of the human or *Drosophila* form of HSPB8 can accelerate mutant FUS degradation using a western blot. We also plan to test if the HSPB8-mediated protective effect is specific for FUS-related neurodegeneration or if it has a general protective effect against any neurodegeneration-causing protein. In summary, we have identified a conserved protective role for HSPB8 in our *Drosophila* model of ALS-associated neurodegeneration, which may be relevant for future therapeutic interventions in ALS and other major neurodegenerative diseases.

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Tulane School of Medicine

Genetic Manipulations of *Salmonella Typhimurium*

Salmonella bacteria cause both acute and chronic disease in people and accounts for over half a million deaths annually worldwide. While it is known that helper CD4 T cells are important in immunity to *Salmonella* infection, less is understood about the mechanisms through which CD4 cells act and their anatomical relationship to the bacteria itself. To address this, we genomically tagged wild-type *Salmonella typhimurium* with a model epitope or a fluorescent protein. We predicted that genetically transforming *Salmonella*, would allow flow cytometric analysis of CD4 T cells and/or microscopic visualization of bacteria in infected mouse organs. Initially, in order to determine if we had attenuated genomically altered *Salmonella* strains, we analyzed their growth kinetics. After confirming that all strains grew equally well, we compared bacterial burden of each strain *in vivo*. Lastly, we targeted a fluorescent protein, tdTomato, to two *Salmonella* pseudogenes under the control of two different promoters. Our results show that while there was no difference in the *in vitro* growth kinetics of our various *Salmonella* strains, 2W1S tagged strains were attenuated *in vivo*. We also successfully fluorescently marked *Salmonella* for later visualization. These results will help to further understanding of the cellular immune responses toward infection and allow visualization of *Salmonella* throughout the time course of infection.

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Chronic Alcohol Differentially Regulates Expression of Genes Involved in Inflammation, Adipogenesis, and Adipokine Signaling in Adipose Tissue of Rhesus Macaques

Alcohol is the most common and costly abused drug in the United States with approximately 14 million American adults (~7%) classified as alcohol abusers. Chronic alcohol abuse results in dysregulation of fat mass, dyslipidemia, and adipokine alterations. We hypothesized that chronic alcohol consumption would produce adipose depot-specific changes in expression of genes related to inflammation, adipogenesis and adipokine signaling.

The purpose of the study was to quantify expression of genes involved in inflammation, adipogenesis and adipokine signaling in chronic *ad libitum* ethanol drinking (ALC; 2.5g-4.1g EtOH/Kg/day) (N=4) and control rhesus macaques (N=4). Subcutaneous, mesenteric, and epididymal adipose tissue, obtained at necropsy, was flash frozen and stored at -80°C. RNA was isolated and RT-qPCR was performed for markers of cellular inflammation and oxidative stress (CCL2, CD68, FOXO1, IFN-γ, IL-6, TNF-α), adipogenesis (C/EBP-α, CREB-1, PPAR-γ), adipokines (adiponectin, resistin, PAI-1), and adipocyte functional markers (FABP4, HSL, RBP4). Results were analyzed using the ΔΔCp method.

Gene expression of CCL2, a monocyte chemotactic protein, and CD68, a macrophage marker, were increased in the mesenteric tissue as were the pro-inflammatory cytokines IL-6, TNF-α, and IFN-γ. CREB-1, a transcription factor necessary for adipogenesis, was significantly increased; however PPAR-γ, the earliest marker of progenitor cell adipogenesis commitment, was decreased in the subcutaneous depot. C/EBP-α was increased in both mesenteric and epididymal depots. Additionally, the adipokines PAI-1and resistin were increased in the mesenteric depot. Finally, all three adipocyte functional markers were upregulated in the mesenteric depot.

These data suggest that chronic alcohol consumption results in differential adipose tissue depot expression of inflammatory, adipogenic and adipokine genes. Furthermore, these results indicate that alcohol modulates transcription factors necessary for adipogenesis in both subcutaneous and mesenteric depots, which may be a central mechanism underlying fat mass redistribution associated with chronic alcohol consumption.

