25th Annual Puerto Rico Neuroscience Conference

Saturday, December 3, 2016

University of Puerto Rico, Río Piedras Campus

San Juan, Puerto Rico

Sponsored By

Endure NIH Blueprint for Neuroscience Research

Neuroscience Research Opportunities to Increase Diversity (NeuroID)

University of Puerto Rico, Río Piedras Campus

Strengthening UPR-RP through the Development of a

Research-Based Academic Culture - Title V - US Department of Education

University of Puerto Rico-Río Piedras Campus

Center for Biomedical Research Excellence Program (COBRE),

Puerto Rico Center for Neuroplasticity

University of Puerto Rico-Medical Sciences Campus

Ponce Health Sciences University

School of Medicine

Specialized Neuroscience Research Program (SNRP)

Universidad Central del Caribe, School of Medicine

Undergraduate Research Education and Training Program (UGREAT)

MBRS-RISE

Universidad del Este, Carolina, School of Science and Technology

Sistema Universitario Ana G. Mendéz

25TH ANNUAL PUERTO RICO NEUROSCIENCE CONFERENCE

Saturday, December 3rd, 2016

University of Puerto Rico-Río Piedras Campus

College of General Studies

Amphitheater #1

Host institution

University of Puerto Rico-Río Piedras Campus

Organizing committee

Carmen S. Maldonado-Vlaar, PhD

President

University of Puerto Rico-Río Piedras Campus

José E. García Arrarás, PhD

University of Puerto Rico-Río Piedras Campus

Devin Mueller, PhD

Ponce Health Sciences University

Astrid Zayas-Santiago, PhD

Universidad Central del Caribe

Jacqueline Flores-Otero, PhD

University of Puerto Rico-Medical Sciences Campus

MESSAGE FROM THE PRESIDENT

Dear Neuroscience Community:

I would like to welcome you to the 2016 Puerto Rico Neuroscience Conference. This year, we are celebrating the 25th anniversary of continuous support of our yearly conference. The University of Puerto Rico-Río Piedras Campus is honored to host this special event. The Puerto Rico Neuroscience Conference is traditionally held every year on the first Saturday of every December and during that day, the event provides a great scientific setting to share and debate new advances that impact the present and future of the Neurosciences. On this 25th Anniversary of the Conference, we are very enthusiastic to share an exciting scientific program and welcome students, researchers, clinicians and everyone that share a strong passion for the study of the Neurosciences.

¡Bienvenidos!

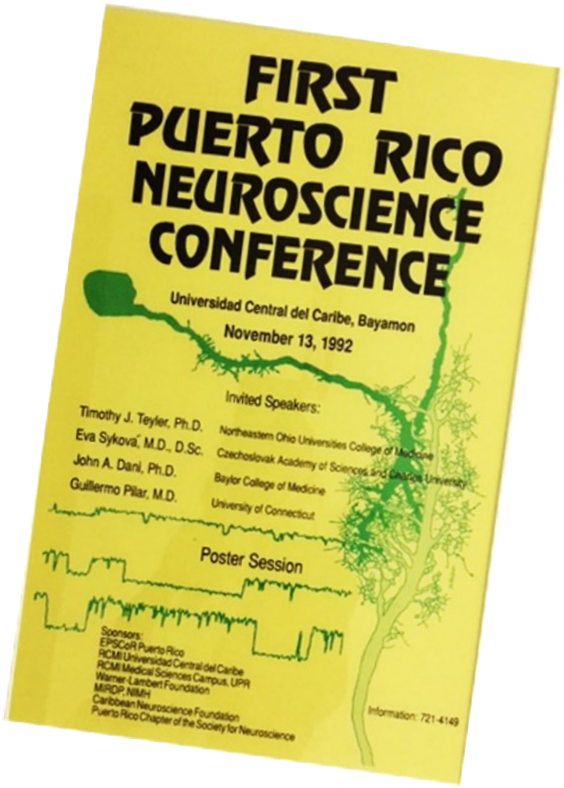
Carmen S. Maldonado-Vlaar, PhD

President

25th Puerto Rico Neuroscience Conference

History of Puerto Rico Neuroscience Conference…25 Years in the Making

Back in 1992, the First Puerto Rico Neuroscience Conference gathered our growing Neuroscience community at the Universidad Central del Caribe in Bayamon, Puerto Rico. Four established neuroscientists in the island, Dr. Vesna Enterovic, Dr. Pedro Ferchmin, Dr. Richard Hann from Universidad Central del Caribe and Dr. Richard Orkand, the Director of University of Puerto Rico Institute of Neurobiology at the time, were the founding members of this event. Since then and every year, Puerto Rico Neuroscience has been the main event that unites established neuroscientists and "neuroscientists in training" in Puerto Rico. Every year, the conference provides local neuroscientists with the scientific venue to present their latest findings, keep up-to-date on the research conducted by their colleagues, establish collaborations and network. The conference invites renowned neuroscientists from a variety of disciplines and premier institutions including Nobel Laureates to talk about their research, making this scientific initiative the yearly highlight event for neuroscientists in the island. In addition, more than 70 posters are presented each year with the majority given by graduate and undergraduate students from different academic institutions. Four university institutions rotate hosting the event each year: Universidad Central del Caribe, Ponce Health Sciences University, University of Puerto Rico-Río Piedras Campus and University of Puerto Rico-Medical Sciences Campus (including the Institute of Neurobiology). This year 2016 we are celebrating our 25 years of continuous support and active development of our Neuroscience community. This year we have reached an impressive record number of 450 participants, an amazing achievement. As always we are expecting an excellent meeting this year and of course look forward to the next 25 years of sustained growth for the Neurosciences in Puerto Rico.



Program

Saturday, December 3, 2016		
7:15AM-8:15AM	Breakfast, Registration and Poster Set-up	Domingo Marrero Navarro Building *Plaza*
8:15AM-9:45AM	**Opening Remarks/First Lecture**	
8:15AM-8:30AM	Carmen S. Maldonado-Vlaar, PhD - President Puerto Rico Neuroscience Conference Carlos González-Vargas, PhD Dean, College of Natural Sciences University of Puerto Rico-Río Piedras	Domingo Marrero Navarro Building Amphitheater #1
8:30AM-9:30AM	**Functional Specificity in the Human Brain** Nancy Kanwisher, PhD - Professor Massachusetts Institute of Technology (MIT) Host: José E. García-Arrarás, PhD University of Puerto Rico-Río Piedras	Domingo Marrero Navarro Building Amphitheater #1
9:30AM-9:45AM	Neuro Blitz Poster Highlights	Domingo Marrero Navarro Building Amphitheater #1
9:45AM-10:00AM	**Break**	Domingo Marrero Navarro Building
10:00AM-11:00AM	**Brain Plasticity-based Therapeutics** Del Castillo Memorial Lecture Michael Merzenich, PhD Posit Science Corporation Host: Jacqueline Flores-Otero, PhD University of Puerto Rico-Medical Sciences Campus	Domingo Marrero Navarro Building Amphitheater #1
11:10AM-1:45PM	**Concurrent activities**	
11:10AM-1:45PM	Poster Presentations (All Themes)	Domingo Marrero Navarro Building *Plaza*

Program

Saturday, December 3, 2016		
11:15AM-1:30PM	NeuroTalks: meet the scientists	Jaime Benítez Building *Plaza*
11:30AM-1:45PM	**Lunch**	Jaime Benítez Building *Plaza*
1:45PM-2:45PM	**PKC Signaling and Enhanced Responding to Stimulant Drugs** Paul Vezina, PhD - Professor The University of Chicago Host: Devin Mueller, PhD Ponce Health Sciences University	Domingo Marrero Navarro Building Amphitheater #1
2:45PM-3:00PM	**Break**	Domingo Marrero Navarro Building *Plaza*
3:00PM-4:00PM	**Astrocyte Functions in Neural Circuits** Baljit S. Khakh, PhD - Professor of Physiology and Neurobiology David Geffen School of Medicine at UCLA Host: Astrid Zayas Santiago, PhD Universidad Central del Caribe	Domingo Marrero Navarro Building Amphitheater #1
4:00PM-4:45PM	**Business meeting**	Domingo Marrero Navarro Building Amphitheater #1
4:45PM-5:00PM	**Closing Remarks** Carmen S. Maldonado-Vlaar, PhD President 25th Puerto Rico Neuroscience Conference	Domingo Marrero Navarro Building Amphitheater #1

25TH ANNUAL PUERTO RICO NEUROSCIENCE
Speakers

Dr. Nancy Kanwisher

Professor, Department of Brain and Cognitive Sciences &
Investigator at McGovern Institute for Brain Research
Massachusetts Institute of Technology (MIT)
Title: *Functional Specificity in the Human Brain*
Host: University of Puerto Rico-Río Piedras Campus

Dr. Paul Vezina

Professor, Department of Psychiatry and Behavioral Neuroscience
The University of Chicago
Title: *PKC Signaling and Enhanced Responding to Stimulant Drugs*
Host: Ponce Health Sciences University

Dr. Baljit S. Khakh

Professor of Physiology and Neurobiology
David Geffen School of Medicine
University of California, Los Angeles (UCLA)
Title: *Astrocyte Functions in Neural Circuits*
Host: Universidad Central del Caribe

Dr. Michael Merzenich

Francis A. Sooy Professor
Executive Chairman, Chief Scientific Officer, Posit Science Corporation
2016 KavliPrize Award with Carla Shatz and Eve Marder
Title: Brain Plasticity-based Therapeutics
Host: University of Puerto Rico-Medical Sciences Campus, School of Medicine

Biosketches

Dr. Michael Merzenich

Dr. Michael Merzenich, PhD, is one of the scientists responsible for our current understanding of brain plasticity–the notion that the brain can change itself at any age. For nearly five decades, he and his colleagues have conducted seminal research defining the functional organization of the auditory and somatosensory nervous systems. Research on cortical plasticity conducted in his laboratory has greatly contributed to our current understanding of the phenomenology of brain plasticity across the human lifetime. Dr. Merzenich has received numerous prestigious awards and prizes for his research and holds nearly 100 patents for his work.

He is a member of both the National Academy of Sciences and the Institute of Medicine. In 2016, Dr. Merzenich was awarded one of the world's top neuroscience prizes, the Kavli Prize, for his achievements in the field of brain plasticity. During the 1980s, initial models of a commercially successful cochlear implant (now distributed by Boston Scientific) were developed in his laboratory at the University of California, San Francisco. Later, driven by a desire to bring scientific discoveries out of the laboratory and into the world at large, to help the most people possible, he extended the fruits of cortical plasticity research into the commercial world by co-founding three brain plasticity-based therapeutic software companies (Scientific Learning, Posit Science, and Brain Plasticity Institute). Those companies have developed and validated neuroscience-based, computer-delivered rehabilitation training programs that have now been applied to more than 4 million impaired children and adults. Their research and treatment targets include developmental impairments that limit the cognitive, reading, and mathematical abilities of school-aged children; perceptual and cognitive impairments in normal aging; preventing and treating schizophrenia, bipolar disorder, depression, and other psychiatric diseases; rehabilitation strategies applied to treat traumatic brain injury and stroke; and the treatment of cognitive impairments arising from brain infections, toxin exposures, hypoxic episodes, and other environmental causes.

Dr. Merzenich has published more than 150 articles in leading peer-reviewed journals (such as Science and Nature) and has received numerous awards and prizes (including the Russ Prize, Ipsen Prize, Zülch Prize, Thomas Alva Edison Patent Award Purkinje Medal and the Kavli Prize, for his achievements in the field of brain plasticity). He and his work have been highlighted in hundreds of books about the brain, learning, rehabilitation, and plasticity. Dr. Merzenich's work is also often covered in the popular press, including the New York Times, the Wall Street Journal, Time, Forbes, Discover, and Newsweek. He has appeared extensively on television, and his work has been featured on four PBS specials, including "The Brain Fitness Program", "Brain Fitness 2: Sight and Sound", "The New Science of Learning," and "Brain Fitness Frontiers." Dr. Merzenich earned his bachelor's degree at the University of Portland and his PhD at Johns Hopkins. He completed a post-doctoral fellowship at the University of Wisconsin in Madison before becoming a professor at the University of California, San Francisco. In 2007, he retired from his long career at UCSF as Francis A. Sooy Professor and Co-Director of the Keck Center for Integrative Neuroscience. He was elected to the National Academy of Sciences in 1999 and the Institute of Medicine in 2008.

Dr. Nancy Kanwisher

Nancy Kanwisher is a Professor in the Department of Brain & Cognitive Sciences at MIT, and an Investigator at MIT's McGovern Institute for Brain Research. After receiving her BS and PhD from MIT, Kanwisher served on the faculty at UCLA and Harvard, before returning to MIT in 1997. Kanwisher has received the Troland Research Award, MacVicar Faculty Fellow Teaching Award, and Golden Brain Award. She is a member of the National Academy of Sciences and the American Academy of Arts and Sciences.

Dr. Paul Vezina

Dr. Vezina received his BA, MA, and PhD at Concordia University, Montreal, Canada. He completed his postdoctoral studies in Neuropharmacology at the College de France in Paris, France. He joined the faculty of the University of Ottawa in 1991 before moving to his present position in the Department of Psychiatry and Behavioral Neuroscience at The University of Chicago in 1993. There, he has served as the Program Director for the NIDA Drug Abuse Training Program and on the editorial boards of several well-recognized scientific journals. Until now, he has published over 100 peer-reviewed articles and book chapters, and has been continuously funded through grants from the National Institute on Drug Abuse since 1995.

Dr. Baljit S. Khakh

Baljit S. Khakh received a PhD degree from the University of Cambridge in 1995. During his graduate studies, he also spent some time at the Geneva Biomedical Research Institute. Dr. Khakh completed a postdoctoral fellowship in the laboratory of Dr. Graeme Henderson at the University of Bristol, followed by a fellowship at the California Institute of Technology. Also, he worked in the laboratories of Drs. Henry A. Lester and Norman Davidson as a Wellcome Trust International Prize Traveling Research Fellow, and Senior Research Fellow in the Division of Biology. In 2001, Dr. Khakh returned to Cambridge in the Division of Neurobiology at the MRC Laboratory of Molecular Biology as a Group Leader. Dr. Khakh joined UCLA in April 2006, where he is now Professor of Physiology and Neurobiology. Between 2014-2016, Dr. Khakh was a Visiting Scientist at the HHMI Janelia Research Campus in Virginia.

2016 ANNUAL PUERTO RICO NEUROSCIENCE POSTERS

Theme: Cognition

Abstract #1

WORKING TOGETHER WITH TEACHERS FROM A NEUROPSYCHO-LOGICAL PERSPECTIVE

Sacha Pérez-Acevedo; Cecilia Marino-Nieto; Wilmarie Díaz-Flores & Edilí Acosta-Ogando

University of Puerto Rico - Río Piedras Campus

The purpose of this poster is to promote teacher's inclusion in a neurocognitive diagnostic model to develop early identification and interventions of students with learning difficulties. Teacher's training in a neurocognitive processes rating scale can provide for early identification and development of interventions. Atendees will obtain conceptual and practical information of the neurocognitive model and the importance of collaboration between teachers and psychologists. Novice or experienced practitioners will most likely want to attend this presentation.

Acknowledgements: Instituto de Investigación Psicológica (IPsi); Collaborators: Judiana M. Seda-Ramírez, B.A. & Mary A. Moreno-Torres, Ph.D.

Keywords: Neurocognitive processes, Learning difficulties, Teachers, Psychologists

Theme: Development

Abstract #2

CHANGES IN EXPRESSION OF DENDRITIC SPINE-ENRICHED NEURONAL PROTEINS IN THE PREFRONTAL CORTEX DURING ADOLESCENCE

Ana Defendini[1]; Allyson Mallya[2] & Ariel Deutch[2]

1. University of Puerto Rico - Río Piedras Campus, Department of Biology
2. Vanderbilt University

Structural and functional alterations in the prefrontal cortex (PFC), including reduced spine density of PFC pyramidal cells, are thought to contribute to the cognitive symptoms of schizophrenia. These are not only debilitating, but do not respond to current treatments. It has been suggested that aberrant developmental synaptic elimination during adolescence contributes to the reduced spine density of PFC pyramidal cells (PCs) in schizophrenia. Recent data proposes that microglia are critically involved in synaptic elimination during early postnatal development. In an effort to better characterize the development of the PFC, which has a delayed and protracted maturation, we will examine the developmental expression pattern of neuronal proteins that are expressed at high levels in dendritic spines. We will use immunoblotting to quantify levels of PSD-95 and spinophilin at postnatal days 30 (peak spine density), 39 and 50 (decreasing spine density). We expect the changes in neuronal protein levels to mirror the developmental pattern of spine density, which in PFC PCs reach their peak density at P30, and thereafter decrease until a mature adult spine density is obtained. Along with data examining the developmental expression pattern of PFC glial proteins at the same postnatal days, we will obtain a better understanding of adolescent PFC development. These studies serve as a foundation for future work aimed at understanding the processes governing developmental PFC synaptic elimination, which is thought to be disrupted in schizophrenia, and which may contribute to cognitive symptoms. Future experiments will examine in the adult the structural and functional consequences of adolescent microglial ablation.

Acknowledgements: This research was supported in part by MH077298 from the National Institute of Mental Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of NIMH/NIH.

