NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

SYMPOSIUM PROGRAM & ABSTRACTS

UF | UNIVERSITY of FLORIDA

College of Medicine

AUGUST 8, 2014
8:00 A.M.
HPNP Building
Room G-201

University of Florida College of Medicine
Office for Diversity and Health Equity

NIH SHORT-TERM RESEARCH TRAINING FOR MINORITY STUDENTS

The program's success is due largely to the active participation of the Faculty mentors listed below:

Jennifer L. Bizon, Ph.D. Associate Professor	Department of Neuroscience, College of Medicine
Barry Byrne, MD, Ph.D. Professor	Pediatrics and Molecular Genetics & Microbiology, College of Medicine
Kate M. Candelario, Ph.D. Post-Doctoral Associate	Department of Neurosurgery, College of Medicine
Kirk Conrad, MD Professor	Department of Physiology & Functional Genomics, College of Medicine
Paul Davenport, Ph.D. Distinguished Professor	Department of Physiological Sciences, College of Veterinary Medicine
Yousong Ding, Ph.D. Assistant Professor	Department of Medicinal Chemistry, College of Pharmacy
Marcelo Febo, Ph.D. Assistant Professor	Department of Psychiatry, College of Medicine
Maureen Keller-Wood, Ph.D. Professor and Chair	Department of Pharmacodynamics, College of Pharmacy
Eric Krause, Ph.D. Assistant Professor	Department of Pharmacodynamics, College of Pharmacy
Tara Sabo-Attwood, Ph.D. Associate Professor	Department of Environmental and Global Health College of Health and Public Health Professions

The Short-Term Research Training for Minority Students program is funded by a grant from the National Institutes of Health, National Heart, Lung and Blood Institute and supported by the University of Florida College of Medicine. The grant's principal investigator, Charles E. Wood, Ph.D., is the Professor and Chair for the Department of Physiology at the University of Florida.

STUDENTS	AFFILIATION
Yamilette Borja	University of Florida
Kristal Gant	Elizabeth City State University, NC
Michael Hamm	Columbus State University, GA
Innah Lachica	University of Florida
Jacqueline Moats	Florida International University
Alice Nakasone	Barry University, FL
Julian Rashid	University of Florida
Steven Summers	Morehouse College, GA
Khalil Thompson	Xavier University of Louisiana
Simeon Walton	University of Florida

SYMPOSIUM PROGRAM

BREAKFAST AND WELCOME 8:00am

Charles Wood, Ph.D., Professor and Chair for the Department of Physiology

Michelle E. Jacobs, M.D., Assistant Dean, Office for Diversity and Health Equity
Professor, Department of Psychiatry, College of Medicine

SESSION I

SESSION CHAIR: Charles E. Wood, Ph.D.
Professor and Chair

Student: Michael Hamm 8:30am – 8:45am
Project: ***Gene Therapy for Friedreich's Ataxia***
Mentor: Barry Byrne

Student: Jacqueline Moats 8:50am – 9:05am
Project: ***"Age-related cognitive impairment in associations with an enhanced GABAergic inhibition to the hippocampus from the basal forebrain, but reduced local inhibitory function within the hippocampus."***
Mentor: Jennifer L. Bizon, Ph.D.

Student: Steve Summers 9:10am – 9:25am
Project: ***"The role of the Melanocortin 4-Receptor in the Paraventricular Nucleus of the hypothalamus: a neuroanatomical study."***
Mentor: Eric Krause, Ph.D.

Student: Yamilette Borja 9:30am – 9:45am
Project: ***Cholecystokinin Expression and Localization in Human Placenta***
Mentor: Kirk P. Conrad, M.D.

Student: Julian Rashid 9:50am – 10:05am
Project: ***Expression and Characterization of an Anti-tobacco Mosaic Virus Protein***
Mentor: Dr. Yousong Ding, Ph.D.

BREAK **10:05am– 10:20am**

SESSION II

SESSION CHAIR: **Michelle E. Jacobs, M.D.**
Assistant Dean, Office for Diversity and Health Equity
Professor, Department of Psychiatry, College of Medicine

Student: Kristal Gant 10:20am - 10:35am
Project: **The Role of Estrogen Receptors in Epithelial to Mesenchymal Transition (EMT) in Idiopathic Pulmonary Fibrosis**
Mentor: Tara Sabo-Attwood, Ph.D.