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Effects of vascular risk factors on brain atrophy in multiple sclerosis

200,000 of the 400,000 Americans that suffer from MS will not be able to walk without aid 10 years after diagnosis. Most of the disability of the disease is thought to be due to axonal degeneration. Since most of the brain volume is comprised of neurons and axons, whole-brain atrophy measured by magnetic resonance imaging may be used to evaluate macroscopic neuro-degenerative disease progression in patients with MS. Brain atrophy occurs in people with MS approximately ten times faster than what occurs with healthy aging and is a good predictor of both current disability as well as disability progression. Thus, atrophy may be a good biomarker for disease severity as it is much more reproducible than clinical measures and it correlates well with them. Furthermore, the open-source software SIENAX has undergone extensive validation and is known to provide reliable estimates of total brain tissue volume normalized for a standard skull size.

We currently do not fully understand the risk factors that predict the progression of disease in patients with MS. Recent studies suggest that vascular risk factors may increase the progression of disability in MS. However, their impact on brain atrophy is unknown. Understanding risk factors for progression can suggest targets for new drugs and recommend behavior changes that can alter the disease course.

Our hypothesis: Vascular risk factors, such as diabetes, hypertension, hyperlipidemia and BMI, and race are independent predictors of progression as measured by brain atrophy. The specific aims of this study are to:

1. Use SIENAX (Structural Image Evaluation using Normalization of Atrophy), a single time-point cross-sectional tool, to measure whole-brain atrophy
2. Collect the information/statistics on vascular risk factors from patients of the LSUHSC Multiple Sclerosis Clinic of New Orleans
3. For this preliminary dataset, determine if the above risk factors predict brain atrophy.

Upon consenting eligible patients, we collected demographic information including age, gender, race, ethnicity, and type of MS. Subject charts were reviewed for a previous history of hypertension, hyperlipidemia, diabetes, smoking habits, history of stroke or transient ischemic attack, coronary disease, myocardial infarcts, weight, and BMI. Questionnaires were mailed to participants for self-reported smoking habits and demographic information. MRI scans were collected for cerebral atrophy analysis using SIENAX. We extracted the brain and registered it to a standard brain and then segmented the brain parenchyma into lesions, grey matter (GM), white matter (WM), peripheral grey matter (PGM), and cerebrospinal fluid (CSF). SIENAX then calculates the whole-brain volume and normalizes it according to the head size; it also estimates the volumes of GM, WM, PGM, and CSF.

To test our hypothesis, we will construct a regression model with brain trophy as the dependent variable, disease deviation as a covariate, and the vascular risk factors as covariates as well.

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Detection and sub-cellular localization of differentiation protein markers in estrogen receptor positive and negative breast cancer cells

Abstract

MCF-7 breast cancer cells are typical of a more differentiated, less invasive breast cancer and express estrogen receptor α that mediates estrogen signaling. Proteins typical of the more differentiated phenotype, such as E-cadherin that mediates cell-cell contact, may be more highly expressed in these cells. MDA-MB-231 breast cancer cells are less differentiated and more invasive and may express proteins typical of the undifferentiated, mesenchymal phenotype such as vimentin, fibronectin and β-catenin. We hypothesized that MCF-7 cells would express higher levels of E-cadherin protein than in MDA-MB-231 cells, and conversely that MDA-MB-231 cells would express higher levels of vimentin, fibronectin, and β-catenin proteins. Western immunoblotting was used to determine protein levels of E-cadherin, vimentin, vibronectin, and β-catenin. Immunocytochemistry (ICC) was used to detect intracellular protein localization. The results supported the hypothesis, as Western Blots revealed a higher E-cadherin protein expression in MCF-7 cells compared to MDA-MB-231 cells, and conversely, a lower expression of fibronectin, and β-Catenin. In addition, ICC results demonstrated E-cadherin membrane localization in MCF-7 cells, and nuclear and cytoplasmic expression of proteins β-catenin and fibronectin, respectively within MDA-MB-231 cells. These results support the hypothesis and confirm two methods of visualizing protein expression and localization within breast cancer cells that have different invasive phenotypes.