Keywords: PFC, PSD-95, Spinophilin, Schizophrenia

Abstract #3

ROLE OF DSCAM ON AXON ORGANIZATION IN THE RETINA

Ortega-Burgos, Yohaniz 1; Bloomsburg Samuel 2, Fuerst Peter G.,Ph.D 2

1. University of Puerto Rico, Humacao Campus, Humacao, PR
2. University of Idaho, Moscow, ID, United States

The retina is the neural tissue of the eye, where light information is received and processed. There are different cells that compose the retina: photoreceptors, which respond to light input, ganglion cells, which are the output neurons to the brain, and bipolar and amacrine cells, which intervene between the photoreceptors and the ganglion cells. One of the important forms of processing light is the separation of information into ON and OFF pathways. This processing ability and therefore the ability to see normally, depends on the structure; it needs to be organized to function adequately. There are many factors that help establish organization; one of them is the protein DSCAM. The goal of this study was to understand the role of DSCAM in the organization of axons of a particular population of bipolar cells, the Type 4 OFF bipolar cells. The methods used included the use of transgenic mice, using 3 different genotypes: Wild type, DscamLOF (full deletion of Dscam gene) and Dscam FF HtrCre (Cre-Lox dependent deletion of the Dscam gene). Retinas sections were stained through immunohistochemistry, using primary antibodies directed at GFP and calbindin, and fluorescent secondary antibodies. The work was based on the hypothesis that the Dscam deletion will lead to a defective axon layering and disorganization of axon territories. After this study, several roles of DSCAM were found. DSCAM is necessary for axon tiling and preventing the overlapping between neurons Also; the deletion of the Dscam produces severe disorganization in neural connectivity.

Acknowledgements: National Science Foundation REU Site award DBI 1460696.

Keywords: Neuroscience Developmental Biology Dscam Neurobiology

Abstract #4

SCREENING DIP-Γ EXPRESSION IN L3 NEURON POSTSYNAPTIC PARTNERS WITHIN THE DROSOPHILA VISUAL SYSTEM

Almaris N. Figueroa Gonzalez[1]; Chundi Xu[2] & Matthew Y. Pecot[2]

1. University of Puerto Rico - Río Piedras Campus
2. Harvard University, Department of Biology

Understanding the molecular mechanisms of synaptic specificity remains a challenging question in neurobiology. In the Drosophila visual system, lamina neurons L1-L5 innervate the medulla, forming stereotyped connections with other neuronal subtypes. Previous studies showed that lamina neurons express unique combinations of Dprs, a subclass of immunoglobulin (Ig)-domain containing proteins. Dpr interacting proteins (DIPs) are expressed in subsets of lamina neuron synaptic targets raising the possibility that Dpr/DIP interactions are involved in the mechanisms that regulate synaptic specificity between neurons within the medulla. L3 neurons express Dprs 15, 16, and 17, all of which interact with DIP-γ. Therefore, we set out to identify which postsynaptic partners of L3 express DIP-γ. Using the GAL4/UAS gene expression system to label known L3 postsynaptic partners, and a fluorescent reporter recapitulating the DIP-γ expression, we found that C3, TM5 and TM20 neurons express DIP-γ in the adult fly brain. These results suggest that L3 terminals may identify C3, TM5 and TM20 postsynaptic dendrites via Dpr-DIP interactions.

Acknowledgements: NIH ENDURE NeuroID Program, Summer Honors Undergraduate Research Program

Keywords: Synaptic specificity, Dprs, DIP-gamma, L3 neurons

Abstract #5

THE MESENTERY NERVOUS SYSTEM AND THE CHANGES IT UNDERGOES DURING INTESTINAL REGENERATION IN AN INVERTEBRATE DEUTEROSTOME

Christian J. Nieves Ríos; Samuel Álvarez Falcón; José E. García Arrarás

University of Puerto Rico, Río Piedras Campus, Department of Biology

Holothuria glaberrima is an echinoderm with the ability to regenerate its internal organs. Previous studies demonstrate that in this species intestinal regeneration depends on the mesentery. The mesenterial tissues play a key anatomical role not only in the connections between the viscera and the body wall but also serve as the physical substrate for nerves connecting the visceral nervous components to the central nervous system. Although the mesenterial nervous system component has been well described in vertebrates, particularly in mammals, a description in other deuterostomes is lacking. It is crucial to describe this component in H. glaberrima in view that the nervous system has been shown to play a role in many regenerative events. Thus, our main focus is to describe the nervous components of the intestinal mesentery in the sea cucumber H. glaberrima, and the changes that it endures during the regeneration progress. We have used immunohistochemistry and tissue whole mounts to describe the nervous components within the mesentery of normal and regenerating animals. Nerve fibers and cells were described and quantified using the RN1 antibody, a neuronal marker. Our results show that the orientations of nerve fibers in both mesotheliums are perpendicular to those in the connective tissue, and that both mesotheliums are anatomically similar regarding their quantity and distribution. Most of the nerve fibers within the mesentery remain during the regenerative process, with two major changes observed during the first days: a disorganization of the nerve fibers and their retraction near the tip of the mesentery. In later stages, a reinnervation process takes place. Our results present descriptive and quantitative evidence of the anatomical distribution of nerve fibers in the mesentery, and provide useful techniques for studies of the nervous system in intestinal regeneration of sea cucumbers. Research was funded by NSF (IOS-0842870), NIH (R15NS01686) and the University of Puerto Rico. JMR was funded by NIH-MBRS (RISE) program of the University of Puerto Rico.

Acknowledgements: NSF (IOS-0842870), NIH (R15NS01686), NIH-MBRS (RISE) program, and the University of Puerto Rico, Río Piedras Campus

Keywords: regeneration, nervous system, echinoderm, Holothuria glaberrima

Abstract #6

DETERMINATION OF TRANSCRIPTION FACTORS IN IRON DEFICIENCY-INDUCED NEURONAL AND HEMATOPOIETIC CELL LINES VIA CRISPR-CHAP-MS TECHNIQUE

Carla M. Cardona[1]; Phu V. Tran, Ph.D.[2]; Michael K. Georgieff, M.D.[2]

1. University of Puerto Rico - Mayagüez Campus
2. University of Minnesota

According to the World Health Organization, iron deficiency affects approximately 30% of the world's population, including 20-30% of pregnant women and their offspring, and causes long-term deficits in learning and socio-emotional behavior despite prompt iron treatment. Although the mechanism is unknown, iron uptake from transferrin transport protein is prioritized among tissues, favoring red blood cells over neurons. Identifying this mechanism is potentially instrumental for developing therapeutic strategies to shuttle iron preferentially to the brain for neurodevelopment, thereby ameliorating long-term negative neurobehavioral effects for at risk fetuses and neonates. Differential regulation of transferrin receptor (TfR-1) gene promoter is proposed and will be tested using a novel CRISPR-ChAP-MS technique. GuideRNAs, targeting various TfR-1 promoter regions encompassing suspected transcriptional factor (TF) binding sites (-1K, -3K and -4K upstream of transcription start site of TfR-1 gene), were inserted into pLE016.6 plasmids, which carry a HALO tag and deactivated CAS9 (dCAS9). Engineered vectors were then used to transform DH5α competent bacterial cells for plasmid amplification. Purified expression vectors will be used to transfect hematopoietic (K562) and neuronal (HT-22) cell lines. Following iron deficiency induction by an iron chelator (Deferoxamine), quantitative Real-Time PCR (qPCR) will be used to determine TfR-1 expression. The promoter-TFs-complexes will then be purified using Halo tags. TF identification will be assessed using protein-sequencing HPLC?MS technique.

Acknowledgements: Life Sciences Summer Undergraduate Research Program; University of Minnesota; National Institute of Health - Heart, Lungs & Blood Institute

Keywords: Iron deficiency, CRISPR-ChAP-MS, Transferrin, neurodevelopment

Abstract #7

INDUCING SODIUM ION CURRENTS TO DETERMINE THE ROLE OF THE NERVOUS SYSTEM IN INTESTINAL REGENERATION

Paola Figueroa Delgado; José E. García-Arrarás

University of Puerto Rico, Río Piedras Campus, Department of Biology

Several studies have shown a role of the nervous system in regenerative processes. Specifically, action potentials in excitable cells are known to be necessary, even to restore functional regeneration after pharmacological inhibition. Most studies on the nervous system's involvement in regeneration have been on amphibian limbs and tail (i.e. Xenopus). However, the involvement of the nervous system in visceral regeneration remains unclear. We have used the sea cucumber Holothuria glaberrima as a model to study nervous system effect in intestinal regeneration. In this model, the regenerating intestine arises as the mesenteries that held the intestinal track thickens, eventually forming the luminal epithelium and lumen. Studies on the model have shown the presence of an enteric nervous system comprised of serosal (mesothelial), connective tissue, and mucosal neuroendocrine plexus. Sodium ion currents were induced by injecting animals 1 day post evisceration (dpe) with monensin A M and sodium gluconate 90mM for 24 hours. Monensin A salt, isolated originally from Streptomyces cinnamonensis, is an ionophore with high-selectivity to transport sodium ions into cells. Thus, directly moderating cellular sodium ion transport. At 5 dpe, mesenteries were fixed and whole mounts and histological slides were prepared. Immuno-histochemical techniques were used to analyze the tissues. Statistical analysis was provided by a nerve fiber quantification method amongst control and experimental animals. Histological findings denote no significant differences between monensin A treated and non-treated animals. Further evaluation and additional experimental phases are required to understand the role of sodium ions transport in H. glaberrima's viscera regeneration capability. Measurement of tissue transmembrane potential by use of DiBAC4 (3) and intracellular sodium presence (CoroNa+ Green fluorescence) will be considered. Furthermore, use of a voltage-gated sodium channel inhibitor is highly suggested as it can provide information as to the importance of increased intracellular sodium in regeneration capabilities. Our study provides an insight on the effects the mesentery system undergoes when sodium transport is directly modulated by use of an ionophore (monensin A) and increasing the Na+ concentration (sodium gluconate).

Acknowledgements: NIH BP-ENDURE Grant (NEURO-ID Research Program)

Keywords: sodium currents, dedifferentiation, regeneration, nervous system

Abstract #8

DEVELOPMENTAL CHANGES IN DENSITY OF INTRINSICALLY PHOTOSENSITIVE RETINAL GANGLION CELLS

Amanda González-Segarra[1]; Franklin Caval-Holme[2]; and Marla Feller[2]

1. University of Puerto Rico - Río Piedras Campus, Department of Biology
2. University of California—Berkeley

The retina is composed of five neuronal cell classes: photoreceptors, horizontal cells, bipolar cells, amacrine cells, and retinal ganglion cells (RGCs). RGCs mediate communication between photoreceptors and visual centers of the brain. Some of the RGCs are photosensitive because they contain the light-sensing pigment melanopsin that allows them to respond to light autonomously. These intrinsically photosensitive retinal ganglion cells (ipRGCs) are crucial for multiple mouse behaviors, including light avoidance in neonatal mice, pupil dilation, and photoentrainment of circadian rhythms. My project is to understand how the population of ipRGCs emerges during development. Changes in ipRGC cell density throughout development have been reported; however, whether this is due to cell death or cessation of melanopsin production is unknown. To address this question, we tracked the change in cell density over development by labeling ipRGCs. Since ipRGCs are the only cells in the retina that contain melanopsin, we used three different techniques to label cells that express melanopsin and consequently ipRGCs through immunohistochemistry. First, we labeled ipRGCs in wild type mice (WT) using antibodies against melanopsin. However, the number of cells labeled by this approach is limited by the sensitivity of the antibody. Second, we labeled ipRGCs in a transgenic mouse line in which Green Fluorescent Protein (GFP) is expressed under the promoter for melanopsin. Third, we used an intersectional transgenic approach in which the fluorescent protein tdTomato is expressed in cells that express melanopsin at any stage of their development. In all three techniques, retinal tissue was immunostained for melanopsin as well as GFP or tdTomato for comparison. Results were visualized using confocal microscopy and cell density was measured. We found that during the first 3 days of development there is a dramatic decrease in ipRGC number. This drop was most dramatic in the intersectional transgenic mouse line, where we were able to track cells that expressed melanopsin at initial stages of development. Since such a large reduction in cell number occurred during a short amount of time, we conclude that apoptosis contributes substantially to this decrease in ipRGC density.

Acknowledgements: Supported by: NIH Grant # R01EY013528 & MARC-NIH Grant # 5T34GM007821

Keywords: retina, retinal ganglion cells, melanopsin, development

Theme: Integrative Physiology and Behavior

Abstract #9

SYSTEMIC ADMINISTRATION OF A GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONIST ATTENUATES COCAINE SEEKING IN RATS

Gian C. Molina Castro[1]; Nicole S. Hernandez[2]; Heath Schmidt[3]

1. Department of Biology, College of Natural Sciences, University of Puerto Rico, Río Piedras Campus, San Juan, PR
2. Neuroscience Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA
3. Department of Biobehavioral Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, PA

Emerging evidence indicates that glucagon-like peptide-1 receptor (GLP-1R) agonists, which are FDA-approved for treating diabetes and obesity, regulate addiction-like behaviors in rodents. For example, administration of a GLP-1 agonist attenuates cocaine-induced self-administration, locomotion stimulation, conditioned place preference and dopamine release in the nucleus accumbens and striatum. However, the role of GLP-1Rs in cocaine craving-induced relapse remains unclear. We hypothesized that systemic administration of the GLP-1R agonist Exendin-4 would attenuate cocaine priming-induced reinstatement, an animal model of relapse. Rats self-administered cocaine (0.25mg/infusion, i.v.) for 21 days on a fixed-ratio (FR5) schedule of reinforcement. Cocaine self-administration was then extinguished by replacing cocaine with saline. Once cocaine taking was extinguished, rats received an acute priming injection of cocaine (10 mg/kg, i.p.) to reinstate drug-seeking behavior. During subsequent reinstatement sessions, rats were pretreated with vehicle or fluoro-Exendin-4 (0.01, 0.1, 0.2, 1.0, 3.0 µg/kg, i.p.) 1 hour prior to a priming injection of cocaine. Immediately following the reinstatement test session, brains were collected to determine whether fluoro-Exendin-4 penetrated the brain. Systemic fluoro -Exendin-4 administration significantly attenuated cocaine reinstatement dose-dependently and immunohistochemical analyses showed colocalization of fluoro-Exendin-4 with astrocytes and neurons in the nucleus accumbens and VTA. Taken together, these results indicate that peripheral administration of a GLP-1R agonist is sufficient to reduce cocaine seeking and that these effects are mediated, in part, by activation of central GLP-1Rs. The provocative findings suggest that GLP-1R agonists could be repurposed for treating cocaine addiction.

Acknowledgements: Schmidt's Lab, University of Pennsylvania, NeuroID Research Program (1R25MH092912-01)

Keywords: cocaine, GLP-1, reinstatement, Exendin-4

Abstract #10

CARDIOMETABOLIC ADAPTATIONS TO TRAUMA IN AN ANIMAL MODEL OF PTSD

Arsenio L. Reyes-Rivera[1]; Julio D. Vega-Torres; Priya Kalyan-Masih[2];
Ivana Alicea-Polanco; Johnny D. Figueroa[2]

1. San Juan Bautista School of Medicine, Caguas, PR
2. Center for Health Disparities & Molecular Medicine, Department of Basic Sciences, Physiology Division Loma Linda University School of Medicine, Loma Linda, CA

Cardiovascular disease (CVD) is the leading cause of death and disability in the world. An increasing body of evidence shows that people suffering from post-traumatic stress disorder (PTSD), a neuropsychiatric disorder observed in both veteran and nonveteran populations, have a high prevalence of hypertension, hyperlipidemia, and coronary heart disease. We have shown significant interactions between dietary fatty acids and endocrine stress responses that may contribute to PTSD vulnerability. However, little is known about how PTSD impairs cardiovascular function and metabolism. The objectives of this study were (1) to determine whether dietary cardiovascular risk factors increase vulnerability to a predator-scent stress (PSS) model of PTSD and (2) to determine the impact of PSS in the expression of stress-related genes in the heart. Lewis rats were fed for eight weeks with either an experimental Western-like high-fat diet (WD) or a control diet. Fear acquisition and extinction behaviors were assessed for one week following PSS using the fear-potentiated startle (FPS) paradigm. The acoustic startle reflex and the elevated plus maze (EPM) were used to determine arousal and anxiety-like behaviors, respectively. The hearts were isolated for Real Time-Polymerase Chain Reaction (RT-PCR) and Western blot analyses. We found that rats that consumed the WD exhibited altered behaviors, as evidenced by reduced acoustic startle responses and impaired associative fear learning. Further, the rats that consumed the WD showed increased anxiety-like behaviors following PSS. Magnetic resonance imaging showed significant hippocampal atrophy (20% reduction) and lateral ventricular enlargement (50% increase) in the rats fed with the WD when compared to controls. These volumetric abnormalities were associated with behavioral indices of anxiety, increased leptin, and reduced hippocampal blood vessel density. Notably, PSS-exposed rats showed a significant reduction (42.9%) in the heart mRNA levels of the glucocorticoid-receptor chaperone FK-506 binding protein (FKBP51) when compared to unexposed controls ($p = 0.024$). We found that the mRNA levels of the fatty acid translocase (FAT/CD36) were significantly reduced (25.0%) in the heart tissue of rats that were exposed to PSS ($p = 0.027$). Altogether, our findings demonstrate that consumption of a WD has a profound impact in stress-related endocrine and behavioral responses. This study identifies FKBP51 and CD36 as potential mechanistic links between traumatic stress and CVD risk.