Student: Innah Lachica 10:40am – 10:55am
Project: ***"The effects of maternal hypercortisolemia during late gestation on the fetal ovine circulatory system"***
Mentor: Maureen Keller-Wood, Ph.D.

Student: Alice Nakasone 11:00am – 11:15am
Project: Stereotaxic Coordinates of the Brain of Guinea Pigs

Mentor: Paul Davenport, Ph.D.

Student: Khalil Thompson 11:20am – 11:35am
Project: ***3,4-MethylenePyrovalerone, A Major Bath Salt Drug, Reduces Functional Connectivity in Rat Brain***
Mentor: Marcelo Febo, Ph.D.

Student: Simeon Walton 11:40am – 11:55am
Project: ***The role of DIAPH3 in glioma tumor cell invasion***
Mentor: Kate M. Candelario, Ph.D.

Lunch **11:55am – 12:30pm**
HPNP Building, Room G-201

PRESENTATION OF THE CERTIFICATES **12:30pm – 12:45pm**

PHOTOGRAPHS IN THE ARB COURTYARD **1:00pm**

ABSTRACTS

Cholecystokinin Expression and Localization in Human Placenta

Yamilette Borja, Robert Boudreaux, M.S., and Kirk P. Conrad, M.D.
Departments of Physiology and Functional Genomics, and of Ob/Gyn,
University of Florida College of Medicine

Background: Cholecystokinin (CCK) is a gastrointestinal hormone that primarily regulates digestion through neural and hormonal pathways. In response to ingested free fatty acids and amino acids, CCK is released into the bloodstream from I-cells located in the duodenal mucosa. It is also secreted through the stimulation of I-cells by two factors, CCK-releasing peptide or monitor peptide. CCK-releasing peptide is secreted into the small intestinal lumen by paracrine cells within the epithelium in response to products of fat or protein digestion. Monitor peptide is released by pancreatic acinar cells and is found in pancreatic juice. Both factors can also be released by neural input, which is significant for initiating pancreatic secretion, preparing the system for digestion of a meal as soon as it enters the small intestine. Presence of these factors stimulates the release of CCK which then travels in the bloodstream to the brain, stomach, gallbladder and pancreas, where CCK receptors, CCK-AR or CCK-BR are expressed. CCK actions include neurotransmission, regulation of gastric emptying, gallbladder contraction, relaxation of the sphincter of Oddi and pancreatic secretion, all of which can contribute to satiety [1].

The placenta is an organ that forms in the uterus during pregnancy and connects to the fetus via the umbilical cord. It functions as a pulmonary, alimentary and excretory system for the fetus. Additionally, it produces a myriad of steroid and protein hormones including progesterone, estrogen and human chorionic gonadotropin. These hormones are vital for implantation and maintenance of pregnancy [2]. Preeclampsia is a condition during pregnancy that is known to cause hypertension, intrauterine growth restriction and proteinuria. It is a leading cause of maternal and fetal mortality and morbidity [3].

A previous microarray experiment from our laboratory on first trimester chorionic villous samples (CVS) revealed expression of CCK, which was markedly up regulated in CVS from women who developed preeclampsia compared to women who experienced normal pregnancy. Subsequent RT-PCR corroborated the expression of CCK and its receptors in first trimester and term placental tissues, as well as in choriocarcinoma (trophoblast-derived) cell lines. ***We hypothesize that CCK protein will be localized to trophoblast in first and third trimester placental tissues.***