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Anatomical Basis of Cognitive Aging

Changes in the brain structure of normal, elderly individuals have been widely reported. Brain "shrinkage" and enlargement of the sulci and ventricles have been thought to be senescent changes in neurologically intact individuals. More recently, neuroimaging studies, including magnetic resonance imaging (MRI), have shown that certain brain regions may be more vulnerable to aging compared with other brain regions. Previous work exploring the neural basis of cognitive decline in normal aging populations have shown differences in the structure of the aging brain that is associated with specific cognitive function.

The purpose of this study is to better understand how changes in brain anatomy may affect cognitive (memory, language, visuospatial) function in healthy and non-healthy aging. Using MRI methods and neuropsychological testing, this study will measure brain volume loss and cognitive function in normal middle aged/elderly individuals.

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Generation of fusion Alu-tag constructs for assay of Alu retrotransposition in culture

Alu elements belong to a group of repetitive DNA elements in the genome known as Short Interspersed Elements or SINEs for short. Alu elements generate new copies of themselves through the process of retrotransposition, in which an RNA intermediate acts as a template for the new copy. New Alu insertion can occur anywhere in the genome. Recent studies have shown that the rate of activity of mobile elements within the human genome is much higher than previously estimated; namely, Alu elements are responsible for the majority of the documented instances of human retroelement insertion-induced disease. Alu activity can generate a large diversity of diseases, such as breast cancer, hemophilia, and diabetes. Despite the implications that Alu element retrotransposition has on human health, the available methods by which Alu activity can be studied are limited. To date, only a neomycin tagged Alu construct is available for the detection of Alu activity in culture. Expanding the repertoire of markers providing other antibiotic selections and/or detection strategies (such as fluorescence) is of critical importance for the study of Alu biology. We propose to create Alu constructs with a fusion reporter gene which allows selection using neomycin resistance and either drug resistance to blasticidin or puromycin or detection using the fluorescent proteins GFP (which fluoresces green) and tomato (which fluoresces red). The designs of these four constructs require that the marker be disrupted by an intron that is removed from the transcript. This guarantees that the marker will only be activated after a retrotransposed copy of the spliced RNA is inserted in the genome *i.e,* activation of the reporter gene indicates the retrotransposition of an Alu. Because Alu are transcribed by RNA Polymerase III, the constructs must be free of stretches of four or more T's which act as transcription termination signals. Also, a self-splicing intron must be present as Alu transcripts are not processed by the spliceosome. We present data demonstrating the construction and evaluation of these new tagged Alu constructs. Our data shows that the creation of the fusion protein sequence does not affect splicing. Initial attempts at the fusion protein construct proved unsuccessful. Possibly, the lack of space between the two proteins of the fusion interfered with proper folding. For this reason, the constructs have been redesigned to include a stretch of amino acids which will act as a spacer between the two fused proteins.

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Invasion of host endothelium by *C. albicans*, but not *C. glabrata*, alters both VEGF and Notch signaling required for wound healing

A number of species of the fungal genus *Candida* are found as opportunistic pathogens causing a variety of human health issues ranging from mild superficial infections to fully disseminated, blood borne disease. In the U.S, *Candida albicans* is the most common cause of fungal infections among immunocompromised patients, with *C. glabrata* a distant second, though emerging rapidly. Of the major distinctions between these species, several traits have emerged as important virulence factors, including the ability of *C. albicans* to form invasive hyphae and the inherent adhesiveness and drug resistance found in *C. glabrata.* As both species are capable of causing disseminated disease, a process requiring transmigration across the vascular wall, we have analyzed the response of vascular endothelial cells (EC) to adherence and invasion by both *C. albicans* and *C. glabrata.* Our data suggests that co-culture with *C. albicans,* but not *C. glabrata*, induces significant changes in endothelial gene expression patterns normally required for efficient repair of vessel injury.