Acknowledgements: Research reported in this poster was supported by the National Institute of Health Disparities and Minority Health of the National Institutes of Health under award number P20MD006988 and Seed Grant Funds. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Keywords: Animal Behavior, PTSD, FKBP51, Neuroimaging

Abstract #11

BDNF IN VENTRAL HIPPOCAMPAL NEURONS PROJECTING TO PREFRONTAL CORTEX IS NECESSARY FOR EXTINCTION OF ACTIVE AVOIDANCE

Valeria Lozada[1]; Luis E. Rosas Vidal[2]; Gregory J. Quirk[2]

1. University of Puerto Rico, Río Piedras Campus, San Juan, PR

2. University of Puerto Rico, Medical Sciences Campus, San Juan, PR

Individuals suffering from Post-Traumatic Stress Disorder (PTSD) and other anxiety disorders show persistent avoidance of people, places or activities associated with a traumatic event. However, little is known about the neural mechanisms that mediate these behaviors. It has been shown in previous studies that prelimbic cortex (PL) is necessary for expression of active avoidance, whereas infralimbic cortex (IL) is necessary for extinction of avoidance. Consistent with this, recent studies suggest that brain-derived neurotrophic factor (BDNF) in IL is sufficient and necessary to induce extinction of conditioned fear, however, its role in avoidance extinction remains unclear. Therefore, in this study we aimed to answer the following questions, (1) Is BDNF released in IL necessary for avoidance extinction? (2) If so, which structure could be the main source? And finally, (3) Is BDNF in this structure necessary for avoidance extinction? Using the platform-mediated avoidance task, we observed that infusions of BDNF binding antibody into IL impaired extinction recall, suggesting that BDNF signaling in IL is necessary for extinction of avoidance. In order to determine the main source of prefrontal BDNF, we used immunohistochemistry (IHC) for BDNF combined with the neuronal marker NeuN to detect extinction-induced increases in neuronal BDNF. This co-labeling approach showed that extinction training significantly increased neuronal BDNF expression only in the ventral hippocampus (vHPC), an input to IL. To confirm if the BDNF produced in vHPC was projecting to IL, we combined BDNF IHC in vHPC with a retrograde tracer infused into IL. Our results showed that extinction augmented the expression of BDNF in vHPC neurons projecting to IL, suggesting that the main source of BDNF during avoidance extinction comes from vHPC. Finally, to determine whether BDNF in the vHPC is necessary for extinction learning, we used CRISPR/Cas9 system to block the production of BDNF in vHPC neurons. Blocking BDNF production in vHPC impaired avoidance extinction recall, suggesting that BDNF production in the vHPC is necessary for extinction of avoidance. Diminished hippocampal volume and activity in PTSD could account for the increased avoidance observed in this disorder.

Acknowledgements: NIMH grants MH058883 and MH081975 to GJQ

Keywords: BDNF; Ventral Hippocampus; Prefrontal Cortex; Extinction

Abstract #12

EFFECTS OF AN ORGANIC EXTRACT OF BROWN ALGAE ON AN ANXIETY RELATED BEHAVIOR IN DROSOPHILA MELANOGASTER

Grisel Robles Del Valle; Anamaris Torres Sanchez; Lee Algarin Sepulveda; Perla Elosegui Rodriguez

University of Puerto Rico - Cayey Campus

Nature has a great diversity of organisms with the potential to study their bioactive compounds. When it comes to aquatic life we find that algae are organisms that are well suited for the screening and identification of bioactive compounds due to their widespread distribution in both salt and freshwater. Our hypothesis is that a crude organic extract of the brown algae Stypopodium zonale can decrease anxiety-related behaviors in Drosophila melanogaster. Stypopodium zonale was collected in the south coast of Puerto Rico and the potential anxiolytic-like effects of the extract were studied in an anxiogenic-like behavioral paradigm in Drosophila melanogaster. This behavior is called centrophobia, and is measured using an Open Field Arena (OFA). Validation of the paradigm gave the expected results as reported in the literature, in which Drosophila exhibits a phobia (avoidance) to remain in the center of the OFA, which corresponds to a behavior with anxiety components. The organic extract was dissolved with Dimethyl Sulfoxide (DMSO). Toxicity tests were performed both for DMSO and the crude organic extract, and none of them showed positive results. To perform the behavioral trials, 1 ml of the crude extract and 5 ml of water were mixed with 1.8 g of Drosophila food. The adult flies were grown in a tube with the extract until a considerable quantity of larvae was observed, and then the adults were removed. These new larvae, once turned into adult flies, were used for the behavioral trials. The behavior of control flies (regular food) and experimental flies (extract-containing food) were recorded with a video camera and the results of the centrophobic behavior were analyzed and compared using quantitative criteria. Both the control and experimental trials were performed in triplicate. The results show that flies grown in food containing the crude extract present a significant reduction in centrophobia compared with flies who grew exposed to regular food (control flies). We also observed similar effects in flies grown with food containing a total protein extract obtained from Styopopodium zonale. In conclusion, our results suggest that the organic crude extract from Stypopodium zonale has anxiolytic-like effects in a Drosophila melanogaster model with anxiety components. We are currently performing Proton-Nuclear Magnetic Resonance (NMR) studies on the crude extracts to identify the most abundant secondary metabolites. Future experiments should include the administration of the crude extracts (or fractions of the most abundant secondary metabolites) to a vertebrate model in to test the effect in a behavior with anxiety components. We are also in the process of developing a preliminary model of possible mechanisms of action of the crude organic extract in the reduction of centrophobia.

Acknowledgements: The Academic Department and Research Institute/BRIC provided our funding and the Biology Department provided us with the space necessary to develop our project. Also, PR-LSAMP provided another funding. For that we thank you. Last, but not least, our gratitude to our mentor and research team whose hard work and dedication made this research project possible.

Keywords: Anxiety; Algae; Open Field Arena; Organic extract

Abstract #13

TIME DEPENDENT SHIFTS IN FEAR SIGNALING RECRUITS DISTINCT LAYERS OF PRELIMBIC CORTEX

Alexis Vega-Medina; Kelvin Quiñones-Laracuente; Fabricio H. Do-Monte; Gregory J. Quirk

Departments of Psychiatry and Anatomy & Neurobiology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico

We have previously shown that recent vs. older fear memories are retrieved by different circuits (Do-Monte et al., 2015). Efferents from the prelimbic cortex (PL) to the amygdala retrieve recent (2 h) fear memories, whereas efferents from PL to the midline thalamus retrieve older (7 d) fear memories. Interestingly, these two efferents originate from neurons located at different layers of PL. Layers 1-5 of PL (superficial) contain amygdala-projecting neurons, and layer 6 of PL (deep) houses thalamic-projecting neurons. Therefore, the time-dependent transition in retrieval circuits could be mediated by transferring fear associations across PL layers. To test this hypothesis, we recorded single unit activity of both superficial and deep neurons, while retrieving a fear memory 2 h, 24 h, and 7 days post-conditioning. Deep neurons showed increased spontaneous activity at all time points when compared with superficial neurons. Superficial neurons developed excitatory conditioned responses for the 2 h (16/36), 24 h (14/36), and 7 d (12/36) time points. In contrast, deep neurons displayed inhibitory conditioned responses after the 24 h time point (6/11), a sustained response that persisted >15 s after the tone offset. Taken together, our results suggest that older fear memories may be signaled via inhibition of PL deep layer neurons.

Acknowledgements: IPAN NSF GRANT 1407977 NIMH R37 MH058883 to GJQ

Keywords: fear conditioning, prelimbic cortex, amygdala, thalamus

Abstract #14

INTRANASAL OT IMPACTS COCAINE CONDITIONED LOCOMOTION AND ELICITS CHANGES IN ENDOCANNABINOID RECEPTORS WITHIN THE MESOLIMBIC SYSTEM

Molina-Castro, G.C.; Figueroa-González, A.N., Loyola-Vélez, A.J., Torres-Hernández, E. & Maldonado-Vlaar, C.S.

University of Puerto Rico-Río Piedras Campus, Department of Biology

Oxytocin (OT) is a neuropeptide secreted by the hypothalamic paraventricular nucleus (PVN) and commonly associated with social behaviors, stress responses and drug-addiction. Previous studies have shown that OT has anxiolytic properties associated with cues in a cocaineconditioning paradigm, but the underlying mechanism remains unknown. This study aims to characterize possible mechanistic interactions between OT and the endocannabinoid system mediating cocaine conditioning and anxiety response, in particular the cannabinoid receptor type 1 (CB1) and the transient receptor potential vanilloid type-1 (TRPV1). Rats were exposed to activity chambers after receiving systemic intraperitoneal injections of cocaine (10 mg/kg) or saline 0.9%. On the last day (D7), rats received intranasal infusions of OT (1 ug/ uL) or vehicle 30 minutes prior being exposed to the cue-associated environment. Our results showed that OT pretreatment impacts cocaine-paired conditioned locomotion. Preliminary western blot analysis showed an upregulation of OT receptors within the hippocampus and amygdala and a downregulation of these receptors within the prefrontal cortex (PFC). Further immunohistochemical analysis will be conducted to determine possible interactions or colocalization between OT, CB1 and TRPV1 receptors. This preliminary data suggests intranasal OT as a novel therapeutic approach of cocaine addiction.

Acknowledgements: NIH BP-ENDURE NeuroID Program (1R25MH092912-01),
Dr. Carlos Gonzalez, María Fernanda, Dr. Manuel Díaz, Dr. Amelia Merced, NeuroImaging Core Center Nikon Center of Excellence (NSF DBI-1337284)

Keywords: Oxytocin; Endocannabinoids; Mesolimbic system

Abstract #15

ON THE ROLE OF TRPV1 RECEPTORS WITHIN THE BRAIN IN ANXIETY ELICITED BY COCAINE CUES

Norze Wickensonn*, MD; Loyola Alejandro, Torres Edgardo and Maldonado-Vlaar, Carmen S

University of Puerto Rico - Río Piedras Campus

Transient Receptor potential Vanilloid (TRPV1) is activated in peripheral terminals of nociceptive fibers by noxious heat, low pH and natural product such as capsaicin, and is expressed also in the brain where it seems to be involved in antinociception, locomotor control and regulation of affective behaviors. Capsazepine is a competitive antagonist of the vanilloid receptor. We investigated the effects of blockade of TRPV1 receptors in eliciting anxiolytic responses following exposure to cocaine related cues in rats. Male Sprague Dawley rats received daily intraperitoneal injections of cocaine (10 mg/kg) for five consecutive (D1-D5) prior to being placed in activity chambers. During the daily 90 min sessions, rats paired visual and olfactory cues with the cocaine treatment. Following one day of abstinence, animals were divided into two groups which received either vehicle or capsazepine (10 ug/kg, ip) and returned to the activity chambers followed by Elevated Plus Maze (EPM) testing. Results showed that cocaine significantly increased locomotor activity and produced behavioral sensitization within the first five days. Animals treated with capsazepine showed no significant difference in locomotor behavior on Day 7 when compared to vehicle treated group ($p<0.05$, T-test). In the EPM, the capsazepine dose had no effect on the total time spent on open and close arms during 5 minutes of testing. Future studies are needed to test several doses of capsazepine in other to characterize a possible therapeutical profile of the drug.

Acknowledgements: Decanato de estudios Graduados e Investigacion (DEGI)

Keywords: TRPV1, cocaine, rats

Abstract #16

ZIRCONIUM PHOSPHATE NANOPARTICLE STUDIES IN VIVO IN DROSOPHILA MELANOGASTER

Jose J. Gorbea-Colón[1]; Norma Rodríguez[1]; Bárbara Casañas[1]; Jorge L. Colón[2]; José L. Agosto-Rivera[1]

1. Department of Biology, University of Puerto Rico, Río Piedras Campus, San Juan, PR
2. Department of Chemistry, University of Puerto Rico, Río Piedras Campus, San Juan, PR

Drug delivery systems aim to circumvent the difficult process of novel drug discovery by modifying the in vivo activity of known compounds. Zirconium phosphate (ZrP) nanoparticles have shown to be a viable non-cytotoxic platform for the delivery of several drugs. Given the potential medicinal use of ZrP, we test the effects of peroral ZrP administration on Drosophila melanogaster sleep behavior, establish a lethal dose for future experimentation using this combination and address whether these behavioral effects can be reversed and mortality reduced by discontinuing regimens. To achieve this, ZrP nanoparticles were synthesized from zirconium (IV) chloride and phosphoric acid and assayed using powder X-ray diffraction. ZrP nanoparticles were then suspended in fly food in ascending concentrations of 0.00 mg/mL, 0.25 mg/mL, 2.50 mg/mL and 25.00 mg/mL. The sleep patterns of wild type Oregon R (OreR) flies were assayed under standard Light/Dark (LD) cycles. Assayed flies had continuous access to food stocks for 1 week before exchanging stocks with new ones containing either the same ZrP concentrations or switching their stocks to new food without ZrP. After acquiring two weeks' worth of sleep behavior data, analyses were run using MatLab, Prism and JMP computer software. From these studies, we report that concentrations of up to 2.50 mg/mL were safe in terms of mortality rate. However, in terms of sleep behavior, we observed alterations suggesting that sleep is a more sensitive measure of toxicity. Non-continuous treatment reversed sleep behavior phenotypes in some cases as well as reduced mortality for some groups. In conclusion, further studies are needed; however lower ZrP concentrations seems to be a promising alternative for the development of new drug delivery strategies, although biodistribution studies are of utmost importance before moving to next steps.

Acknowledgements: NIH - BP ENDURE Program (NeuroID)

Keywords: sleep drosophila zirconium-phosphate nanoparticle

Abstract #17

REGULATION OF SLEEP HOMEOSTASIS BY THE TRANSLATIONAL REPRESSOR PUMILIO IN DROSOPHILA MELANOGASTER

Melina Perez; Luis de Jesus; Jonathan Aleman; Jose Agosto

University of Puerto Rico - Río Piedras Campus

Numerous studies have shown that sleep deprivation increases synaptic proteins; nevertheless, the effects of reducing the translation of synaptic proteins on sleep behavior have not been thoroughly explored. Pumilio (Pum) is an RNA binding protein involved in neuronal homeostasis that repress the translation of many synaptic proteins and decrease neuronal excitably during chronic patterns of neuronal activity. Here we show that knocking down Pumilio in Drosophila melanogaster timeless neurons, abolishes sleep rebound. Our data shows that Pumilio is recruited during sleep deprivation and prevent the uncontrolled synthesis of synaptic proteins at the translational level. Consistent with the idea that these effects are due to exaggerated translation of synaptic proteins, oral administration of the translational blocker, rapamycin, completely rescues the sleep rebound in Pum knockdown flies. Conversely, exaggerating synaptic translation by overexpressing the eukaryotic initiation factor 4E (eIF4E), which is a known target of Pum repression, decreased the sleep rebound. These results suggest that unregulated translation impairs sleep rebound, making translation of synaptic proteins an important regulator of sleep homeostasis.

Acknowledgements: We thank Dr. Tugrul Giray, Dr. Adrienel Vazquez, Dr. Manuel Giannoni, Dr. Adrian Avalos, Lizangie Cueto and Alejandro Medina for their support; and the students from the Genetics 3350 lab course for their contribution on RT-PCR data generation. This work has been partially supported by RISE grant # 2R25GM061151-13 and the NSF REU-CRIB Program Grant 1156810. We like to thank Dr. Rosbash's Lab for various fly lines. This work was also partially funded by the University of Puerto Rico, Río Piedras Campus (Seed Money) and by the REU-CRIB Summer Program (1156810).

Keywords: Translation, Pumilio

Abstract #18

POTENTIAL ANXIOLYTIC EFFECTS OF BROWN SEAWEED ORGANIC EXTRACTS FROM THE COASTS OF PUERTO RICO IN DROSOPHILA MELANOGASTER

Alejandra Ferrer-Díaz; Heity A. Fonseca-Cruz; Bianca Correa-Rivera, Génesis Rivera-Carrasquillo; Jesica Vicente-Reyes; Ricardo Chiesa

University of Puerto Rico - Cayey Campus, Department of Biology

Individuals with anxiety disorders are usually treated with benzodiazepines. The main function of benzodiazepines is to reduce anxiety by modulating gamma-aminobutyric acid type A receptors. Long term exposure to this pharmacological agent can cause dependence and tolerance, meaning that individuals need to use them on a daily basis. Our research focuses on determining potential anxiolytic effects of crude organic extracts from brown seaweed Stypopodium zonale using the Dark/Light box test as a behavioral anxiety-related paradigm in Drosophila melanogaster. The dark/light box can be used as an anxiety paradigm because Drosophila prefers to spend more time in the dark side of the box, which is an indicative of anxiety, while crossing or exposing part of its body to the illuminated side of the box is a measure of exploratory (less anxiety) behavior. We hypothesize that whole organic extracts obtained from brown algae might possess metabolites with the potential to decrease anxiety-related behaviors. In order to test our hypothesis, we performed an organic extraction from the algae Stypopodium zonale and dissolved it with DMSO 23%. We administered the extracts to wild type Drosophila via food intake throughout their developmental cycle and then, exposed them to the Dark/Light box. The control group consisted of flies exposed to regular food. Behavioral assays lasted for a period of 30 minutes, in which the first fifteen minutes were for habituation and the resting fifteen were used to analyze how the flies behaved in the apparatus. The variables we took into consideration were: total amount of time the fly spent on both sides and the number of transitions the fly made from one side to another. We measured two types of transition: partial and full. A full transition is when the fly passes from the illuminated side to the dark side or vice versa. A partial transition is when the fly stays between the dark side and the illuminated side. Preliminary results demonstrate that flies exposed to the whole organic extract of Stypopodium zonale present less anxiety-related behavior in the dark/light box test in comparison to the control group. In conclusion, though we have to further validate these preliminary results, Stypopodium zonale whole organic extract seems to exert anxiolytic-like effects in a Drosophila melanogaster model of anxiety. Overall, this project help us elucidate the role of organic extracts from brown seaweed in anxiety-related behaviors present in Drosophila melanogaster.

Acknowledgements: Dra. Claudia Ospina - Chemistry Department, UPR Cayey Collaborator), III - UPR Cayey, BRIC Program - UPR Cayey, Academic Deanship - UPR Cayey, Biology Department - UPR Cayey.

Keywords: anxiety, dark-light box assay, Stypopodium zonale

Abstract #19

FEAR CONDITIONING AND EXTINCTION ALTER VENTRAL HIPPOCAMPUS ACTIVATION OF NMDAR CURRENTS IN INFRALIMBIC NEURONS

Omar Soler-Cedeño; OrlandoTorres-Rodríguez; Anixa Hernandez; James T. Porter

Pharmacology and Physiology, Ponce Health Sciences University - Ponce Research Institute - Ponce, PR.