Methods: Paraffin-embedded blocks of first trimester placentae were kindly provided by Dr. Post Uiterweer (Utrecht Netherlands). The placentae were acquired from elective terminations under approval of The Medical Ethical Review Board of the University Medical Center Utrecht. These tissues contain both fetal chorionic villous and maternal decidual tissue. A third trimester placenta was acquired under approval of University of Florida IRB #147-2009, and tissue samples of the decidual basal plate and chorionic villous were obtained and fixed in 4% formaldehyde overnight at 4°C. They were then embedded in paraffin, 4μm sections cut and mounted on slides that were used for immunohistochemical localization of CCK. Two CCK antibodies were used, one directed against a C- and another against a N-terminal peptide. The tissues sections on the slides were permeabilized for 10 minutes with 0.3% Triton X-100 in PBS, treated with 1% hydrogen peroxide for 3 minutes in methanol to quench endogenous peroxidase activity, and then blocked for 20 minutes at RT with 1% normal horse serum in PBS. The sections were then immunostained overnight at 4°C with the primary antibodies goat anti-CCK C-20 (2μg/mL), goat anti-CCK58 N-19 (2μg/mL), mouse anti-cytokeratin (1μg/mL) or control isotype normal goat IgG (2μg/mL). This was followed by addition of the corresponding biotinylated secondary antibodies horse anti-goat (1μg/mL) or biotinylated horse anti-mouse (3μg/mL) for 30 minutes at RT, and then ABC reagent for 1 hour at RT. Sections were finally developed using DAB substrate. Subsequently the slides were mounted with Cytoseal and observed with a Nikon bright field microscope. Images were then obtained using an Olympus BX41 system microscope and an Olympus DP71 digital imaging system.

Results: CCK was localized to syncytiotrophoblast and extravillous trophoblast cells in both first trimester and term placenta. This was concluded based on anatomical location or parallel sections of CCK and cytokeratin antibody, the latter staining trophoblast cells. When using blocking peptides with the corresponding CCK antibody a diminished signal was observed, and when the blocking peptides were used with the opposite CCK antibody there was little or no signal reduction.

Conclusions: These findings suggest that CCK is expressed by trophoblast, but its role in normal pregnancy or preeclampsia is presently unknown, and requires further study. Trophoblast CCK may be secreted serving an endocrine, paracrine, or autocrine function. We will investigate the possibility that CCK is secreted into conditioned medium by cultured trophoblasts and villous explants, and attempt to manipulate its secretion with somatostatin and CCK-releasing peptide. Other future goals include examination of additional placenta, explorations of CCK receptors via *in situ* hybridization (antibodies that work appear not to be available), investigations of cell signaling, as well as of potential roles for CCK in trophoblast migration and syncytialization using pharmacologic and molecular strategies.

The Role of Estrogen Receptors in Epithelial to Mesenchymal Transition (EMT) in Idiopathic Pulmonary Fibrosis

Kristal L. Gant, BS[1], Cody Smith, MS[2], and Tara Sabo-Attwood, PhD[2]
[1]Elizabeth City State University, Elizabeth City, NC; [2]Department of Environmental and Global Health and Department of Physiological Sciences,
University of Florida, Gainesville, FL

Idiopathic Pulmonary Fibrosis (IPF) is a fatal lung disease in which an unknown injury to alveolar epithelial cells initiates various mechanisms that yield an excess deposition of extracellular matrix components. This leads to a build-up of scar tissue and causes lung dysfunction. Epithelial to mesenchymal transition (EMT) is one such mechanism that involves a change in phenotype of alveolar epithelial cells to fibroblastic cells, the effector cells of fibrosis. Estrogen's role in the development of lung fibrosis and in particular EMT isn't well-known, but gender-based differences in the prevalence and incidence of IPF suggest estrogen and estrogen receptors may be involved in pathogenesis. Understanding estrogen's role in EMT is important in further understanding the cause of lung fibrosis and in possibly controlling its progression. The aim of this research was to develop a model of EMT in which to test the effect of estrogen receptor modulation. In this model, exposure of lung epithelial cells (BEAS-2B) to transforming growth factor beta 1 (TGF-β1), a key modulator of fibrosis, causes a decrease in expression of epithelial marker genes (E-cadherin, CDH1) and an increase in expression of fibroblast marker genes (α-smooth muscle actin, ACTA2, and vimentin, VIM) thus indicating EMT and a change in cellular phenotype. Our hypothesis was that cells exposed to TGF-β1 would adopt the mesenchymal/fibroblastic phenotype and that the expression of estrogen receptors (ESR1, ESR2, and GPER) and EMT markers would increase suggesting a feed-forward mechanism where increased ER expression further increases EMT. For these experiments, BEAS-2B cells were exposed to variable concentrations of TGF-β1 (0.1 ng/µl, 1 ng/µl, and 5 ng/µl), and cells were harvested after 0, 24, 48, 72, 96, and 120 hours of exposure. Next, mRNA was purified, reverse transcribed, and qPCR was performed with validated primers specific to the EMT markers and estrogen receptors. Data analysis indicated a general trend at each dose tested supporting the original hypothesis. The expression of ACTA2 and VIM and ESR2 increased while the expression of ESR1 and EMT marker CDH1 decreased indicating that EMT occurred and that ESR activity may have changed. It is yet to be determined whether the change in expression of ESRs affected EMT. Expression changes in estrogen receptor GPER were unclear but also trended toward increased expression at the highest dose tested. The data obtained is preliminary and further testing and exposures must take place before prominent conclusions can be reached.