Previous studies in our lab have shown that *C. albicans* inhibits endothelial migration and proliferation; key components of angiogenesis and the wound healing process dependent upon the coordinated regulation of both Vascular Endothelial Growth Factor (VEGF) and Notch signaling pathways. Quantitative gene expression analysis reveals significant up regulation of VEGF mRNA in *C. albicans*, but not *C. glabrata*-treated endothelium. Interestingly, we find down-regulation of VEGF co-receptors NRP1 and NRP2 and no change in expression of a number of VEGF targets including several matrix metalloproteases (MMPs). These data suggests that VEGF signaling is non-functional in these cultures. We also find rapid induction of the Notch ligand Dll4 and of the Notch targets Hey1 and Hey2. Inhibitor studies suggest that these changes in expression are not entirely VEGF or Notch-dependent. Immunocytochemical analyses show that EC grown in control (EC alone) or infected (EC: *C. albicans* co-culture) conditioned media (CM) are able to internalize recombinant VEGF protein. Conversely, for EC grown in infected CM, VEGF remains primarily cell-surface bound and internalization is limited. Lastly, in a Matrigel tube-forming assay we find that EC grown in infected CM are less efficient at forming mature vessels when compared to control.

In summary, endothelial cells infected with *C. albicans* have altered gene expression patterns, including changes in VEGF- and Notch-related genes. These changes may result in the inability of neighboring cells to respond to normal wound healing signals, possibly facilitating the development of systemic candidiasis. Understanding how endothelial cells respond to invasion by C. *albicans* will allow for more rapid development of new treatments for systemic fungal disease.

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The Histological Effects of Corneal Cross-linking in a Bacterial Keratitis Model

Purpose: To determine and visualize the structural changes induced by UV crosslinking in the corneal stroma of infected rabbit corneas.

Methods: The bacterial keratitis model entails infecting the cornea by interstromal injection of 1000 CFU Pseudomonas Aeruginosa and allowing the infection to progress for 37 hours prior to intervention. Sixteen New Zealand white were infected in both eyes. Corneal crosslinking was performed in one eye of each rabbit by removal of the epithelium, additional of riboflavin drops for 30 mins, and then irradiation by UVA 370nm. The contralateral control eye was treated with antibiotic. Animals were sacrificed at a half hour, 4 and 8 hours post-crosslinking. At the time of sacrifice, the corneas were harvested and frozen in OCT media for sectioning. Sections were stained with Hematoxylin and Eosin.

Results: Significant differences were found between non-infected, infected and crosslinked eyes in terms of corneal thickness, interstitial spaces between the stroma collagen fibrils, and amount of infection present. Specifically, there was significantly less edema in the interstitial space in the crosslinked cornea compared to the non-crosslinked cornea. However, the structural effects were diminished over the experimental period.

Conclusion: Corneal crosslinking may be useful for maintaining corneal integrity in severe infections.

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Trisomy 21 Karyotype without the Down Syndrome Phenotype: Molecular Cytogenetic Analysis of the Down Syndrome Region

According to the Center for Disease Control, Down Syndrome is one of the most common birth defects, with an estimated 6,037 cases occurring annually in the United States. It is generally characterized by the presence of an extra chromosome 21 (trisomy 21). A contiguous gene region, called the Down Syndrome Critical Region (DSCR), contains most of the genes associated with the Down Syndrome clinical features and is located on 21q22.11-22.2. This study focuses on a female who was found to have trisomy 21 by routine chromosome analysis (karyotyping), but did not have features typical of Down Syndrome. The 24-year old female patient had a short stature, mental deficiency, strabismus and abnormal dermatoglyphics, none of which (with the exception of the mental deficiency) are typical hallmarks of Down Syndrome. The patient's Eptein Barr Virus-transformed lymphoblasts were cultured and analyzed by karyotyping and fluorescence *in situ* hybridization (FISH) to examine the DSCR.