After completing exposure therapy, some patients with posttraumatic stress disorder (PTSD) relapse when trauma-associated cues are experienced outside of the therapeutic context. Similarly to humans, fear extinction in rodents, which mimics human exposure therapy, is context-dependent such that an extinguished fear association to a cue renews when presented in a different context to that where the extinction occurred. Recent evidence suggests that synaptic plasticity between the ventral hippocampus (vHPC) and the medial prefrontal cortex (mPFC) could be mediating the context-specificity of fear extinction memory. Therefore, we wanted to investigate if extinction in the same conditioning context causes different synaptic ch anges in vHPC synapses in the mPFC than extinction in a different context. To address this, we assigned male Sprague Dawley rats to one of the following experimental groups: pseudo-conditioning, fear conditioning, same context extinction, and different context extinction. We did whole-cell patch-clamp recordings of mPFC's prelimbic (PL) and infralimbic (IL) pyramidal neurons to assess AMPA and NMDA receptors mediated excitatory postsynaptic currents (EPSCs) evoked by optical stimulation of channelrhodopsin expressing vHPC axons. Surprisingly, we found in IL that AMPA to NMDA ratios were significantly increased after fear conditioning. Moreover, fear extinction reversed the decrease induced by fear conditioning when the extinction training was conducted in the same conditioning context. Interestingly, increased AMPA to NMDA ratios were also observed in animals that received same context extinction but had unsuccessful extinction recall compared to animals with successful extinction recall. Further analysis indicated that the changes observed in the AMPA to NMDA ratios after fear conditioning and same context extinction were due to alterations in NMDA receptor-mediated EPSCs. These synaptic alterations were not observed in vHPC connections to PL. Taken together, these preliminary data suggest that fear conditioning induces weakening of vHPC synapses in IL, and that same context extinction induces synaptic strengthening of the weakened synapses. The inability of different context extinction in inducing similar strengthening of vHPC synapses in IL might account for fear renewal, which could explain relapse in certain patients with PTSD.

Acknowledgements: This work was supported by F31 MH106288, MBRS-RISE R25 GM082406 to OSC, R15 MH101700 to JTP, and G12 MD007579 Brain Research Integrative Neuroscience Core.

Keywords: Psicology Neu rociense Master Degree

Abstract #20

DEVALUATION OF ALCOHOL REWARD AND IDISCO BRAIN MAPPING

Hector L. Figueroa[1]; Adam Kimbrough[2]; Olivier George[2]

1 University of Puerto Rico - Cayey Campus

2 The Scripps Research Institute

Alcoholism is a disorder where the individual incurs in excessive alcohol consumption. Alcoholism is a 3-staged process consisting of; binge or intoxication, withdrawal and, craving. This three stages are characterized by the type (positive or negative) of driving force in alcohol consumption and its effects on the human body. In order to further understand alcohol's brain effects this study contemplates two objectives; first the establishment of a model for devaluation of compulsive alcohol drinking via LiCl injection and second, the acquisition of preliminary data for iDisco brain mapping. In order to accomplish the first objective rats were conditioned for alcohol preference. Alcohol preference conditioning was achieved by lever pressing training. After the rats acquired lever pressing, their administered liquid was changed from water to ethanol. In order to induce alcohol dependency in the rats, alcohol vapor chamber sessions were administered at 10 hours a day for 2-3 weeks. Then they were exposed to an alcohol reward devaluation treatment via LiCl injections in their stomach region at day 1 and faced with, 2-choice selection of water and ethanol for 33 days. To accomplish the second objective the iDisco procedure for brain clearing and immunolabeling was followed as explained in "The iDisco Method". In this study the rats devalued with LiCl significantly reduced their alcohol intake, however they recovered their alcohol intake after several 2-choice sessions post-devaluation. The iDisco brain mapping preliminary data suggests it can be used to study and unveil pathways in behavioral research.

Acknowledgements: NIH, UPR-Cayey, The Scripps Research Institute, SURF

Keywords: devaluation, iDisco, alcohol, behavioral

Abstract #21

NEURAL BASIS OF MATERNAL BEHAVIOR

Joyce Mendoza-Navarro; Naomi López-Caraballo & Robert Froemke

University of Puerto Rico - Río Piedras Campus

The peptide hormone oxytocin modulates social behavior, and is crucial for forms of parental care such as parent-child bonding or responding to infant distress calls. Oxytocin also is reported to improve social cognition in autistic persons, and is being studied as a therapeutic drug in a wide range of psychiatric disorders. By recording neural activity from the paraventricular nucleus (PVN), where oxytocin is synthesized, we are able to get insight into how oxytocin is released during maternal behavior. We have been examining the role of oxytocin for a form of maternal behavior known as pup retrieval. When a pup is out of the nest, and the mother retrieves the pup and takes it back to the nest. Ultrasonic vocalizations emitted by isolated infant mice are what prompt the mother to fetch the pup and return it safely to the nest. In contrast, a naïve virgin female mouse does not perform this maternal behavior, but after several days of being cohoused with a mother and her litter, virgins begin retrieving and interacting with pups also. Previous work in our laboratory has shown that oxytocin release accelerates the onset of maternal behaviors in these co-housed virgins. What are the factors or social interactions that naturally lead to oxytocin release and neuroplasticity during initial maternal experience? To determine what episodes or social interactions activate oxytocin neurons at PVN, we built a new system to monitor continuous, week-long real-time neural activity while simultaneously video recording. Chronicling these week-long recordings of behaving animals provides us the chance to monitor oxytocin neurons over the entire duration of experience with postnatal pups. This novel hybrid behavioral-neural monitoring system that we have constructed allows us to detect potentially rare but important changes in neural firing patterns that might enable neural and behavioral changes. In our recordings, we observed that after about a day of co-housing, the mother brought pups to the virgin instead of placing them in the nest. This interaction then increased spiking in PVN neurons, and has lead us to hypothesize that the mother is activating the virgin's PVN neurons. In conclusion, by understanding how oxytocin release modulates maternal behavior, potential therapeutic drugs for post-partum depression could be developed.

Acknowledgements: NIH Grant 3R01DC012557-02S1

Keywords: oxytocin; PVN; maternal behavior

Abstract #22

METABOLIC ALTERATIONS TRIGGERED BY THIAMINE DEFICIENCY IN NEURONAL SH-SY5Y CELLS

Francisco Barat Nazario; Gabriela Colon Cosme; Nataliya E. Chorna.

PR-INBRE Metabolomics Research Core, University of Puerto Rico, School of Medicine, San Juan, PR; Department of Biology, University of Puerto Rico, Río Piedras Campus, San Juan, PR; Department of Biochemistry, University of Puerto Rico, School of Medicine, San Juan, PR

Thiamine deficiency (ThD) affects all organ systems, and particularly the cells of the nervous system resulting in neuronal cells loss through several mechanisms that include impairment in carbohydrate metabolism, energy failure, excess levels of free radicals and lactic acidosis. To date, many mechanisms have been proposed to explain the nature and molecular pathways associated with ThD including neurodegeneration. However, all metabolic consequences of ThD remain incompletely understood. Experimentally, ThD is induced by a depletion or decrease of thiamine level using thiamine antagonist – oxythiamine (OT). Human SH-SY5Y cells differentiated into neurons were used in this study. To model the conditions of ThD, cells were treated for 72 h with 1mM OT. GC/MS was used to identify differentially changed metabolites. We detected significant reduction in the levels of intermediates of the tricarboxylic acid (TCA) cycle and amino acids while the level of lactic acid was significantly elevated. Evaluation of changes in canonical pathways was performed using Ingenuity Pathway Analysis. As expected treatment of SH-SY5Y cells with OT triggered impairments in the flux of the TCA cycle and energy metabolism. Detected decreases in L-glutamate along with 5-oxo-proline and glycine limited their availability as intermediates of the γ-glutamyl cycle. In addition, decreases in the contents of L-aspartate, L-glutamate, glycine and fumarate triggered reduction in the flux of the purine nucleotide biosynthesis. Reductions in L-alanine and oxaloacetate limited their availability for pyruvate utilization as well as its conversion into acetyl-CoA due to the inhibition of pyruvate dehydrogenese by OT. Taken together our data suggest that the main metabolical consequences that were not previously identified for ThD in neuronal cells are (1) deficiency in amino acid transport inside/outside the cell due to malfunction of the flux of the γ-glutamyl cycle; (2) accumulation of reactive oxygen species due to impairment of the γ-glutamyl cycle and its linked pathway - biosynthesis of glutathione; (3) development of lactic acidosis due to impaired pyruvate utilization, via carboxylation to oxaloacetate and transamination to alanine that will lead to its conversion mostly into lactate increasing lactic acidosis.

Acknowledgements: This work was supported by INBRE-PR NIH Grant 8P20GM103475

Keywords: Thiamine deficiency; neuronal cells; metabolism

Abstract #23

THE EFFECT OF XYLAZINE ON THE INTRINSIC EXCITABILITY OF RAT PREFRONTAL CORTEX PYRAMIDAL CELLS

Priscila Sanabria[1]; Katiria Colon; Nicole Moreira; Sergio Alegre; Alex Fret; Juan Rojas; Natalia Betancourt; Jovan Ortiz and Carlos A. Jimenez-Rivera[3]

1. Department of Physiology., Universidad Central Del Caribe, Bayamon, PR
2. Univ. of Puerto Rico-Bayamon Campus, Bayamon, PR
3. Department of Physiology., Univ. of Puerto Rico-Medical Sci. Campus, Río Piedras, PR,
4. Department of Biology, UPR, Río Piedras Campus.

Xylazine is a veterinary non-opiate sedative that has been used as an adulterant to drugs of abuse in Puerto Rico since early 2000. The potential addiction profile of xylazine is unknown but users have described increased withdrawal symptoms after intravenous use of heroin and xylazine preparations. Currently, little is known of how xylazine acts in the CNS in humans and in particular the prefrontal cortex (PFC). The PFC is an important brain area for decision and working memory, and is also part of the neuronal circuits implicated in the initiation and development of addiction. Evidence in recent years has pointed to significant alterations of intrinsic properties of neurons upon exposure to drugs of abuse. In this study we tested, using whole-cell recordings, the effects of xylazine on the intrinsic excitability of ex vivo pyramidal cells from 300 μm coronal slices of rat medial prefrontal cortex. Layer V pyramidal cells in male and female Sprague-Dawley (100-150g) rat prefrontal cortex were visually identified by their shape and presence of apical dendrites and confirmed by biocytin staining. These pyramidal cells were intentionally targeted because they possess projection fibers to subcortical structures involved in the generation of behavioral responses. Pyramidal cells were injected in current-clamp mode with 1s current pulses from -100 to 350 pA with 50 pA steps, with an intertrial interval of 2 s. Cells were grouped in two major types based on the firing pattern evoked by current stimuli: regular spiking and inactivating burst types. The effect of xylazine was evaluated at 10 and 100 μM. The results showed that 5 min superfusion with xylazine produced an increase in the DC gain of regular spiking neurons as indicated by a increase in action potential frequency (higher f-I slope). This result was not observed for the inactivating burst type cells. Future studies will include assessment of fast, medium and slow afterhyperpolarizations, which are affected by changes in intrinsic excitability. These results constitute the first efforts to understand the effect of this adulterant at the level of the neuronal circuits involved in addiction.

Acknowledgements: 5 G12 RR 003035-27 (PS); SC1GM084854-06 & (NSF) OISE-1545803 (CAJR).

Keywords: xylazine, cocaine, pyramidal neurons, prefrontal cortex

Abstract #24

EXTINCTION OF MORPHINE PLACE-PREFERENCE AND NEUROPLASTICITY TRANSCRIPT PROFILE OF THE VENTRAL STRIATUM

Lloret Mario; Barreto-Estrada Jennifer; Martinez Freddyson

School of Medicine of the University of Puerto Rico

The main objective was to characterize brain injuries among OEF/OIF/OND VACHS veterans using functional Nuclear Medicine neuro-imaging. Aims: (1) Describe size and location of TBI lesions using Tc99m-ethyl-cysteinate-dimer-SPECT/CT and F-18-Fluorodeoxyglucose-PET/CT; (2) Assess symptomatology; (3) Estimate association between brain perfusion and metabolic impairment versus symptomatology; (4) Create a socio-demographic and health-characteristics profile of Puerto Rican Hispanic veterans diagnosed with TBI. Methods: This was a retrospective-descriptive study. CPRS records of all subjects that underwent a SPECT/CT and/or PET/CT for TBI during 01/01/2007-03/30/2012 were reviewed. Objectives 1, 2 and 3 variables included: results of SPECT/CT, PET/CT, CT and MRI, trauma mechanism, symptoms, motor FIM score, Barthel Index, Cognistat Assessment and Mental Status Exam. Objective 4 variables included: age, gender, marital status, race/ethnicity, income, education, TBI severity, comorbidities, hospital admission, bed days of care, Occupational/Physical/Speech Therapy use, clinic nurse/doctor visits and prosthetic device use. Results: 150 records were eligible for the study. Preliminary results on the first 100 records analyzed (94%-males, average age-40 years/old, 73%-mild TBI, 80%-White/Hispanics, 9%-Black/Hispanics and 11%-no reported race/ethnicity) showed that the most common physical, cognitive and psychological symptoms were headaches, forgetfulness and irritability, respectively. 96 subjects had only SPECT/CT, 4 had only PET/CT and 3 had both. 39% of the SPECT/CT studies were abnormal. Most common location for TBI lesions was the frontal lobe. 51% did not have MRI or CT and 49% had CT and MRI all with normal results or showing minor abnormalities. Socio-demographic trends showed that 76% of veterans received some level of college education (51% did not graduate), 76% were married, and the average annual-income was $27,949. Service utilization during the first year after TBI diagnosis confirmation showed: hospital/admission-31%, average bed days of care-15, Occupational/Physical/Speech Therapy usage-88%, 85% and 46% respectively, average visits/year to providers-53, and prosthetics usage: eyeglasses-73%, dressing equipment-45% and bath equipment-51%. Implications: SPECT/CT and PET/CT have an add-value in the diagnosis of TBI. The study provided a profile of Post-deployment population of Puerto Rican veterans with TBI. Impacts: This line of research provides the basis to develop new predictive TBI model-systems and proposing algorithms to target rehabilitation interventions.

Acknowledgements: This material is the result of work supported with resources and the use of facilities at the VA Caribbean Healthcare System. The contents of this presentation do not represent the views of the Department of Veterans Affairs or the United States Government.

Keywords: Traumatic Brain Injury (TBI), Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET), Neuroimaging

Abstract #25

EFFECTS OF DOPAMINE RECEPTOR BLOCKING ON DECISION MAKING ON APIS MELLIFERA CAUCASICA

Janpierre Alemán[1]; Jenny Acevedo[1]; Darimar Loubriel[1]; Meredith Johnson[3]; Olivia Niedzialek[2]; Peter Cruz[1]; Nadiyah Folks[2]; Sarah Anderson;[3,4] Dillon Travis[3]; J.L. Agosto-Rivera[1]; Devrim Oskay[2] Charles I. Abramson [3,4] & Tugrul Giray[1]

1. Universidad de Puerto Rico, Recinto de Río Piedras
2. Namik Kemal Universitesi
3. University of Central Oklahoma
4. Oklahoma State University

Dopamine receptors are responsible for many neurological processes including pleasure, motivation, learning and memory. To understand and evaluate how these neuromodulators can control decision making we used foragers of Apis mellifera caucasica and let them forage on an artificial "flower patch" of white and blue "flowers" with long and short pins that simulate stamens. These "flowers" contained different concentrations of sucrose solution (0.5M in short pins/ 2M in long pins) in order to test how blocking their dopamine receptors, using a flupentixol/coconut oil mix, would affect their choice of work and reward. We found that untreated bees took the expected route. They preferred to forage in the high reward "flowers", even though this meant navigating the significantly harder long pins. On the other hand when bees were exposed to the flupentixol/coconut oil mix their behavior shifted drastically. They were more likely to forage in easier "flowers" even though these contained significantly less reward. This could be due to the flupentixol blocking the subject's dopamine receptors and effectively lowering the individual's motivation for navigating the harder long pins even though doing so would provide a better reward. This and subsequent experiments provide can provide a better understanding of how dopamine receptors affect an individual's motivation and decision making not just In bees, but also in humans.

Acknowledgements: We thank the honey bee technicians at the Namik Kemal Universitesi bee yard for maintaining all hives used to make this experiment posible. We also thank the National Science Foundation for NSF-REU Award #1560389 and NSF-PIRE #1545803.

Keywords: Apis mellifera caucasica; dopamine; flupentixol; flower patch

Abstract #26

HYPERPOLARIZATION-ACTIVATED CATION CURRENT (IH) CHANNEL SUBUNITS HCN2: ROLE ON THE DEVELOPMENT AND EXPRESSION OF COCAINE BEHAVIORAL SENSITIZATION

Victoria Encarnación López; Karl Y. Bosque Cordero; Rafael Vázquez Torres; Ana Vaquer Alicea; Cristina María Ríos; Alan Montiel Ramos; Bermary Santos Rivera; Carlos A. Jiménez Rivera

University of Puerto Rico - Río Piedras Campus

Cocaine abuse alters the Mesocorticolimbic system (MCL); a set of interconnected brain regions responsible for regulating pleasure, motivation and reward. Neuroadaptions induced by cocaine can modify neuronal excitability in the MCL system and can be involved in the development and expression of cocaine sensitization. The cocaine behavioral sensitization model is characterized by the progressive escalation of psychomotor response as a result of repeated administration of the psycho-stimulant drug. The hyperpolarization-activated cation current (Ih) has a potential regulatory role in neuronal excitability and is subjected to the expression of Hyperpolarization-Activated Cyclic-Nucleotide gated channel (HCN). Ih may play a significant role in the behavioral responses to cocaine. We focused our study on the total protein expression of the HCN2 channel subunits in four regions of the MCL system: Ventral Tegmental Area (VTA), Hippocampus (HIP), Nucleus Accumbens (NAcc), and Prefrontal Cortex (PFC). Previous investigations conducted in our laboratory demonstrated that HCN2 subunits expression, was increased in the VTA, NAcc, HIP, and PFC after the development of cocaine sensitization. After a withdrawal period, normal total protein expression of the HCN2 subunit is reinstated in the VTA, NAcc, and PFC, whereas in the HIP the HCN2 subunit expression is decreased. In the present study we explore the initial changes in the expression of the HCN2 subunit at two different timepoints: after an acute cocaine injection and following two consecutive cocaine exposures. Sprague Dawley male rats (250g) received intraperitoneal cocaine (15mg/kg) or 0.9% saline injections for one or two days, and locomotor activity was recorded for one hour. Afterwards, rats were sacrificed and tissue micro-punches from VTA, NAcc, HIP and PFC were collected and subjected to protein extraction and western blot analysis. Our preliminary results show that acute cocaine injections do not induce changes in total protein expression of the HCN2 subunit in the PFC and HIP. Protein expression of HCN2 in the VTA and Nacc after an acute injection are still being explored. After two days of cocaine injections, a significant increase in HCN2 subunit total protein expression was observed only in the VTA. There were no significant changes in HCN2 subunits expression in the HIP, PFC, or NAcc regions. Future studies will continue to help characterize the sequential changes this subunit undergoes after cocaine exposure and how it participates in the addictive process.