Gene Therapy for Friedreich's Ataxia

Michael Hamm, Thiago Love-Lenoir, GA

The lab that I participated in was Dr. Barry Byrne's lab. He has been involved in many gene therapy projects. In the project I was involved in, we hypothesized that introducing a functional FXN gene via gene therapy would increase frataxin production. My specific project was to aid in the cloning of the expression and viral plasmid. Friedriech's Ataxia (FRDA) is a neuromuscular genetic disorder named after Nicolaus Friedreich, which has many symptoms affecting coordination, vision, hearing, etc. It is caused by inheriting two recessive alleles for the FXN gene and it makes a GAA expansion in the FXN gene expand greatly. Frataxin is found in the mitochondria, so lack of frataxin leads to lack of energy production and possibly buildup of oxidative stress and iron in the mitochondria. After putting the pcDNA 3.1 expression backbone into the viral pTR-UF12 plasmid, we transformed the plasmid, picked 11 colonies, and grew them up in 5ml LB buffer. After confirming the insert with NsiI/SpeI and SmaI, we grew with LB overnight for a maxi prep. The last step I was involved in was confirming the single and double-stranded clone.

The effects of maternal hypercortisolemia during late gestation on the fetal ovine circulatory system

Innah Lachica, Andrew Antollic and Maureen Keller-Wood, Ph.D.

Background: Cortisol is a glucocorticoid hormone secreted by adrenal cortex and regulates many aspects of metabolism. During the period of late gestation, cortisol facilitates maturation of many fetal organs in order to prepare the fetus for extra uterine life. In addition, glucocorticoids are administered to mothers who are suspected of pre-term pregnancy. However, studies have shown that repeated treatment with glucocorticoids during pregnancy has adverse effects on the offspring later in life. Specifically, individuals that were given the treatment developed hypertension and abnormal functioning of the HPA axis. Other studies have shown that excess glucocorticoids result in enlargement of the ventricular walls of the heart and heart weight-to-body ratio. Even more so, a study in our laboratory shows that increased maternal cortisol in ewes in late gestation resulted in a high incidence of stillbirth.

Objective: Previous studies found that chronic maternal hypercortisolemia resulted in a significant increase in fetal demise and changes in gene expression within the fetal heart. This project sought to explore the changes between maternal uterine blood flow, fetal heart rate and fetal pressure in cortisol-infused and control groups of ewes.

Methods: Data from pregnant ewes and their fetuses were used. Ewes had been continuously infused with cortisol from day 115 of gestation until delivery. Four ewes were not treated and served as controls. The delivery occurred at day 135-142 in cortisol-infused ewes and day 142-148 in control ewes. Continuous recording of the uterine blood flow data was collected using an implanted Transonics Flow Probe. Meanwhile, the fetal heart rate and pressure data was collected using an implanted DSI radiotelemetry device throughout pregnancy. Maternal and fetal data were analyzed on gestational days 130, 140 and the day of birth. The same variables were looked at 1 hour before birth and during periods of contraction. Statistical analysis was performed using IBM SPSS software package.