Preliminary results indicate that the patient has three 21 chromosomes, one of which has a small deletion. Since it is the presence of an extra DSCR and not and extra chromosome that causes Down Syndrome, it is theoretically possible to have trisomy 21 and not exhibit any phenotypical Down Syndrome features. If one of the three Down Syndrome critical regions is deleted or simply inactivated, the individual is not going to have the typical appearance and conditions associated with Down Syndrome as in the present case. This occurrence would be extremely rare, requiring both a non-disjunction event in meiosis and a deletion of the DSCR.

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Disputing a topographical representation of contralateral musculature in the Parkinsonian Subthalamic Nucleus through multiunit cell analysis

Parkinson's disease is a neurodegenerative disease characterized by hypokinesia, resting tremor, and rigidity. The preferred method of treatment currently is deep brain stimulation to the subthalamic nucleus. However, the specific targeted neuronal populations within the subthalamic nucleus are more speculative among physicians based on neural activity associated with contralateral limb movement encountered during surgery. To adjust the location of electrode placement during surgery, physicians move medially for lower extremity or laterally for upper extremity cell populations based on an assumed subthalamic nucleus homunculus.

Current research claims a somatotopic organization of the Parkinsonian subthalamic nucleus exists among humans. This study aims to apply a more rigorous method of data analysis to determine if a homunculus exists within the Parkinsonian subthalamic nucleus. In this study, microelectrodes are used during deep brain stimulation (DBS) to determine the geographic location of the STN and therefore proper electrode placement. 5 microelectrodes are placed over a near horizontal plane in Parkinsonian patients within the subthalamic nucleus (STN). The underlying neurophysiology of neurons allows the electrodes to locate neuronal populations, or multi-unit neuronal activity corresponding to specific contralateral musculature. The electrodes can simultaneously sample a 4mm by 4mm area over a specific time that is locked to when limb movement occurs. The relationships derived from data analysis provide insight to increasing and decreasing multi-unit neuronal cell activity within specific regions of the STN, allowing us to determine if a homunculus exists.

Data from four patient surgeries have been analyzed to date. Thus far, no evidence of a homunculus within the subthalamic nucleus of Parkinsonian patients has been found. The results of this study intend to provide clinicians a more accurate topographic representation of the subthalamic nucleus during DBS.

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Chloride Dynamics and the Generation of Hypochlorous Acid within Neutrophils

Cystic Fibrosis (CF) is an inherited recessive genetic disease which is caused by a mutation of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, a cAMP-activated ATP-gated chloride channel within the membranes of many epithelial tissues in the body, including the lungs. The mutation of the gene impairs the ability for the CFTR channel to transport chloride across these membranes, causing thickening of mucus. This mucus plays a role in decreasing the ability of the body to fight bacterial infections; however, neutrophils may play an important part as well, leading eventually to the fibrosis characteristic of CF. Neutrophils phagocytose bacteria within the lungs and annihilate them with oxidative species such as hypochlorous acid (HClO). Chloride anion concentrations within these neutrophils are absolutely critical for the production of HClO. An investigation into the cytosolic chloride dynamics of these neutrophils has been made through an expressed dual fluorescent protein probe which is sensitive to chloride concentration within differentiated promyelocytic cells, PLB-985 and PLB-X-CGD, which have been tranduced through a lentiviral vector to express the protein probe. These promyelocytic cells were differentiated into neutrophil-like cells for 4-5 days by culturing the cells in DMF with low serum then analyzed through two-color flow cytometry.

Neutrophils experience an efflux of cytosolic chloride when stimulated by various agents such as phorbol 12-myristate 13-acetate (PMA). The expressed fluorescent protein probe demonstrated that both types of differentiated cells underwent an efflux of chloride after being stimulated by PMA. The efflux of chloride from the wild-type, differentiated PLB-985, neutrophils culminated after 10-15 minutes, giving way to a recovery influx of chloride to resting levels for the next 10-15 minutes. Both wild-type cells that were treated with drugs to inhibit NADPH oxidase and the NADPH oxidase-deficient X-CGD cells did not recover to their resting cytosolic chloride concentrations after PMA-induced chloride efflux. A possible player controlling chloride flux across the plasma membrane is membrane potential. Various ionophores were used for its investigation which suggested that its dynamics may be driven by a voltage-gated chloride channel. With these two types of cells differing only in their rate of recovery of cytosolic chloride concentrations to resting levels after PMA stimulation, the influx of the chloride may be driven solely by the NADPH oxidase which is absent in the X-CGD cell line.