Acknowledgements: This project was supported in part by grants from NIHGM (2CSC1GM084854-05A1), the NSF-PIRE (OISE-1545803), the National Center for Research Resources (5R25GM061838-15, 2G12-RR003051) and the National Institte on Minority Healt and Health Disparities (8G12-MD007600) from the National Institue of Health.

Keywords: hyperpolarization-activated cation current (Ih), HCN2 subunits, mesocorticolimbic system

Abstract #27

ANALYSIS OF CONVOLVULACEAE CIRCADIAN RHYTHM AND SYSTROPHA VISITATION RATES

Loubriel Grajales, D.[1]; Johnson, M.[2]; Niedzialek, O.[3]; Hranitz, J.[4]; Giray, T.[5]; Barthell, J.[6]; Agosto Rivera, J.[7]; Gonzalez, Vh.[8]

1,5,7 University of Puerto Rico at Río Piedras, 2. Dickinson College; 3. Bard College, 4. Bloomsburg University of Pennsylvania, 6 University of Central Oklahoma, 8 University of Kansas

Spiral-horned bees belong to the genus Systropha and are morphologically and biologically interesting organisms. Based on previous data, one can hypothesize a strong association of Systropha with Convolvulaceae because Systropha are thought to be oligolectic on Convolvulaceae flowers, mainly on species of Convolvulus with tricolporate pollen grains. However, despite these morphological divergences in the host plants, the available data suggest an association between Systropha and Convolvulaceae flowers. The comparison between Systropha and Convolvulaceae is studied to understand the circadian rhythm of Systropha. Five 1 x 1 meter patches of Convolvulus were selected at Namık Kemal Üniversitesi in Tekirdağ, Turkey. The visitation numbers of four categories of bees, Systropha planidens, Systropha curvicornis, Apis mellifera, and "other bees", were recorded in each quadrat for two minutes. Although Convolvulus opened at approximately 7:00 and closed by 14:00, the nectar levels occurred in a smaller time frame, peaking at 8:00 and zeroing approximately at 11:00. Systropha exhibited peak visitation levels that lasted from approximately 9:00 to 11:00. The closing of the flowers was not related to a decrease in light intensity or temperature, which were at peak levels at that time. This finding suggests that closing time is determined by another environmental factor or by the intrinsic clock of the flower. Meanwhile, the peak of Apis mellifera visitations varied on each day of the study. The early visitation time of Systropha may be to avoid competition with other bees for the nectar from Convolvulus. Light intensity increases as nectar volume decreases. The correlation of temperature and nectar volume presents a negative relation between temperature and the nectar volume. Light intensity increases as nectar volume decreases. We can infer that light intensity is an influential factor in Convolvulaceae rhythmic behavior.

Acknowledgements: The authors would like to thank Olivia Niedzialek, Melina Pérez Torres, Alexander M. Melendez Moreno, Janpierre Alemán Ríos, Ana Mosier and Bree Cooper for assistance in bee and data collection utilized in these experiments. We especially will like to thank John Barthell for his input support, and insights on solitary and generalist bees.
This work is supported by the National Science Foundation's REU program (DBI 1263327).

Keywords: Convolvulaceae, Systropha, Circadian rhythm, visitation

Abstract #28

PUMILIO REGULATION OF SLEEP HOMEOSTASIS IS FACILITATED THROUGH EIF4E INHIBITION

Richard Nieves; Luis de Jesus; Jose Luis Agosto

University of Puerto Rico-Río Piedras Campus

The eukaryotic initiation factor 4E (eIF4E) is a core component of the translation machinery that mediates the initiation of cap-dependent translation. Recent studies have shown that eIF4E is important for synaptic homeostasis and that its overexpression leads to exaggerated translation of synaptic proteins and increased spine density. More importantly, synaptic translation of eIF4E is directly suppressed by the translational repressor Pumilio (Pum) in both Drosophila and rodent models. Previous studies in our lab has shown the role of pumilio in sleep homeostasis. However, it is unknown if eIF4E-mediated synaptic translation is triggered by sleep deprivation and whether it plays a role in sleep homeostasis. We hypothesized that knockdown of eIF4E within circadian wake-promoting neurons will decrease synaptic translation and increase sleep recovery. To test this hypothesis we manipulated the expression of eIF4E in timeless (tim) circadian neurons, and examined its effects on sleep recovery after chronic sleep deprivation in Drosophila melanogaster. The results showed a significant reduction of sleep rebound in flies overexpressing eIF4E. These results suggest that synaptic translation is a core component of the sleep homeostatic system.

Acknowledgements: The authors would like to thank the University of Puerto Rico Río Piedras Campus for providing the facilities and equipment for the experiment to develop. Also, would like to thank the lab team for the support and interest they gave throughout the experiment.

Keywords: Pumilio, Translation, eIF4E

Abstract #29

MECHANISM OF EXTRACELLULAR ZINC INDUCED RELEASE IN NEUROHYPOPHYSEAL TERMINALS

Sonya Malavez-Cajigas[1]; Edward Custer[2]; Sonia Ortiz-Miranda[2]; José Lemos[2]

1. Department of Biology, University of Puerto Rico Río Piedras, San Juan, Puerto Rico
2. Department of Microbiology and Physiological Systems, University of Massachusetts Medical School, Worcester, Massachusetts

Zinc is a trace metal with many important roles in the human body. Recently it has been shown that it is important for processes in the nervous system including at synapses modulating neurotransmission and plasticity. Studies demonstrate that, among other receptors, there is a G-coupled protein receptor known as GPR39, that when it interacts with zinc triggers intracellular calcium release. This receptor has been evaluated in different cell models and based on calcium responses produced, it has been determined that GPR39 desensitizes during a long zinc exposure and that works at its best when exposed to a pH of 7.4. Because zinc and this receptor have been found in terminals from the neurohypophysis (NH) or posterior lobe of the pituitary and its mechanism is not well understood, we studied its role in oxytocin (OT) and vasopressin (AVP) release. Experiments evaluated possible desensitization, pH effects, and importance of extracellular calcium on OT and AVP release. Both OT and AVP are hormones that can be found stored and when necessary released at the NH. These hormones play important roles, such as water and sodium balance, in the human body. OT is also important for sexual behavior and female and male reproductive physiology. Furthermore, both hormones have important roles in social behavior, possibly mitigating diseases such as ASD. For this purpose, we isolated neurohypophyseal terminals (NHT) and perfused them to evaluate OT and AVP release when induced by extracellular zinc. Hormone release was determined with an ELISA that is selective for OT vs. AVP. Results demonstrated that there is both a decrease and an increase of hormone release when pH is altered, showing that it is pH-dependent. Extracellular calcium is not necessary for zinc-induced AVP or OT release. Finally, that there is a decrease in both AVP and OT release when NHT were exposed multiple times to zinc, suggesting that GPR39 is involved in the mechanism of action.

Acknowledgements: This project was made possible by NIH Neuroscience Research Opportunities to Increase Diversity (NeuroID) Program Grant: NIH 1R25MH092912-01, NIH grant 1R01 NS093384-02 to JRL & SOM and the Summer Research Fellowship Program at UMMS.

Keywords: Vasopressin, Oxytocin, Neurohypophysis, Zinc

Theme: Motivation and Emotion

Abstract #30

OPPOSING INHIBITION IN PRELIMBIC PREFRONTAL NEURONS IMPAIRS ACTIVE AVOIDANCE

Diehl, Maria M.; Bravo-Rivera, Christian; Rodriguez-Romaguera, Jose; Pagan-Rivera, Pablo; Lozada-Miranda, Valeria; Quirk, GJ.

University of Puerto Rico, Medical Sciences Campus, Departments of Psychiatry and Neurobiology & Anatomy, San Juan, PR.

There is increasing interest in the neural mechanisms of active avoidance. We previously found that pharmacological inactivation of prelimbic prefrontal cortex (PL) impairs the expression of platform-mediated avoidance in response to a conditioned tone (Bravo-Rivera, et al., 2014). However, optogenetically silencing PL using Archaerhodopsin (AAV-CaMkIIa-eArchT3.0-eYFP) during the tone did not impair avoidance (Diehl et al., 2015 SFN abstract), suggesting that excitatory PL activity may not be necessary for avoidance. Following up on our previous results (Bravo-Rivera, et al., 2014 SFN abstract), we recorded 329 PL neurons during avoidance expression and found that excitatory tone responses did not correlate with avoidance, as similar proportions of excitatory responses were observed in avoidance-conditioned and naïve rats in the same environment (13.4% in avoidance group (n=44/329); 12.6% in naïve group (n=11/87). Instead, we found that inhibitory tone responses were correlated with avoidance (13.4% in avoidance group (n=44/329); 3.4% in naïve group (n=3/87), $p<0.01$, Fisher Exact test). Neurons showing inhibitory responses were classified as putative pyramidal neurons, based on low baseline firing rate (<15 Hz) and broad spike width (>225 μs; see Sotres-Bayon, et al, 2012 for classification method), and had an average baseline firing rate of 3.86 Hz. Most neurons (n=39/44) returned to baseline firing rate 15 sec after tone onset, and were confined to rostral PL (rPL). To test whether PL inhibitory tone responses are necessary for avoidance, we used Channelrhodopsin (ChR2; AAV-CaMkIIa-hChR2(H134R)-eYFP) to oppose the inhibitory responses by stimulating at 4 Hz throughout the 30-sec tone. Photoactivation of rPL at 4 Hz impaired avoidance compared to eYFP controls (ChR2=36.31% time on platform n=10, eYFP=87.46% n=11, $p<0.01$). In contrast, no effect was observed with photoactivation of rPL at 2 Hz, or with photoactivation of caudal PL. Interestingly, avoidance was most impaired during the first 15 seconds of the tone, when inhibitory responses were greatest. The present findings suggest that prolonged inhibitory tone responses in rPL are essential for initiating and in some cases maintaining the expression of active avoidance. Inhibitory responses in rPL neurons could serve to disinhibit striatal neurons to drive avoidance, similar to prefrontal disinhibition of striatum during cost-benefit decision-making (Friedman et al., 2015).

Acknowledgements: NIH grants R37-MH058883 and P50-MH086400 (Conte Center) to GJQ; F32-MH105185 to MMD

Keywords: prefrontal cortex, fear, optogenetics, channelrhodopsin

Abstract #31

PKCI/Λ AND PKMZ PROTEIN EXPRESSION IN MESOCORTICOLIMBIC AREAS DURING COCAINE BEHAVIORAL SENSITIZATION

Vaquer-Alicea, Ana[1]; Ortiz-Ithier, Tara[2]; Dominguez-González, Tania[3]; Vázquez-Torres, Rafael.[1]; Sacktor, Todd [4]and Jiménez-Rivera, Carlos[1]

1. Department of Physiology, Medical Sciences Campus, University of Puerto Rico, San Juan, P.R.
2. Department of Natural Sciences, University of the Sacred Heart, San Juan, P.R.
3. Department of Biology, Río Piedras Campus, University of Puerto Rico, San Juan, P.R.
4. Neural and Behavioral Science, State University of New York Downstate Medical Center, Brooklyn, New York

Cocaine addiction induces long-lasting alterations in the mesocorticolimbic system, some of which may be mediated by the mechanisms of long-term potentiation (LTP). Persistent phosphorylation by protein kinase M zeta (PKMζ), a brain-specific atypical isoform of PKCζ, mediates late-LTP maintenance. This notion was recently challenged when PKC/PKMζ knock-out mice showed intact LTP and reversal of LTP by infusion of its inhibitor ZIP. This suggested that LTP can develop independently of PKMζ, and ZIP may block another atypical PKC. PKCi/λ is another atypical PKC isoform whose marked homology (88%) to PKMζ makes it a potential candidate for participation in the LTP when PKMζ is not present; and could possibly compensate for PKMζ loss in the knockout mice. Here, we aim to investigate the role PKMζ, and PKCi/λ may play in cocaine-induced LTP, using the cocaine behavioral sensitization model. To this end, we examined the total protein expression profile of PKMζ and PKCi/λ in naïve animals and at different time points of the cocaine behavioral sensitization: 24 hours after an acute exposure, 24 hours after 5 days of cocaine sensitization, and 24 hours after a 7 day withdrawal period following 5 days of cocaine sensitization. Sprague Dawley male rats (250g) received intraperitoneal cocaine (15mg/kg) or 0.9% saline injections for 1 or 5 consecutive days, and locomotor activity was recorded for 1hr. The rats were sacrificed and tissue micro punches of the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC), and hippocampus (Hipp) were subjected to protein extraction and western blot analysis. Results show no significant difference in the expression of PKCi/λ and PKMζ between all four brain areas of naïve animals. After a single cocaine exposure, PKCi/λ protein expression was decreased in the PFC. In cocaine sensitized animals PKMζ protein expression and not PKCi/λ was significantly increased in the NAc and Hipp. After a 7 day withdrawal period, PKCi/λ protein expression was significantly decreased in the hippocampus. These results suggest that PKMζ protein increase in NAc might precede important changes for the expression of sensitization; possibly playing a role in NAc LTP. PKC i/λ protein expression decrease in the PFC after an acute cocaine exposure and after a 7 day withdrawal period may result from a compensation to increased PKMζ activity. Future measurement of phosphorylated PKCi/λ and PKMζ (activated state) will allow us to ascertain their role in cocaine sensitization and LTP formation. This may shed light into the pathological mechanisms of cocaine-related plasticity during the addiction process.

Acknowledgements: This project was supported in part by grants from NIHGM (2SC1GM084854-05A1), the National Center for Research Resources (5R25GM061838-15, 2G12-RR003051) and the National Institute on Minority Health and Health Disparities (8G12-MD007600) from the National Institutes of Health.

Keywords: Cocaine-mediated plasticity, PKMζ,PKCi/λ, Mesocorticolimbic system

Abstract #32

EXTENDING AVOIDANCE CONDITIONING DISRUPTS EXTINCTION LEARNING AND INCREASES PERSISTENT AVOIDANCE

Freddyson J. Martínez-Rivera[1]; Marcos J. Sánchez-Navarro[2]; Gregory J. Quirk[1]

1. Departments of Psychiatry and Anatomy & Neurobiology, University of Puerto Rico, School of Medicine, San Juan, PR.
2. Universidad Metropolitana, San Juan, PR.

Obsessive-compulsive disorder (OCD) is characterized by compulsive urges resembling avoidance of perceived danger, and is treated with extinction-based therapies. OCD compulsions have been correlated with habit formation that can occur following excessive repetition (Gillan et al., 2015), but little is known about the effects of repetition on extinction of avoidance. We used an active avoidance task, in which rats are trained to avoid a tone-signaled foot shock by stepping onto a platform. The tone-shock association is then extinguished by preventing access to the platform with a barrier (Extinction with Response Prevention, Ext-RP). We previously reported that a minority of rats persists in their avoidance following Ext-RP (Rodríguez-Romaguera et al., 2016). A possible factor contributing to persistent avoidance is the development of response habits over extended periods of training. To investigate this, we trained two groups of rats with either 8 days (8d) or 20 days (20d) of avoidance conditioning, followed by four days of Ext-RP and a subsequent test without the barrier. Our preliminary results show that the 20d group was impaired in its extinction of freezing during Ext-RP (repeated measures ANOVA; $F(1, 58) = 17.25$, $p=0.001$). Furthermore, rats in the 20d group showed a higher percentage (75% ± 4.5) of persistent avoidance at test, compared to the 8d group (44% ± 6.8; t-test; $t\ 58 = 4.18$, $p<0.0001$). Together, these results suggest that repeated expression of avoidance-like compulsions could reduce the effectiveness of extinction-based therapies, and suggest that the necessity of better and early clinical interventions for OCD.

Acknowledgements: This work was supported by NIMH Conte Grant P50 MH086400 and R37 MH058883 to GJQ; Science Scholarship UMET to MSN. Authors want to thank Hector Bravo Rivera for his support and ideas. We also thank Coraly Velázquez and Estefania Medina for their technical help.

Keywords: Avoidance, Extinction, OCD, Overtraining

Abstract #33

DROSOPHILA PERSIMILIS' POSTURAL DISPLAY: SIMULTANEOUS DANCE, YOGA AND COURTSHIP FEEDING BEHAVIOR

Mónica Vega-Hernández; Caroline Cecile, Gabrielle Fabre

In this research we measure and describe the Postural Display of Drosophila persimilis males during courtship. Drosophila persimilis courtship is similar to Drosophila melanogaster's in that males exhibit traditionally studied behaviors like wing fluttering, licking, following and touching. However, they differed notably in that Drosophila persimilis performs a distinct Postural Display. The Postural Display entails synchronous, specialized movements of the abdomen, the head, the forelegs, the wings and the mouth. Remarkably, the mouth movement peaks when the male feeds the female a drop of regurgitated food. Employing a variety of techniques to measure vibrations, sound and visual cues, we asked what signals foster copulation. We found that no air borne (sound) signals are generated during the performance. Interestingly, the abdomen produces substrate borne vibratory signals that correlate with female immobility, a signature of receptivity to copulation. Moreover, we found that one of the variables triggering the behavior in males is linked to the nutritional state of the females.