Results: No changes in uterine blood flow were observed at 130d ($p=0.262$) or 140d ($p=0.342$) of gestation. Similarly, no changes in fetal heart rate were observed at 130d ($p=0.754$) or 140d ($p=0.632$) of gestation. Likewise, no changes in fetal pressure were observed at 130d ($p=0.494$) or 140d ($p=0.095$) of gestation. However, 1 hour before birth, a significantly lower fetal heart rate was observed ($p=0.0269$) as well as a significantly lower fetal pressure ($p=0.0466$) in fetuses of cortisol-infused ewes as compared to controls. Because of considerable maternal contractions during this period of time, we isolated individual contractions and found significantly lower fetal heart rate ($p=0.0251$) and fetal pressure ($p=0.0486$) in the cortisol-infused fetuses compared to controls.

Conclusions: The results show that there are significant changes that occur near the time of birth and during periods of contractions between cortisol-infused and control groups. The average fetal heart rate and fetal pressure an hour before birth and during periods of contraction is much lower in cortisol-infused group than the control group. The significantly low difference in the average seen in fetuses of cortisol-infused ewes may suggest that maternal hypercortisolemia contributes to the weak response of the fetus during stressful activities at the time of delivery. In addition, instances of hypoxia during labor, the ensuing bradycardia and inability to maintain pressure may negatively effect the distribution of blood and nutrients to essential metabolically active organs, such as the brain and heart. Prospective study of the strength of contractions and fetal ECG may provide more insight on the critical role of increased cortisol in fetal circulatory system during late gestation and especially during labor.

"Age-related cognitive impairment is associations with an enhanced GABAergic inhibition to the hippocampus from the basal forebrain, but reduced local inhibitory function within the hippocampus."

Jacqueline M. Moats, Joseph A. McQuail, Ph.D., Cristina Bañuelos, Ph.D., Jennifer L. Bizon, Ph.D.. Department of Neuroscience, McKnight Brain Institute, University of Florida 3261

Abstract

Memory declines with advancing age, but the neural correlates of this decline are unknown. Normal memory requires the intact function of the hippocampus and ascending inputs arising from the medial septum/basal forebrain. Due to its prominent degeneration in Alzheimer's disease, early studies hypothesized that loss of MS/BF cholinergic neurons were the cause of age-related memory impairments. However, recent studies of normally aging rodents have shown no loss or at least no relationship between cholinergic neuron number and memory. In contrast, normal aging differentially modulates GABAergic neurons in the basal forebrain and hippocampus. Whereas there is an *increase* in basal forebrain GABAergic projection neurons in memory impaired aged rats, there is a *decrease* in local GABAergic interneurons within the hippocampus of aged rats. As GABA is the predominant inhibitory neurotransmitter throughout the brain, altered GABAergic networks may lead to age-related memory impairments. Specifically, we hypothesized that age-related memory impairment is associated with enhanced inhibitory input to the hippocampus from the basal forebrain, but reduced local inhibitory function within the hippocampus. To test this hypothesis, we prepared basal forebrain and hippocampal homogenates from young (6 months) and aged (22 months) rats that were characterized as aged-unimpaired (AU; similar to young) or aged-impaired (AI; outside the range of young) according to performance on a hippocampal-dependent behavioral task. These homogenates were then analyzed for expression of a variety of markers of GABAergic function via quantitative Western blotting. Consistent with our hypothesis, there was a trend towards greater expression of GAD-67, a marker of all GABAergic cells, in the basal forebrain of AI rats and a concurrent decrease in GAD-67 in the hippocampus of AI rats. Despite the general reduction in hippocampal GAD-67, expression of calcium binding proteins that identify subclasses of hippocampal interneurons, were not changed with age or cognitive status. These findings are consistent with the notion that aging differentially modulates GABAergic indices in a brain region-dependent fashion with significant consequences for cognition. Future studies will employ neuroanatomical methods to evaluate the status of GABAergic septohippocampal synapses on specific hippocampal interneuron populations.