Another study, which was conducted on opsonized zymosan-stimulated human neutrophils, suggested the importance of two proton channels within the phagolysosome membrane. These two membrane proteins, the voltage-gated HV1 channel and the vesicular-ATPase proton pump, contribute in varying degrees to the influx of protons into the phagolysosome in order to generate the microbicidal agent HClO. The study was performed through inhibition of the respective proteins by means of zinc (500 μM) for VG-HV1, and concanamycin A (500 nM) for V-ATPase. A fluorescent probe, dihydrorhodamine 123 was used at a concentration of 5 μM to detect levels of NADPH oxidase activity. Zinc and concanamycin A inhibited NADPH oxidase activity by approximately 65.2 % ± 4 % and 20 % ± 5 %, respectively.

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Frequency Analysis during Epileptogenesis

Epileptogenesis is the pathological process that leads to epilepsy. This process involvs the transformation of a neural network into one that is chronically hyperexcitable. It usually refers to a period of months to years between a traumatic accident such as a head injury, and the first seizure. Neuroprotectin D1 (NPD1) is a docosahexaenoic acid-derived lipid mediator that protects the brain against oxidative stress. Our laboratory has discovered that NPD1 inhibits apoptosis and promotes cell survival in epilepsy. We now study how NPD1 sustains the neuronal network integrity. We hypothesize that NPD1 counter-regulates the neuronal alterations during epileptogenesis.

Temporal lobe epilepsy is the most common form of epilepsy, usually originating from the medial or lateral temporal lobe, that causes simple partial seizures (without loss of awareness) and complex partial seizures (complete loss of awareness). We use multi-array electrodes (silicon probes) implanted in the hippocampi of rats and mice with experimentally-induced temporal lobe epilepsy treated with NPD1 or vehicle. The local field potentials recorded by the probes during physiological conditioning were selected and filtered, and the power and correlation of theta (4-8 Hz), gamma (21-40 Hz), and high frequency (100-300 Hz) oscillations were analyzed using power spectral density and coherence analysis.

There is increased theta band connectivity related to a higher number of epileptic seizures. We observed that the power of theta oscillations modulates gamma frequencies and the high frequency band. In addition, NPD1 modulates the theta wave and, therefore, modulates gamma and high frequency oscillations. These preliminary results show that during epileptogenesis there is a modification of the neuronal network, and NPD1 reestablishes the normal function of the brain by decreasing cell apoptosis and damage to brain tissue.

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Blockade of over-expressed Na^+/K^+ pumps in breast cancer cells paired with activation of Na^+ channels using veratridine causes targeted osmotic cell lyses

Modern day methods of treating cancer such as radiotherapy and chemotherapy focus largely on eliminating cancerous tissue before treatment becomes fatal to the patient. Therefore, developing a method of eliminating cancer while sparing healthy tissue would be desirable. Many metastatic cancer cell lines have been shown to significantly over-express voltage gated sodium channels (VGSCs), which could serve as an important pharmacologic difference between metastatic tissue and normal tissue. We have hypothesized that in cells that over-express sodium channels, activation of the VGSCs, combined with pharmacological blockade of Na^+,K^+-ATPase (sodium pumps), will cause excess intracellular sodium. Consequently, water will follow the sodium across an osmotic gradient, causing cellular swelling and lysis. It has been shown in mice with allographs of the invasive MDA-MB-231 breast cancer line that blockade of Na^+/K^+ pumps using the cardiac glycoside ouabain, combined with electrical activation of VGSCs, can produce targeted osmotic lysis of the cancerous tissue with preservation of normal mouse tissue (unpublished). Because it is impractical to electrically stimulate many locations on the body in which metastatic tumors can arise, it would be useful to develop a technique involving a pharmacological agent to stimulate VGSCs. Our study has focused on the use of veratridine, a well-known sodium channel activator, as a replacement for electrical stimulation. We hypothesized that veratridine would serve as a useful substitute for electrical stimulation in its use during targeted osmotic lyses of the MDA-MB-231 breast cancer cells. *The final portion of this abstract will contain the results of using veratridine as a sodium channel activator and will be included when data is collected in the upcoming weeks.*

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Determining the Mechanism of Regulation of INSM1 by Achaete-Scute homolog 1 in prostate cancer.