Acknowledgements: Thank you to P. A. Lawrence, B. Hedwig and M. Burrows for their support; the UC San Diego Drosophila Stock Center for flies; T. Bailey and C. Spottiswoode for scientific discussion. The work is supported by the Welcome Trust Investigator Awards to P. A. Lawrence WT096645MA and WT107060AIA.

Keywords: biotremology, drosophila, persimilis, courtship, behavior, abdomen, tremulation, substrate-borne vibrations, female stationary, quivering, feeding, copulation

Abstract #34

NUTRITIONAL OMEGA-3 FATTY ACID DEFICIENCY INTENSIFIES ANXIETY- AND DEPRESSION-LIKE BEHAVIORS AFTER WITHDRAWAL IN RATS SUBJECTED TO INCUBATION OF COCAINE-CRAVING

Serrano-Torres S; Lopez-Alonso AT; Rodriguez-Sosa N; Geste JR; Maldonado-Vlaar CS
University of Puerto Rico - Río Piedras Campus

In recent years, n-3 polyunsaturated fatty acids (PUFAs)—more commonly known as omega-3 (ω-3)—have been gaining attention due to their ability to modify the physiology of several neurotransmitter systems; and thus supporting a potential involvement with cognitive and emotional processes. These PUFA-induced modulatory neurotransmissions may have a direct impact on addictive-related behaviors, drugs' addictive potential, and/or withdrawal symptoms severity. To address this issue, we evaluated whether nutritional ω-3 deprivation from pre-puberty to adulthood would lead to changes in cocaine addictive behaviors. In addition, emotional behavioral alterations of early and late withdrawal after chronic cocaine self-administration were investigated. Male Sprague-Dawley rats (P21) were fed either a standard rodent lab chow (CON) or deficient ω-3 rodent lab chow (DEF). Animals were trained to self-administer cocaine (6 h/day with 0.5 mg.kg/ infusion) paired with a tone-light cue for 8 days. We observed incubation of cue reactivity between ID1 (Incubation Day 1) and ID40 of forced abstinence. Also, anxiety-like and depression-like behaviors were evaluated after 10 weeks of food/only consumption (FO), WD1 (Withdrawal Day 1) and WD35; using the elevated plus maze test (EPM) and the Forced Swimming Test (FST). Interestingly, dietary ω-3 depletion reduced lever pressing activity during the first two days, and diminished movement episodes throughout the self-administration sessions. On ID40, the DEF group had lower cue-induced cocaine-seeking behavior compared to the CON group. Conversely, there was a significant robust incubation of cocaine seeking in CON group at ID40 vs. ID1, in contrast to the DEF group, which was not significant. In the EPM, starting from WD1, the DEF group showed a gradual reduction in the time spent on the open-arm and an increased time spent in the closed-arms. During the FST test, the DEF group demonstrated greater immobility time on WD1 compared to the CON group, whereas no difference was observed in FO or WD35. This study demonstrated that dietary absence of ω-3 could intensify the symptoms of anxiety and depression during withdrawal periods after an extended access of cocaine self-administration. Furthermore, it also suggests that this deficiency can modify sensitivity to rewarding effects of cocaine. We can speculate that these dietary-induced perturbations in PUFA homeostasis can deregulate the dopaminergic and serotoninergic systems, leading to mood behavioral changes and impaired responsiveness to positive events. Future molecular experiments will address this assumption to achieve a more definite conclusion.

Acknowledgements: PRSTRT2016-00157, NIH Grant Numbers 1SC2GM102040-01A1, 8G12MD007583-27, R25GM110513, Title V grant number P031S130068, US dept. of Education

Keywords: Gliomablastoma, Microglia, Invasion

Abstract #35

MOTIVATIONAL AND EMOTIONAL FACTORS IN ADOLESCENTS WITH TYPE 1 DIABETES: THEIR RELATIONSHIP WITH SELF-CARE BEHAVIORS

Marah S. Trifilio-Martínez; Ashley Figueroa-Rivas; Yetzaida Márquez-Torres; Valeria Vilomar-Sanabria; Lisangely Burgos; Martha Quiles-Jiménez; Johanna Rodríguez-Beato; Natasha Ramos-Ayala; Valerie Asencio-Torres; Natasha Santiago-González; Valeria González; Camille Cruz-Martinez; Eduardo Cumba-Avilés

University of Puerto Rico - Río Piedras Campus

Background: Adolescents with Type 1 Diabetes (T1D) have a very strict treatment regimen that includes daily insulin use, frequent glucose monitoring, meal plan guidelines, and a regular exercise routine. Research suggests that motivational and emotional factors may interfere with their ability to comply with self-care recommendations to achieve glycemic control and improve health. Objective: We examine the relationship of motivational and emotional factors with self-care behaviors presented by adolescents from Puerto Rico with Type 1 Diabetes and depressive symptoms. Method: Participants were 51 Latino youth (29 women) aged 12-17 years old, who enrolled in a depression treatment study conducted at the University of Puerto Rico (IRB#1112-005). Among the measures completed by adolescents were the following: Children's Depression Inventory, Beck Anxiety Inventory, Self-efficacy for Diabetes Scale, Escala de Autoeficacia para la Depresión en Adolescentes, Diabetes Social Support-Family, and Diabetes Quality of Life-Youth. Clinical evaluators (graduate students) rated adolescent depression using the Children's Depression Rating Scale-Revised. We used Pearson product-moment correlations to examine the relationship of motivational and emotional factors with self-care behaviors. Results: Among motivational factors significantly related with T1D self-care in youth was self-efficacy (for depression and for diabetes), anhedonia, and satisfaction with life, with absolute values for coefficients ranging from .24 ($p \leq .05$) to .50 ($p \leq .001$). Clinician-rated youth depressive symptoms ($r = -.29$, $p \leq .05$), and self-reports of adolescents on how did they feel about their family social support behaviors regarding the management of T1D (particularly with insulin use and glucose monitoring) were the main emotional factors related to self-care behaviors (with coefficients that ranged between .38 and .45). All significant correlations observed were in the expected direction. A non-significant correlation was observed between youth self-reports of anxiety and diabetes self-care. Conclusion: Both motivational and emotional factors (particularly those related to family social support with the T1D regimen) appear to play an important role in adherence to self-care behaviors among T1D adolescents from Puerto Rico. Findings suggest that psychosocial interventions that provide skills developing to promote a sense of mastery, combined pleasant activity scheduling and cognitive restructuring, and include strategies to improve family social support with diabetes management, may enhance outcomes related to youth adherence to T1D treatment regimen.

Acknowledgements: This research was supported by the National Institute of Diabetes and Digestive Kidney Diseases of the National Institutes of Health under Award Number R03DK092547 and by Award Number 2U54MD007587 from the National Institute on Minority and Health Disparities.

Keywords: Motivation, Emotion, Type 1 Diabetes, Adolescents

Abstract #36

THE ROLE OF NUCLEUS ACCUMBENS BETA-CATENIN EXPRESSION IN ALCOHOL CONSUMPTION

Roberto A. Aponte-Rivera[1]; Efrain A. Ribeiro[2]; Ezekiel Mouzon[2]; Eric J. Nestler[2]; & Cristina Velázquez-Marrero[3]

1. University of Puerto Rico Río Piedras Campus, San Juan, PR
2. Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY
3. Institute of Neurobiology, University of Puerto Rico Medical Sciences Campus, San Juan, PR

The nucleus accumbens (NAc) is a brain region that is involved in regulating rewarding and aversive behaviors. Dysregulation of β-catenin expression and function in NAc has been implicated in psychiatric diseases such as depression, anxiety, and drug addiction. Recent evidence suggests that β-catenin within the NAc may be involved in the development of alcohol tolerance via alterations in the Wnt/ β-catenin signaling pathway. Therefore, to understand the role of NAc β-catenin on alcohol consumption, we utilized β-catenin floxed mice and viral-mediated gene transfer to conditionally knockout β-catenin expression in the NAc. Based on previous data, we hypothesized that knocking out β-catenin from the NAc would decrease ethanol consumption. To test this, mice were given intermittent access to 20% ethanol in a 2-bottle choice paradigm (IAE), which has been shown to escalate ethanol drinking in a robust manner. We also provided a separate cohort of transgenic mice with a 6-hour 20% ethanol/saline or saline pretreatment via intraperitoneal injections before IAE. We validated the localization of our viral infection to the NAc via confocal imaging of Green Fluorescent Protein expression and confirmed β-catenin knockdown using qPCR. Our results showed that conditional knockout of β-catenin expression in the NAc of mice results in a trend towards reduced ethanol preference. On the first day, ethanol pretreated mice showed a decreased ethanol preference, which disappears over time regardless of β-catenin knockout.

Acknowledgements: This research would not have been possible without the opportunity provided by the Summer Undergraduate Research Program (SURP) from the Icahn School of Medicine at Mount Sinai. Microscopy was performed at the Microscopy CORE at the Icahn School of Medicine at Mount Sinai. Supported by: NIH NIGMS 5P20GM103642 COBRE Center for Neuroplasticity grant awarded to Cristina Velazquez-Marrero, Ph.D. at the Institute of Neurobiology, Old San Juan. NIH 1R25MH092912-01 Neuroscience Research Opportunities to Increase Diversity (NeuroID) program grant awarded to Roberto A. Aponte-Rivera at the University of Puerto Rico Río Piedras Campus.

Keywords: Alcohol, Beta-Catenin, Drinking, Preference

Abstract #37

SEX SPECIFIC EFFECTS OF MORPHINE IN FEAR EXTINCTION IN RATS: POSSIBLE ROLE OF MAPK SIGNALING CASCADE

Ashlie Maldonado; Camille Chardón; Emily Pérez; John Alvarado; Annelyn Torres[2]; Edwin Santini[2]; Dinah L. Ramos-Ortolaza[1]

1. Pontificia Universidad Católica de Puerto Rico, Recinto de Ponce
2. Ponce Health Sciences University, Ponce, P.R.
3. Nova Southeastern University, Ponce, P.R.

Morphine is an opioid commonly used to treat chronic pain. Patients under long term morphine treatment are at risk of developing dependence. Knowing that drug abuse and dependence often co-occur with anxiety disorders, we wanted to determine the extent to which morphine dependence would affect fear responses, as fear is one of the main traits of these disorders. Our previous study showed that morphine dependent male rats display persistent fear to a tone previously associated with a footshock, suggesting an impairment in fear extinction. Interestingly, this effect is sex-specific, as morphine dependent female rats showed normal fear extinction. For our current study, we wanted to explore possible brain mechanisms underlying the observed behavioral profiles. We decided to focus specifically on proteins of the mitogen-activated protein kinase (MAPK) signaling cascade. Previous studies have shown that decreases in phosphorylation of members of this cascade in the hippocampus of morphine-dependent mice are associated with anxiety- and depressive-like behaviors. We, therefore, hypothesized that a similar decrease in phosphorylation levels of MAPK proteins could underlie the impairment in fear extinction in our morphine dependent male rats. In order to test our hypothesis, we dissected the hippocampus of our morphine-dependent animals and performed a series of Western blots for the following MAPK proteins: pERK, pJNK, and p-p38. Preliminary results show different phosphorylation levels of these proteins between morphine dependent animals and saline controls supporting a role of this signaling cascade in the behavioral effects of morphine dependence. Additional studies are being conducted to determine whether significant changes in phosphorylation of these proteins could account for the behavioral differences observed between morphine dependent male and female rats.

Acknowledgements: PUCPR Scientific Research Funds, NSU HPD Research Grant, PHSU Behavioral Core Facility NIH-G12RR003050

Keywords: dependence, withdrawal

Theme: Motor Systems

Abstract #38

LOCALIZATION OF ALLATOTROPIN-LIKE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF BIOMPHALARIA GLABRATA, AN INTERMEDIATE HOST FOR SCHISTOSOMIASIS

Maldonado-Alers, J.; Hernández-Vázquez, A.; Rolón-Martínez, S.; Miller, M. W.
University of Puerto Rico - Río Piedras Campus

Approximately ten percent of the world's population lives at risk of contracting the parasitic disease, schistosomiasis, commonly known as "snail fever". The digenetic trematode worm species Schistosoma mansoni that causes the most common form of intestinal schistosomiasis requires the freshwater snail Biomphalaria glabrata to serve as its primary intermediate host, where it proliferates and develops into its cercariae form that can infect humans. As infection of pulmonate snails by larval trematodes has been shown to alter neuropeptide gene expression, a neural transcriptomics approach was undertaken to determine precursor prohormones that could encode neuropeptides in Biomphalaria. A transcript (1616 nucleotides) was found to encode a putative precursor (316 aminoacids) that could liberate a single copy of B. glabrata allatotropin (GFRMNSASRVAHGYa). For this investigation, an antiserum (rabbit polyclonal) generated against Cys-GFRMNSASRVAHGY conjugated to BSA was used to localize allatotropin-like immunoreactivity in the central and peripheral nervous systems of B. glabrata. Allatotropin-like immunoreactivity was observed throughout the central nervous system (CNS) with distinct neurons and clusters on the ventral and dorsal surfaces of each major ganglion. Allatotropin-like cells of smaller diameter were present on the dorsal and ventral surfaces of the buccal ganglion (18.8 ± 2.79). In addition, dispersed clusters of small diameter cells were observed in the cerebral (14.7 ± 8.84) and pedal ganglia (25.4 ± 18.6). However, in the pleural ganglia no allatotropin-like neurons were present. Within the left parietal (4.00 ± 2.83) and visceral ganglia (2.40 ± 1.02), clusters of small prominent cells were observed. These results suggest that allatotropin could regulate behaviors related to feeding and reproduction that are altered during the course of infection in this host-parasite system.

Acknowledgements: National Institutes of Health (USA): RCMI MD007600.
National Science Foundation (USA): DBI 1337284, and OISE 1545803.

Keywords: Biomphalaria Glabrata, Allatotropin, Schistosomiasis, Central Nervous System

Abstract #39

THE MODULATORY EFFECTS OF CAFFEINE ON THE INTRINSIC PROPERTIES OF SPINAL LATERAL MOTONEURONS: EVIDENCE FOR ITS DEPENDENCE ON ADENOSINE A1-DOPAMINE D1 RECEPTOR HETEROMERS

Rivera-Oliver Marla; Álvarez-Bagnarol Yocasta; Ayala-Santiago Christian; Moreno Estefanía; Seale Garrett; Pérez-Portocarrero Luis; Acevedo-Arús Omar; Casadó Vicent; Ferré Sergi & Díaz-Ríos Manuel

University of Puerto Rico - Río Piedras Campus

Caffeine is a known non-selective adenosine receptor antagonist whose actions include multiple sites within the brain, mostly by binding to adenosine A1 and A2A receptors (A1R and A2AR). It produces similar behavioral effects as other classical psychostimulants including increased motor activation, arousal, and having reinforcing effects related to indirect dopaminergic mechanisms that depend on heteromerization of A1R and A2AR receptors with dopamine D1 and D2 receptors (D1R and D2R) in the striatum, respectively. We recently performed extracellular recordings of ventral nerves from the lumbar cord of mice in the presence of serotonin (5-HT), NMDA and dopamine (DA), which are known to elicit locomotor activity in mammals, and showed that caffeine stimulates motor activity by blocking A1R, by potentiating the ability of dopamine to activate D1R. Also, perforated patch clamp recordings in lumbar cord slices showed that the effects of caffeine are specifically targeted at modulating the intrinsic membrane properties of spinal lateral motoneurons (MNs). We then investigated if these properties of caffeine depended on the existence of A1R-D1R heteromers within spinal MNs. Thus, we proceeded to assess the presence of A1R-D1R complexes within spinal lateral MNs using electrophysiological and histological techniques. Basal concentrations of DA or NMDA perfusion in the presence of synaptic blockers of inhibitory and excitatory neurotransmission depolarized the membrane potential of most MNs reversibly. The addition of caffeine, in the presence of DA or NMDA, significantly depolarized the membrane potential, decreased the action potential after-hyperpolarization (AHP) and increased the firing frequency by 90% of the recorded MNs. Also, we were able to support our theory that caffeine exerts its neuromodulatory effects on the spinal lateral MNs via A1R -D1R heteromers when the perfusion of an A1R agonist before a D1R agonist completely blocked the modulatory effects of the D1R agonist. Finally, potential A1R-D1R heteromer in MNs were localized anatomically through immunohistochemistry and with the use of a proximity ligation assay using antibodies directed toward the A1R and the D1R. Our experiments suggest that the primary target for the neuromodulatory effects of caffeine in the lumbar region of the spinal cord are the lateral MNs and that the excitatory effects produced by caffeine onto this neuronal population is dependent on A1R-D1R heteromers.

Acknowledgements: We would like to thank Dr. Thomas Cleland (Cornell University) for providing the scripts for electrophysiological data analysis. This work is supported by COBRE Center for Neuroplasticity (NIH NIGMS 1P20GM103642), National Institute of Drug Abuse intramural funds, Nikon A1R confocal microscope (NSF DBI-1337284), RCMI (NIMHD 8G12-MD007600), RISE Program (R25GM061151-13) and Spanish Ministerio de Ciencia y Teconolgía (SAF2014-54840-R).

Keywords: Spinal Cord, Caffeine, Motoneuron, Heteromer

Abstract #40

LOCALIZATION OF CAUDODORSAL CELL HORMONE (CDCH)–LIKE IMMUNOREACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF BIOMPHALARIA GLABRATA, AN INTERMEDIATE SNAIL HOST FOR INTESTINAL SCHISTOSOMIASIS

Ramirez, C.; Hernandez, A.; Maldonado, J.; Rolon-Martinez, S.; Miller, MW[1].