Stereotaxic Coordinates of the Brain of Guinea Pigs

Alice Nakasone, Hsiu-Wen Irene Tsai , Paul W. Davenport
Department of Physiological Sciences, University of Florida

There is no current literature that provides stereotaxic coordinates of the brain of a guinea pig. The purpose of this project was to study stereotaxic coordinates for future experiments, specifically for locating regions such as the amygdala, periaqueductal gray (PAG), and nucleus tractus solitarius (NTS). The amygdala is part of the limbic system and is involved in the perception of our emotions and motivations. PAG is involved in homeostatic regulation of salient functions such as pain, anxiety and autonomic function. NTS is the site of integrating and processing peripheral afferents such as respiratory and cardiovascular afferents. Two guinea pigs were sacrificed and perfused. Brains were harvested and fixed in 4% paraformaldehyde for 3 days and then transferred into a solution of 30% sucrose in phosphate buffer solution (PBS). The fixed brains were coronally and sagittally sectioned into 40 μm thick with a microtome for cresyl violet staining. The obex was used as a rostrocaudal zero reference point for the coronal sections and the midline was used as a dorsoventral zero reference point for sagittal sections. The results showed that the stereotaxic coordinates for amygdala (coronal: +0.825 to +1.340 cm; sagittal: -0.488 cm), PAG (coronal: +1.636 to +2.104 cm; sagittal: -0.248 cm), and NTS (coronal: -0.008 to +0.408 cm; sagittal: -0.248 cm). The results will be used to define the locations of amygdala, PAG and NTS in guinea pigs for multi-electrode array recording during swallow and cough stimuli.

Expression and Characterization of an Anti-tobacco Mosaic Virus Protein

Julian Rashid, Yousong Ding
Department: Medicinal Chemistry

Discovery and development of new drug molecules remains an urgent need to combat existing and emerging diseases. Historically, small molecules from Nature have contributed the majority of drugs, and natural products remain a preeminent, fertile source of new drug leads. In this project, we exploited mushrooms as an important but underappreciated resource for discovery and development of therapeutics. Specifically, we aimed to express and characterize a protein (Y3) produced by *Coprinus comatus*. This protein has shown anti-tobacco mosaic virus and anticancer activities. However, detailed physiochemical properties of this important protein remain to be explored. Under the guidance of Dr. Yousong Ding, were successful in expressing Y3 in yeast cells. After purification of the produced recombinant glycosylated protein, we tested its activity against human liver cancer cell lines. Further activity testing will be done against several other cancer cell lines and viruses. Other areas for future investigation include the characterization of the Y3 structure as well as engineering of this protein to understand its structure-activity relationship. This work will lay the groundwork for further exploration of bioactive peptides from mushrooms as drug leads.

"The role of the Melanocortin 4-Receptor in the Paraventricular Nucleus of the hypothalamus: a neuroanatomical study."

Steven Summers, Lei Wang, Justin A. Smith, Helmut Hiller, Annette D. de Kloet, Jacob Ludin and Eric G. Krause. Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL USA.

Central melanocortin 4 receptors (MC4-R) are known to play a role in the regulation of body weight and appetite but efforts to target these receptors for weight loss therapy have been hindered by the hypertensive effects of MC4-R agonists. The paraventricular nucleus of the hypothalamus (PVN) is a brain region that integrates neural signals to coordinate various homeostatic processes such as autonomic and endocrine responses to stress. In this regard, the PVN is known to express MC4-R as well as a variety of neuronal phenotypes that regulate body weight and cardiovascular function. In particular, the PVN contains neurons that express corticotropin-releasing hormone (CRH) and oxytocin (OT), peptides that influence body weight by regulating food intake and energy expenditure. This study tests the hypothesis that MC4-R are expressed on CRH and OT neurons within the PVN. To identify neurons that express CRH and MC4-R transgenic mice that express enhanced green fluorescent protein (eGFP) under the control of the promoter for MC4-R were bred to mice with Cre-driven expression of red fluorescent protein in neurons that produce CRH. Subsequently, these mice were perfused and extracted brains were coronally sectioned through the PVN. Immunohistochemistry was conducted on brain sections through the PVN to identify OT neurons and amplify eGFP signal. Quantitative fluorescent microscopy revealed that MC4-R are expressed on CRH and OT neurons in the PVN; however, these co-localizations more frequently occurred in the posterior portion of the PVN. Interestingly, the posterior of the PVN contains neurons that project to the rostral ventral lateral medulla (RVLM) and control blood pressure. To determine whether MC4-R are expressed on neurons in the PVN that control blood pressure, mice were delivered the retrograde neuronal tract-tracer, flurogold (FG), into the RVLM. One-week later, mice were perfused, brains were extracted and PVN sections were processed for FG immunohistochemistry. Indeed, MC4-R were found to co-localize with FG indicating that these receptors are expressed on RVLM projecting neurons that control blood pressure. Collectively, our results suggest that the effects of MC4-R agonists on body weight may be through actions on CRH and OT neurons; however, these same neurons also are known to control blood pressure and may contribute to the negative cardiovascular effects associated with MC4-R based therapies.