Prostate cancer (PC) is the second most frequently diagnosed and the second leading cause of cancer related deaths in men in the United States. Androgen ablation is initially beneficial to nearly all PC cases. Unfortunately, patients can relapse with androgen-independent PC. Androgen independent PC is resistant to cell death and acquired neuroendocrine differentiation (NED). Human achaete-scute homolog 1 (Asc1), a bHLH transcription factor is highly expressed in NED and has been suggested to be instrumental in the development of neuroendocrine PC. Insulinoma-associated protein 1 (INSM1) is a transcription factor that is present and necessary in the embryonic stage of development. However, in adults; INSM1 is expressed only in neuroendocrine forms of cancers such as pancreatic cancer, small cell lung carcinoma, pituitary tumors, and thyroid cancer. INSM1 is turned on by the INSM1 promoter region located upstream of the INSM1 coding region. Using adenoviral transfected Achaete-scute homolog 1 (Ascl1) LNCAP cells, Ad-Ascl1 induced INSM1 mRNA expression. In this project, we are determining the relationship between INSM1 and Ascl1; examining Ascl1's ability to regulate INSM1 at the promoter level. We will use a downstream luciferase reporter gene to measure the activity of INSM1 promoter in the presence of Ascl1. We will use different regions of the INSM1 promoter to determine how Ascl1 induces INSM1 in LNCaP cells. Currently, we have cloned different INSM1 promoter region that will be used to transfect LNCaP cells, which are non-neuroendocrine forms of prostate cancer cells, along with the Ascl1. Understanding the factors that regulate the neuroendocrine differentiation in prostate cancer could lead to the development of targeted treatment for this aggressive disease.

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Biomarkers of IL-17 and IL-17R signaling in Cigarette Smoke Induced Emphysema

Rationale: Tobacco smoke exposure is one of the principal risk factors for emphysema. It has been shown that interleukin (IL)-17+ cells are increased in Chronic Obstructive Pulmonary Disease (COPD) lung tissue and IL-17R signalling is required for ectopic lymphoid follicle formation which is a prominent pathologic feature of COPD. It has also been shown recently that cigarette smoke is a mucosal Th17 adjuvant and that IL-17R signalling is required for smoke induced emphysema. However, this was only shown to be true for a long term effect (6 months). As of now, the mechanisms of early emphysema still remain unclear. To understand the early stage of emphysema, we studied the biomarkers of IL-17 and IL-17RA in cigarette smoke in 2 weeks.

Methods: IL-17 A KO, IL-17 RA KO, and wild type (WT) control mice were exposed to 2 cigarettes a day, five times a week for 2 weeks, while control mice were exposed to room air. Serum and Bronchoalveolar lavage (BAL) were collected. Lung tissues were then extracted from mice. Afterward, the tissues were thoroughly homogenized and their RNA extracted. Using the extracted RNA, reverse transcription was performed and a multitude of cDNA from each sample was produced. The cDNA was then amplified using Real-Time (RT) PCR on Qiagen's RT^2 Profiler PCR Assay to obtain data and analyze gene expression in the lungs. Cytokine and chemokine in BAL and lung homogeny fluid were measured by bio-plex.

Results: Mice lacking IL-17RA showed no substantial change in macrophage recruitment in the BAL as compared to WT and IL-17A KO mice, which experienced increased macrophage numbers after smoke exposure. In addition to macrophage recruitment, there were significantly lower amounts of MCP-1, IP-10, KC, and IL-10 in the lung tissues of IL-17 RA KO mice as compared to the tissues of the control mice. IL-17 RA KO smoke mice had lower Th1 and Th17 cell responses and independent responses in Th2 cell response. IL17-RA KO mice also experienced decreased transcripts for Matrix metalloproteinase (MMP) 2, 3 and 8 after two weeks of smoke exposure.