1. Institute of Neurobiology, UPR-Medical Sciences Campus
2. University of Puerto Rico, Río Piedras Campus

This parasitic disease schistosomiasis (snail fever) affects more that 200 million people in tropical countries worldwide. The pulmonate snail Biomphalaria glabrata serves as a major intermediate host for Schistosoma mansoni, the trematode worm that causes human intestinal schistosomiasis. It was observed previously that there is a dramatic reduction in the production of eggs in infected snails, and suggested that the parasites are able to redirect energy allocation toward their own transformation and multiplication. However, little is known about how parasitism may alter the reproductive behaviors of B. glabrata. Bilateral clusters of caudodorsal cells (CDCs) are central neurons that primarily control female reproductive processes and behaviors in freshwater snails. In other species, CDCs were shown to control egg-laying by discharging an ovulation hormone termed caudodorsal cell hormone (CDCH). We propose that the reduction in egg-laying observed in infected snails could reflect parasite-induced decreased levels of CDCH. As little is presently known about CDCH in B. glabrata, this study utilized standard immunohistochemical procedures to localize the peptide in the CNS. CDCH-like immunoreactive (CDCH-li) cells were located in specific ganglia and nerves of the B. glabrata. The majority of the cells were located in clusters in the right and left cerebral ganglia (R Ce g., L Ce g.). Some cells were present in the right parietal ganglion (R Par g.) and pedal ganglia (Pd g.). Prominent CDCH-li fibers were observed in the cerebral commissure (C-c.), a known neurosecretory region between the two cerebral hemiganglia. The localization of these CDCH-li fibers is consistent with previous observations in other models, indicating that this hormone is secreted into the circulation and involved in the control of reproductive behaviors. Understanding the localization of CDCH in B. glabrata will contribute to our knowledge of parasite-host interactions in this major biomedical model.

Acknowledgements: Supported by the National Institutes of Health: MD007600 (RCMI), NIGMS MBRS: GM-08224; National Science Foundation: DBI-0932955, HRD-1137725, OISE-1545803, and DBI -1337284; APS STEP-UP Summer Research Fellowship: 1R25DK095492-01.

Keywords: Schistosomiasis, CDCH. Biomphalaria glabrata, CNS

Abstract #41

EFFECTS OF INFLAMMATION ON MUSCLE MECHANICAL PROPERTIES

Genesis Lopez[1]; Shreejit Padmanabhan[2]; Adam Abtahie[2]; Katherine Wilkinson[2]

1 University of Puerto Rico - Río Piedras Campus
2 San Jose State University, CA Department of Biological Sciences

Inflammation is a process by which the body can protect itself from infection. Our lab found decreased sensitivity in muscle sensory neurons that sense muscle stretch following 18 hrs of systemic inflammation induced by an injection of lipopolysaccharide (LPS) (0.5 mg/kg LPS in 200μL sterile saline), a gram negative bacterial coat protein. These sensory neurons are known as muscle spindle afferents and are mechanoreceptors that contribute to the sense of body position in space, or proprioception. Changes in muscle elasticity may affect their function, leading us to hypothesize that systemic inflammation will decrease muscle elasticity. To test this hypothesis, we used an in vitro isolated mouse muscle nerve preparation to compare muscle elasticity between control (200 μL saline) and LPS mice. The extensor digitorum longus muscle and sciatic nerve were dissected and placed into an oxygenated bath. Optimal muscle length (Lo) was determined for each muscle, and stretched to 2 stretch lengths (2.5% and 5% of Lo; 40%Lo/s ramp speed). We measured the baseline tension, peak tension during stretch, and plateau tension before the release of stretch and used the following equation to measure muscle elasticity: $E=(\Delta F/CSA)/(\Delta L/Lo)$. If our hypothesis is supported, we expect to see a smaller increase in tension during stretch and a reduced elasticity in LPS muscles. Decreased muscle elasticity could decrease the mechanical forces on the muscle spindle afferents and prevent the firing of action potentials. These results would suggest a mechanism for the decreased muscle spindle afferent sensitivity we observe following systemic inflammation.

Acknowledgements: This research was supported by the National Science Foundation (NSF). Grant number: IOS1355202 to Genesis V. Lopez. Research Experience for Undergraduates (REU) program in San Jose State University

Keywords: Muscle spindle afferent, Ese, Epe, inflammation

Abstract #42

IDENTIFICATION AND CHARACTERIZATION OF THORACIC NEURAL CIRCUITS IN THE MAMMALIAN SPINAL CORD

Ernesto Cabezas-Bou[1]; Manuel Díaz-Ríos[2]

1 University of Puerto Rico - Río Piedras Campus
2 Institute of Neurobiology, UPR-Medical Sciences Campus

The control of our trunk-related muscles is essential in order to coordinate locomotion in limbed and limbless animals. Limbless animals are able to move via a longitudinal coordinated wave of muscle contractions combined with segmental alternating contractions (half-center neural circuit organization). With the evolution of limbs, trunk neural networks had to now interact with these new limb-related networks and with sensory feedback in order to produce fluid movements. Studies regarding the control of movement in vertebrates have been mostly focused on limb-related neural networks. But the organization of trunk-related neural networks have been largely unexplored. Thus, fundamental questions remain unanswered: Have the trunk neural networks of limbed vertebrates preserved elements of their segmentally -organized limbless ancestors? How is the trunk-related neural circuitry of limbed mammals organized? And, how does this network coordinates motor activity with or without limb-related networks? Studies have shown that the lumbar network entrains the thoracic network during locomotion suggesting a passive role of this trunk-related circuitry. We hypothesize that the trunk-related circuitry can have a principal role during posture and locomotion. Our results show that the thoracic cord coordinates with the lumbar cord during locomotor-like activity displaying a motor output with similar temporal dynamics (parameters). More interestingly, the isolated thoracic spinal cord can produce synchronous or alternating patterns of motor activity independent of lumbar (limb-related) neural networks and the motor output displays much slower temporal dynamics suggesting postural/balance-related control of movement. Alternating activity was elicited in the presence of a high-divalent solution suggesting that these rhythmic output was mostly coordinated through monosynaptic connections. Moreover, the use of blockers for inhibitory neurotransmission (strychnine and picrotoxin) disrupted this rhythmic alternating pattern. These findings support our overarching hypothesis that trunk-related motor output is at least partly produced by a half-center circuit organization which is likely evolutionarily conserved from limbless vertebrates. Further experiments will be directed toward identifying the organization of the trunk-related neural network, and the role of sensory feedback in thoracic motor control.

Acknowledgements: COBRE Program

Keywords: Thoracic, CPG, Circuits, Spinal Cord

Theme: Neural Excitability, Synapses, and Glia

Abstract #43

REDUCTION OF KIR4.1 POTASSIUM CHANNEL EXPRESSION IN DIABETIC MICE: RELEVANCE TO STROKE

D.E. Rivera-Aponte; M. Mendez-Gonzalez; L. Miranda; L. A.Cubano; S.N. Skatchkov; & M.J. Eaton

Universidad Central del Caribe

Diabetics are at greater risk for stroke when compared to non-diabetics and elevated blood glucose concentration during stroke is associated with poor outcome. Astrocytes play a critical role in protecting neurons by maintaining extracellular homeostasis and preventing neurotoxicity through glutamate uptake and potassium buffering. These functions are aided by the presence of potassium channels, such as Kir4.1 inwardly rectifying potassium channels, in the membranes of astrocytic glial cells. We have previously shown that Kir4.1 expression and function is down-regulated in cultured astrocytes grown in high glucose compared to astrocytes grown in normal glucose. The purpose of the present study was to extend these findings from cultured astrocytes to brains of diabetic mice. In this study we used the db/db mouse model of Type 2 diabetes and the heterozygous db/+ control mice. The db/+ control mice have mean fasting blood glucose levels of 96.1 ± 4.8 mg/dl, whereas the db/db diabetic mice have mean 230.6 ± 15.9 mg/dl glucose levels. We first evaluated the protein and gene expression of Kir4.1 using Western blot and q-PCR. We found a 40% reduction of Kir4.1 protein levels and a 30% reduction in Kir4.1 mRNA levels in db/db mice as compared to db/+ control mice (n=3 for all groups). We next examined the consequences of ischemic stroke on performance of a sensorimotor task by control and diabetic mice. We performed a focal photothrombosis in the sensorimotor cortex of db/db and db/+ mice via the Rose Bengal method and evaluated sensorimotor function using the rung ladder walk behavioral test. The rung walk measures sensorimotor function particularly after stroke. After obtaining baseline data, db/db mice and db/+ mice received the focal lesion and 24 hours later their behavioral performance on the rung test was re-evaluated. Limb placement accuracy was rated on a scale from 0 to 6 with 6 being a perfect step and 0 being a total miss of the rung as described by Farr et al., (2006). We found no difference between the ability of control (n=4 for sham; n=5 for control with surgery) or diabetic (n=5) mice to perform on the rung walk prior to the receiving the focal lesion. 24 hours after the surgery, the control (non-diabetic) mice had apparent deficits in placing the right front limb/paw on the rungs. In addition, the diabetic mice had more severe deficits than the control mice in both the right front and right back limb/paw placement 24 hours after surgery. Our results indicate that Kir4.1 channels are down-regulated in brains of Type 2 diabetic mice and this correlates with greater sensorimotor deficits after ischemic stroke.

Acknowledgements: NIH-NIGMS-SC1-GM088019, NIH-NINDS-R01-NS065201, NIH-NIMHD-G12-MD007583, NIH-NIGMS-R25-GM110513, NIH-NIGMS-SC2-GM095410 and Dept. of Education Title V PPOHA P031M105050

Keywords: Diabetes, ischemic stroke, astrocytes, behavior

Abstract #44

SYNAPSIN PKA PHOSPHO-DOMAINS ARE NECESSARY FOR SHORT-TERM SYNAPTIC MEMORY AND CONFERS AN INHIBITORY FUNCTION IN NERVE-EVOKED VESICLE FUSION

Jose Guzman-Gutiérrez; Agustín Gonzalez-Ruiz; Bryan Melendez; Ramón A. Jorquera

Universidad Central del Caribe

Synapsin (Syn), a family of phosphoproteins are found abundantly in neurons of both vertebrate and invertebrate systems. Syn is located in the nervous terminals and regulates the availability of synaptic vesicles by reversible association with the actin cytoskeleton and other vesicles via phosphorylation and dephosphorylation cycle. Syn deficiencies has been found in several mental disorders including Bipolar and Maniac Spectrum Disorders, Epilepsy, and short-term memory deficiencies. Evidence indicates that the development of short-term memory requires the phosphorylation of Syn domains via Protein Kinase Activated by cAMP (PKA) during nerve activity. However, the neurophysiological effect of the PKA phosphorylations of Syn in synaptic transmission and plasticity is not clear. We determined the effects of PKA-phosphorylation domains of Syn in function at Drosophila neuromuscular junction of third-instar larvae. For this, we scrutinize the synaptic transmission of wild type, Syn KO and its neuronal rescues with normal Syn and with a PKA phospho-incompetent mutant. Transgenic animals containing the UAS-Syn constructs were expressed under the control of the neuronal driver elav-Gal-4. Synaptic transmission activity was measured by electrophysiological recording of the post synaptic compartment by two-electrode voltage-clamp. Synaptic responses were evoked by paradigms of nerve stimulation controlled by a programmable stimulator. Data analysis and signal processing was done using Igor Pro and/or Clampfit programs. Statistical and graphical analysis was generated using OriginPro and/or Microsoft Excel. Our work in Drosophila indicates that Syn is required for normal synaptic transmission and short-term synaptic memory. Interestingly, Syn may operate at the last step of vesicle fusion process by inhibiting the fusion via PKA phosphorylation and promoting it in it non phosphorylated form.

Acknowledgements: NIH 1U54NS083924

Keywords: Synapsin, Vesicle, Plasticity, Synaptic Memory

Abstract #45

MORPHOLOGICAL ANALYSIS OF SYNAPTIC COMPETITION DUE TO INCREASED ACTIVITY OF ONE INPUT AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

Francis Reilly Andújar; Nicole Aponte Santiago; J. Troy Littleton

University of Puerto Rico - Río Piedras Campus

Although extensive studies regarding activity-dependent developmental plasticity of neuronal connections have been performed on vertebrate models, the mechanism behind the process of synaptic competition is still unknown. To begin to characterize how structural plasticity is controlled in developing neural circuits, we are using the Drosophila melanogaster neuromuscular junction (NMJ) as a simple model system due to the strong evolutionary conservation of synaptic molecular components and the excellent genetic accessibility to manipulate activity during development. Most larval muscles are innervated by two motorneurons, a Ib "tonic" and a Is "phasic" motorneuron. We have been able to create hyper-excitable activity in single motorneurons by expressing either the heat activated transient receptor potential channel (TrpA1) or the blue light activated channelrhodopsin (ChR2). We then, through the use of immunohistochemistry were able to visualize the Ib and Is motorneurons at muscles 1 and 2 of third instar Drosophila larvae. Through the use of confocal microscopy, we were able to assay how the change in activity of one of the two motorneurons alters the normal pattern of innervation from both inputs. Our analysis reveals changes in the morphology of the NMJ due to increased activity of one input, with only one remaining motorneuron being present, with structural similarities to the typical Ib synaptic terminal. We observe significant increases on the number of "Ib like" boutons, at muscle 1, on two of our hyper-excitable phenotypes (p. values of 0.0229 and 0.0141) and noticeable increases on the other hyper-excitable phenotypes, when normalized to muscle area and compared to our controls. Also, "Is like" boutons have been either greatly reduced or not present in our hyper-excitable phenotypes. Muscle 2 innervation did not appear to be affected by the increased activity of either input. Based on our results, we are testing three possible scenarios by which activity is altering competition at developing NMJs. One possibility is that the increased activity of the Ib neuron is sufficient to outcompete the Is neuron, completely driving its elimination from the muscle. Another possibility is that the increased activity of the Ib motorneuron is leading to excitotoxicity and death of the Ib neuron. As a consequence, the Is motorneuron is changing its morphology to resemble the larger and more extensive innervation typically seen with the Ib motorneuron. Finally, our third scenario is that both motorneurons are undergoing morphological changes due to increased activity of one of the inputs. Overall, these studies indicate that the Drosophila NMJ displays structural plasticity following genetic alterations of excitability in one of the two innervating motorneurons.

Acknowledgements: J. Troy Littleton Lab; The Bernard S. and Sophie G. Gould Fund

Keywords: Drosophila NMJ; Synaptic Competition; Channelrhodopsin; TrpA1

Abstract #46

THE BLACK WIDOW SPIDER VENOM -ALPHA-LATROTOXIN- REVEALS INCREASED RESIDUAL CA2+ SENSITIVITY OF ASYNCHRONOUS MODES OF VESICLE FUSION IN COMPLEXIN NULL

Gonzalez-Ruiz, A.; Jorquera, RA

Universidad Central del Caribe

Synaptic vesicle fusion is a crucial process of neuronal communication determined by the release of neurotransmitter during synaptic vesicle (SV) fusion. The last step of SV fusion is controlled by Complexin (Cpx), an amphipathic soluble protein enriched in the nervous system and in other cellular system with Ca2+ regulated exocytosis. Cpx has a dual function in SV fusion, inhibits spontaneous and promotes the evoked by action potential. Decreased Cpx levels correlate in patients with schizophrenia and Parkinson's diseases, and as well with alcohol exposure during pregnancy or after anesthesia early in newborns, epigenetic risk factors in maniac development. Nevertheless, the role of Cpx in neurological condition is unknown. Cpx deficiency may increases the asynchronous fusion with more uncouple SVs at the terminal. Alternatively, Cpx may decrease the sensibility of the asynchronous fusion to intracellular resting Ca2+. Combining electrophysiology, genetic and chemical manipulation, we study the synaptic transmission at the Drosophila larval neuromuscular junction. We increased intracellular Ca2+ levels with the alpha-Latrotoxin which increases the intracellular resting Ca2+, and the alien ion Sr2+ which promotes the asynchronous fusion as it is slowly cleared from terminals. Here we found that in Cpx null synapses alpha-Latrotoxin and Sr2+ largely promoted the asynchronous SV fusion evoked during single or paired nerve stimulation. Our work shows that there is a high sensibility of the asynchronous SV fusion in Cpx null synapses. Intracellular Ca2+ buffering with permeable BAPTA or EGTA suppressed this phenomenon confirming our findings. The altered Ca2+ sensibility in synapses with Cpx deficiencies may be associated with the synaptic pathophysiology of related mental illness.

Acknowledgements: 1U54NS083924-01, NIH-NINDS and UCC Start-UP

Keywords: Complexin, Synaptic Vesicle, Fusion, alpha-Latrotoxin

Abstract #47

THE HIV SHELL PROTEIN GP120 STIMULATES U87 GLIOMA CELL PROLIFERATION

G. A. Valentin-Guillama[1], S. Lopez[1], J.Perez[1], N. Chorna[2], N. Boukli[1], L. Kucheryavykh[1]

1. Universidad Central del Caribe 100 Laurel Ave, urb, Santa Juanita, BAYAMON, PR 00960
2. Universidad De Puerto Rico, Recinto de Ciencias Medicas San Juan, 00921

Patients infected with HIV are more prone to develop cancers, including glioblastoma brain cancer (GBM). The median survival of GBM patients with HIV is significantly smaller compared to HIV-negative GBM patients, despite that they receive the same treatments. This indicates that HIV infection is associated with more aggressive behavior of tumor and treatment resistance. Taking in account that HIV itself is not found in GBM specimens, the nature of GBM-HIV relationship is not understood. Here we demonstrated that GP120, a glycoprotein found in HIV shell, provides the stimulatory effect on GBM cells growth and chemotherapeutic resistance. The purpose of this study is to reveal the mechanisms GP120-stimulated glioma growth and treatment resistance. U87 glioma cells were used for the investigation. Using viability and cytotoxicity assays, based on trypan blue staining, we demonstrated that U87 cells treated with GP120 show better viability and survival in response to chemotherapeutic treatment with temozolomide (100μM). Proteomics studies combined with western blot and metabolomics approaches revealed activation of glycolysis in U87 cells treated with GP120: up-regulation of expression of enolase 2, pyruvate kinase, hexokinase, and glyceraldehyde 3-phosphate dehydrogenase and increased production of pyruvate have been identified. Additionally, increased protein and lipids synthesis together with reduction of protein degradation has been identified. In a conclusion we can state that GP120 cause activation of glycolysis in U87 cells, resulting in increased protein and lipids synthesis and promoting cell proliferation and resistance to chemotherapies.