3,4-MethylenePyrovalerone, A Major Bath Salt Drug, Reduces Functional Connectivity in Rat Brain

Khalil Thompson, Dr. Marcelo Febo and Dr. Luis Colon-Perez

Through March 2011-2013 poison control centers representing 45 states and DC reported cases of bath salt intoxication[15]. Bath salt drugs are potent stimulant and hallucinogen polysubstance drugs and their use has significant potential to impair mental health. The dangers of these chemicals are exacerbated by the public perception that these are safe and legal alternatives to illegal drugs such as cocaine, methamphetamine (METH) and methylenedioxy-methamphetamine (MDMA). The reported negative adverse effect of bath salts consumption, particularly at high doses, are of great concern. These effects include insomnia, depression, aggressiveness, muscular tremor and seizure, paranoia, and psychotic symptoms and hallucinations days and even weeks after abuse. Although there are emerging research reports on the neurochemical mechanisms of action and behavioral effects in rodents, there is an urgent need for more investigation on the alteration of functional connectivity in the brain of patients. Locating neural circuit pathways detrimentally affected by the drug will help lead the way to discover effective treatments for MDPV abuse. The hypothesis was that following bath salt exposure there would be a progressive, time-dependent increase in anxiety and aggressive behavior with diminished cognitive function that is characterized centrally by a dramatic reduction in the brain's intrinsic functional connectivity. We used functional magnetic resonance imaging (fMRI) to measure drug-invoked brain BOLD (Blood Oxygen Level Dependent) activation following MDPV administration. A quad transmit/receive coil tuned to 470.7 MHz (^{1}H resonance) was used for B_1 excitation and signal detection. Functional images were collected using a 2-shot spin-echo echo-planar imaging (EPI) sequence. At 0-0.3mg/kg MDPV dosage we saw an immediate significant increase in connectivity between the nucleus accumbens and frontal cortical areas such as insular and orbital cortices. However after 1 hour these areas showed decreased connectivity. At 1-3mg/kg MDPV dosage we saw significant and dramatic decrease in global connectivity in the brain coupled with a strong connectivity between the prefrontal and amygdala areas of the brain. The prefrontal-amygdala area is a common connective region that is sensitive is mood and emotional disorders such as anxiety, depression and schizophrenia. Because of the similar functional changes seen in MDPV abuse and schizophrenia and because of the similar negative cognitive and behavioral effects we hope to use MDPV as a model for schizophrenia research in the future.

The role of DIAPH3 in glioma tumor cell invasion

Simeon Walton and Kate M. Candelario, Ph.D.
Department of Neurosurgery

Glioblastomas are the most common subtype of primary brain tumors and are characterized as the deadliest of human cancers. There are no effective agents that have emerged for the treatment of glioblastomas. Here, Diaphanous related formin-3 (DIAPH3) was investigated as a possible candidate for the pathogenesis of glioblastoma. Several groups have shown that the genomic loss at DIAPH3 is associated with aggressive or metastatic disease. In prostate cancer, DIAPH3 loss enhances tumor cell invasion and induces mesenchymal features. These results suggest that DIAPH3 may act to suppress oncogenic behavior. Two recombinant plasmids, one that encodes only an empty vector and enhanced green fluorescent protein (EGFP) and another that encodes DIAPH3 and EGFP, were amplified in Z-competent E.*coli* cells. Polyacrylamide gel electrophoresis and western blot were used to confirm plasmid of interest. Furthermore, to examine the role of DIAPH3 in an invasive glioma cell line derived from a glioma patient were transfected with the recombinant plasmid to increase DIAPH3 expression. To further exam this, immunocytochemistry was conducted to visualize how many cells were transfected. Finally, the invasiveness of DIAPH3 in glioma cell was examined using an in vitro invasion assay. The results from this assay show potential for further investigation to determine the role of DIAPH3 in glioblastoma cells.