Conclusion: Cigarette smoke is a potent mucosal Th17 adjuvant and induction of IL-17 and IL-17R signaling is required for the elaboration of MCP1, macrophage recruitment, and tissue emphysema. MMP and the Th1 and Th17 cytokines are biomarkers for early stage of emphysema. These data suggest a novel immune pathway that may be a target of intervention for COPD.

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Analyzing the Viral Diversity in Rhesus Macaques Infected with SIVmac251 as a Model of HIV Pathogenesis

Human Immunodeficiency Virus (HIV) is a significant health threat around the world and the virus continues to evolve. Simian Immunodeficiency Virus (SIV) is a useful tool for understanding HIV, and rhesus macaques (*Macaca mulatta)* infected with the isolate, SIVmac251, have proven to be an ideal model for evaluating the various aspects of HIV pathogenesis. Utilizing the rhesus model of HIV provides many experimental opportunities that cannot be studied in humans. With special emphasis on the *gag*, *pol* and *env* genes of SIVmac251 we sought to evaluate the levels of diversity observed in the viral genome. Variations in *gag* (Group Specific Antigen) have been shown to be important in evasion of innate immune responses and cell-mediated immune responses. Variations in *env* (Envelope/attachment protein) are highly important in immune evasion and can affect the types of cells in which the virus can efficiently enter. Drug resistant viruses develop due to mutations in *pol* (Polymerase) through the use of Antiretroviral Treatments (ART). In this study samples of the SIVmac251 virus inoculum as well as serial blood samples obtained from infected rhesus macaques were sequenced to determine the level of diversity in these regions and monitor the development of new genotypes over the course of infection and the use of ART. These results were correlated with clinical measures of disease including plasma viral load. When additional animal samples are analyzed, a more detailed picture of how diversity develops over time and how treatment with ARTs affect the rate of diversity and its impact on disease progression will emerge. These studies of the *gag*, *env*, and *pol* regions of the virus genome will allow us to devise more effective treatments and provide insight to specific regions of the virus driving disease progression.

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Characterization of the IL-22 Soluble Receptor IL-22Ra2

Interleukin-22 (IL-22) is a T-cell-derived cytokine of the IL-10 family that has complex pro and anti- inflammatory effects. IL-22 initiates a response through binding to the receptor complex of IL-22Ra1 and IL-10R2 which induces signaling through the STAT-3 pathway. IL-22 has been shown to be beneficial in the clearance of bacterial pathogens and is important in epithelial repair. While there is a growing body of evidence demonstrating the importance of IL-22, there is currently little understanding of its regulation.

One potential mechanism of IL-22 regulation is through the induction of IL-22Ra2, otherwise known as IL-22 binding protein (IL-22BP). IL-22BP is a soluble protein with a high affinity for binding with IL-22. The binding protein is important because it binds to the same region on IL-22 as the primary receptor thus acting as a competitive inhibitor for IL-22. While inhibition has been shown in vitro, very little is known about the physiologic role of Il-22BP and its expression throughout the body.

The purpose of this study is to characterize the expression of IL-22 binding protein. Il-22 is induced in the liver after LPS administration where it serves a hepatoprotective role. We have found that, while mice undergoing ethanol-induced inflammation have increased IL-22 after LPS administration, they also have significantly increased expression of IL-22BP. More importantly, histological analysis shows mice receiving both LPS and ethanol had extensive necrosis and inflammation when compared to ethanol fed control mice or LPS alone. These data lead to the hypothesis that IL-22 binding protein is up-regulated in conditions of chronic stress resulting in a reduced ability of IL-22 to bind to the primary receptor and thus preventing Il-22 from aiding in tissue repair. These studies, as well as ongoing studies in the lung, will allow us to characterize the expression of IL-22BP and its physiologic role in infection and repair.