Acknowledgements: PRSTRT grant #2016-00157, NIH Grant Numbers: 15C2GM102040, 8G12MD007583-27, R25GM110513, U.S. Department Of Education Grant P315130068

Keywords: Glioma, HIV, GP120, U87

Abstract #48

ROLE OF ENDO/LYSOSOMAL SYSTEM IN SYNAPTIC FUNCTION

Melendez, B.; Santiago, I.; Jorquera, RA

Universidad Central del Caribe

The Endo/Lysosomal System (ELS) is a complex intracellular organization of multiple membranous compartments including the plasma membrane, auto-phagosome, lysosome and the recycling, early and late endosomes. The ELS controls most of the cellular functions where vesicles and/or endosomes traffic and fuse with each other in order to sort and/or modify their cargoes. The ELS includes trans-golgi network (TGN), endoplasmic reticulum (ER), plasma membrane exo/endocytosis and recycling, organelle biogenesis, autophagy and cellular degradation in lysosomes. Even though the ELS is crucial for several cellular processes, its role in synaptic function is unknown. Interestingly, TGN and ER are not present in the presynaptic terminals, however, other components of the ELS have been found. To understand the ELS role at synapses we compared the localization of several proteins of the ELS with others known synaptic protein. For this purpose, we do live imaging of the neuromuscular junction of Drosophila transgenic animals expressing fluorescently tagged proteins. In addition, we are using several animal lines that disrupt or decrease the expression of ELS proteins to scrutinize their effects on synaptic transmission. Our work suggests novel functions for some proteins of the ELS at the plasma membrane and confirms the role of others.

Acknowledgements: 1U54NS083924-01, NIH-NINDS

Keywords: Endo/Lysosomal System, Synapses

Abstract # 49

CHLOROQUINE ALTERS PROTEIN LOCALIZATION AND NEUROMUSCULAR PHYSIOLOGY

Burgos, C.; Melendez, B.; Santiago, I.; Jorquera, RA

1. Neuroscience Department, School of Medicine, Universidad Central del Caribe, Bayamón, PR
2. STEP-UP Program, University of Nevada, Las Vegas and University Gardens High School
3. Program of Physiology and Biophysics, Institute of Biomedical Sciences, Universidad de Chile, Chile

Vesicle Associated Membrane Protein 7 (VAMP-7 or Ti-VAMP), is a SNARE protein insensitive to tetanus toxin and is thought to participates in constitutive membrane fusion in all types of cells. VAMP-7 has been involved in lysosomal and late endosomal fusion. In addition, VAMP-7 has been observed in autophagosomes and multi vesicular bodies, a pathway used during malaria infestation, a worldwide disease. Chloroquine, the most effective treatment for malaria, affects lysosome acidification and alters endosomal protein trafficking. Hence, elucidating the molecular pathway involving chloroquine action will help in understanding malaria treatment. Here we analyze the effects of chloroquine at the muscle of the Drosophila larvae by two-photon imaging of VAMP-7 tagged with GFP. Our work shows that VAMP-7 is localized at the plasma membrane and in intracellular compartments around the nuclei. In addition, VAMP-7 is localized in punctae with different morphology. Chloroquine treatment increases the number and size of VAMP -7 punctate, in turn, it decreases the localization at the plasma membrane. Our result is consistent with VAMP-7 function in intracellular membrane trafficking and lysosome function. Finally, electrophysiological recordings of animals with chloroquine treatment reveal altered neuromuscular physiology. A myopathy induced by acute or chronic chloroquine treatment is discussed.

Acknowledgements: 1U54NS083924-01, NIH-NINDS and STEP-UP University of Nevada, Las Vegas

Keywords: Chloroquine, Endo/Lysosomal System, VAMP7, Drosophila

Abstract #50

VAMP7 DEFICIENCY MODIFIES BASAL SYNAPTIC TRANSMISSION AND IMPAIRS TETANIC AND POST-TETANIC SHORT-TERM PLASTICITY

Santiago, ID; Meléndez B; Littleton, JT; Jorquera, RA.

1. Neuroscience Department, Universidad Central del Caribe, Bayamón, PR, USA
2. Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA, USA
3. Instituto de Ciencias Biomedicas, Universidad de Chile, Santiago, Chile

Tetanus insensitive (Ti)-VAMP or VAMP7 is a v-SNARE found in many if not all Eukaryotic cell types and it is involved in endosome fusion with lysosomes and membranes. In neurons, VAMP7 regulates neurite outgrowth and has been implicated in synaptic vesicle (SV) trafficking and spontaneous neurotransmitter release. However, the relationship between VAMP7 functions in endosome fusion and neurotransmitter release is not clear. VAMP7 gene polymorphisms alter the expression of the protein and have been found in bipolar disorder patients. Nevertheless, the role VAMP7 in neuropathological mental disorder is not clear. Combining available genetics tools, fluorescent imaging and electrophysiology we investigated VAMP7 localization and function at the Drosophila neuromuscular synapses. Null animals (VAMP7-/-) generated by imprecise p-element excision are adult lethal, survive until third instar larva and have larger number of synaptic contacts. However, spontaneous EPSCs and nerve-evoked EPSCs were not increased proportionally to the increased number of synaptic connections. Additionally, VAMP7-/- synapses display decreased tetanic potentiation and abolished short-term synaptic memory. Presynaptic loadings with FM 1-43 dye revealed increased exo/endo-cycling pool of vesicles and altered reserve pool formation in VAMP7-/-. VAMP7-GFP expression displays punctae morphology similar to endosomal and plasma membrane markers at motor neuron soma, nerve terminals and in postsynaptic muscle, indicating localization of VAMP7 to plasma membrane and intracellular compartments in both pre and post synaptic sites. Additionally, VAMP7 also localizes under the presynaptic terminals at the post synaptic site suggesting a participation in retrograde signaling or nerve terminals remodeling. Our work is consistent with the idea that VAMP7 inhibits synaptic transmission at the active zones, fostering vesicle delivery in an activity dependent manner and with a post synaptic role of VAMP7 in nerve-terminal pruning.

Acknowledgements: 1U54NS083924-01 NINDS-NIH and UCC Start-Up

Keywords: VAMP7, Vesicle, STP, Synaptic-memory

Abstract #51

CHARACTERIZATION OF A FMRFAMIDE-GATED CHANNEL FROM BIOMPHALARIA GLABRATA, AN INTERMEDIATE HOST FOR SCHISTOSOMIASIS

Laura Vicente-Rodríguez; Solymar Rolón, MS; Nuria Vázquez, PhD; Joshua Rosenthal, PhD; Mark Miller, PhD

Institute of Neurobiology, UPR-Medical Sciences Campus

Schistosomiasis, "bilharzia" or "snail fever" is a major global concern. It is the second most prevalent parasitic disease and it may affect 240 million people annually. Schistosoma mansoni is one of the trematode species that causes the most widespread form of intestinal schistosomiasis, which may result in abdominal bleeding, hepatosplenomegaly and intestinal damage. Important developmental stages of the Schistosoma mansoni life cycle occur within fresh water snails, specifically from the genus Biomphalaria, the parasite's primary intermediate host. Neuropeptides act on specific receptors to regulate a wide range of cellular functions such as cardiac activity, synaptic transmission and locomotion. Accordingly, their receptors are promising molecular targets for pesticides and parasiticide drug development. In a recent study, the organization and immunohistochemical localization of two B. glabrata FMRFamide precursors was described. However, the role of this peptide in the B. glabrata's neural signaling remains unknownWe hypothesize that a FMRFamide gated receptor triggers a neural response involved in B. glabrata's vital functions such as feeding and sexual behavior and that disruption of this receptor could reduce the snail's ability to survive. Using transcriptome data, full-length cDNAs for a FMRF-amide receptor were amplified by RT-PCR, cloned and sequenced. RNA from these clones was synthesized in vitro and injected in Xenopus laevis oocytes. Protein expression in oocytes was monitored through western blot and channel's properties were characterized using electrophysiology. In future work, the specific location of channel expression within B. glabrata's nervous system will be determined by in-situ hybridization. This study aims to confirm the available data supporting the participation of FMRF-amide in the regulation of vital physiological and behavioral processes in gastropods and will explore the FMRF-amide peptide signaling as target for snail control.

Acknowledgements: This research is supported by: NSF HRD-1137725-Puerto Rico Center for Environmental Neuroscience & MBRS-RISE- R25GM061838

Keywords: Schistosomiasis, Peptide-gated channel, FMRFamide

Theme:Neurodegenerative Disorders and Injury

Abstract #52

A THREE DIMENSIONAL IN VITRO CULTURE PLATFORM FOR NEUROMUSCULAR JUNCTIONS IN A PHYSIOLOGICALLY RELEVANT SETTING

Roy A.J. Brooks Rivera[1]; Onur Aydin[1]; and M Taher Saif[2]

[1] Polytechnic University of Puerto Rico; [2]University of Illinois at Urbana-Champaign

Long term potentiation (LTP) is a key mechanism for learning and memory, its dysfunction is thought to underlie memory loss. By designing an in vitro culture platform where neuromuscular junctions (NMJ) can be studied in a physiologically relevant setting, we can elucidate the mechanisms of LTP in NMJs. A NMJ is a chemical synapse in which the motor neurons send signals to the muscle, and subsequently the muscle contracts. It is at this NMJ location that we desire to study LTP to enable a further understanding of memory. This culturing method could be used to study the stimulation and decay of LTP, resulting in a model that could be used to study memory loss. Using a 3D culture, mouse myoblast (C2C12) derived skeletal muscle, and motor neurons derived from mouse embryonic stem cells (mESC), we could attain a NMJ through the placement of skeletal muscle and motor neurons in a micro-fabricated device. With a 3D culture, we'd be able to closely mimic tissues in a natural setting, and form a microenvironment. To exercise muscle growth in this device, we used a micro-fluidic platform in which we seeded C2C12 and formed a muscle strip. This strip was able to create tetanic force (uN/m) with values of: 0.297, 0.702, 1.053, 1.404, and 2.457 across two days; it is expected that these values reach 10-15 if allowed to compact further. When stimulated it is expected to produce contractions. When used in a neuromuscular network, this technique could be used to study neurodegenerative diseases.

Acknowledgements: Financial support was provided by the NSF under NSF Award Numbers: 0939511 & 1460995 as part of EBICS & EBICS VITA REU, respectively.

Keywords: Microfluidic; Neuromuscular-junction; Neurodegenerative

Abstract #53

HEREDITARY NEUROPATHY ASSOCIATED MUTATIONS IN HSPB1 FAIL TO REGULATE NF-KB PATHWAY

Ken R. Malavé Ramos; Patrick L. Heilman; Stephen J. Kolb

1. Biology Department, University of Puerto Rico, Mayaguez, Puerto Rico
2. Dept. of Pharmacology and Biological Chemistry, The Ohio State University, Columbus, Ohio
3. Dept. of Neurology, The Ohio State University, Columbus, Ohio

Neuroinflammation is one of the contributors of neurodegeneration leading to certain pathological conditions such as Amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and Multiple sclerosis (MS). The Nuclear Factor Kappa Beta (NF-κβ) pathway regulates the expression of pro and anti-inflammatory cytokines, and activation of NF-κβ pathway is associated with p65 translocation from the cytoplasm into the nucleus. The small heat shock protein 27 (HSPB1) is a molecular chaperone that displays neuroprotective properties in many diseases. HSPB1 regulates the activation of NF-κβ signaling by interacting with multiple upstream protein complexes. In HeLa cells, overexpression of HSPB1 reduces TNFα-mediated NF-κβ by binding IKKβ and downregulating its activity. Studies have been conducted on HSPB1's role in the NF-κβ pathways whereas the mutant HSPB1's role in the NF-κβ pathway is still unknown. Our lab has verified these results and further shown that overexpression of wild Type B1 (WTB1) reduces the rate of IκBα degradation. Interestingly, mutations in HSPB1 cause hereditary neuropathy resulting in muscle weakness, and sensory loss. Overexpression of distal Hereditary Motor Neuropathy (dHMN) mutant HSPB1 does not suppress TNFα-mediated NF-κβ activity. In this study, we continued our examination of HSPB1's modulation of this signaling pathway. We monitored the nuclear translocation of p65 in cells overexpressing wild type or mutant HSPB1 by both immunofluorescent and western blotting techniques. We will determine what effects, if any, wild type and dHMN-linked mutant HSPB1 overexpression have on the translocation of p65 in response to TNFα induced stress. We anticipated that overexpression of wild type, but not dHMN-linked mutant HSPB1, will inhibit the translocation of p65 into the nucleus of transiently transfected HeLa cells stimulated with TNFα. Our results show that overexpression of wild type HSPB1 in transiently transfected HeLa cells inhibits p65 translocation into the nucleus when induced with TNFα. Understanding how overexpression of dHMN-linked mutant HSPB1 alters p65 translocation into the nucleus will result in a better understanding of its effects on important molecular pathways, providing critical information for the future development of therapies and treatments for multiple neurodegenerative diseases.

Acknowledgements: I would like to acknowledge Dr. Arthur Burghes' Lab for the use of their microscope, Dr. Stephen J. Kolb for allowing me to conduct research at his lab, Patrick L. Heilman for his guidance and the Summer Research Opportunities Program at the Ohio State University for funding my research.

Keywords: dHMN-linked mutant HSPB1, NF-κβ pathway, HeLa cells, Hereditary Neuropathy

Abstract #54

A BRAIN SLICE MODEL OF GULF WAR ILLNESS ALLOWS FOR MECHANISTIC STUDIES AND THE SEARCH FOR ANTIDOTES

P. A. Ferchmin[1]; D. Perez[1]; M. Carrasco[1]; M. Perez Lassalle[1]; H. A. Martins[2]; V. A. Eterovic[1]

1. Univesidad Central del Caribe, Bayamón, PR
2. University of Puerto Rico, Medical Science Campus, San Juan, PR

Gulf War Illness (GWI) is a neurological syndrome afflicting about 25% of veterans from the Gulf War (1990-1991). The etiology of the GWI are the neurotoxicants to which the veterans were exposed. The relevant neurotoxicants include traces of sarin, pyridostigmine (Py), N,N-diethyl-meta-toluamide (To), and permethrin (Pe). Here diisopropylfluorophosphate (DFP), a surrogate of sarin, was included instead of sarin. The objective of the present project was to study the mechanism of GWI with the aim of developing antidotes to alleviate the GWI. The method used was an ex-vivo model which is faster and less costly than classical in vivo models. The neurotoxicity and neuroprotection were assessed by recording population spikes (PS) from acute hippocampal slices. PSs are the sum of the synaptically elicited axon potentials of a population of neurons. Axon potentials are all-or-none robust responses produced by functional neurons. The loss of PSs is an early event that precedes neuronal death which can be prevented by neuroprotective compounds. Remarkably, the neuroprotective and neurotoxic effects observed in slices have repeatedly been confirmed in vivo confirming the usefulness of this method. All the chemicals implicated in the GWI showed toxicity in this hippocampal slice model. The GWI drugs were tested by application to the slices for 2 hours in μM concentrations followed by 1 hour of the presumed antidotes. GWI drugs decreased the PS in a concentration-dependent manner. However, the application of edelfosine, flupirtine or (1S,2E,4R,6R,7E,11E)-cembra-2,7,11-triene-4,6-diol (4R) restored 80-90% of the initial PSs. Edelfosine is an inhibitor of phospholipase C (PLC-β3), flupirtine is an activator the Kv5.2-7 channels and 4R is a neuroprotective, and anti-inflammatory cembranoid developed by our group. Our data suggest that the pivotal component of the GWI neurotoxicity is the muscarinic over-activation of PLC-β3 and inhibition of Kv5.2-7 channels. In slices, this causes glutamatergic excitotoxicity and inflammation. A chronic in vivo exposure to GWI drugs will likely cause a pathological lingering glutamatergic excitotoxicity, and inflammation later expressed as a neurological deficit. Paradoxically the symptoms of the GWI continue in the absence of the neurotoxic agents indicating that the GWI is perpetuated by a vicious cycle. We suggest that the GWI symptoms could be ameliorated by reactivation of the Kv5.2-7 channels and decreasing inflammation.

Acknowledgements: U54NS083924; 5 G12 RR 003035-28; 8G12 MD 007583-28

Keywords: Gulf War Illness; Edelfosine; Flupirtine, Cembranoid

Abstract #55

FMRP LOCALIZES ADJACENT TO MITOCHONDRIA IN SYNAPTIC SPINES OF RAT HIPPOCAMPAL NEURONS

Nicole M. Cruz Reyes [1,4,] Nikita Mehta [2,4], Han-A Park [3], Paige Miranda [3], Nelli Mnatsakanyan [3], Elizabeth Jonas [3,4]
1University of Puerto Rico, Cayey, PR, 2University of Chicago, Chicago, IL, 3Yale University, New Haven, CT, 4Marine Biological Laboratory, Woods Hole, MA

Fragile X Mental Retardation Protein (FMRP) is a well-studied mRNA binding protein necessary for the repression of protein translation in neurons. In Fragile X syndrome, an X-linked neurological condition (FXS), CGG repeats in DNA shut down transcription of the Fmr1 gene through a process called methylation. As a consequence, FMRP production either stops or is very low, resulting in abnormally high protein synthesis of a large number of synaptic proteins. FXS causes intellectual disability and autistic behaviors, among other impairments. Although the learning deficit experienced by the patients is not well understood, it may result at least in part from the inability to produce Long-Term Potentiation (LTP) of synaptic transmission in the hippocampus and areas of the neocortex. Previous findings suggest that, in addition to its role as an mRNA binding protein, FMRP may be localized to, or associated with, mitochondria and may act similarly to B-cell lymphoma-extra large (Bcl-xL) to protect neurons from stress by increasing mitochondrial efficiency. This mitochondrial effect may also reduce unnecessary protein translation. Our investigation focuses on finding evidence to support subcellular localization of FMRP. Using immunocytochemistry of isolated rat hippocampal neurons we localized anti-FMRP antibody to sites overlapping with the mitochondrial antibody COX IV. Microscopy was performed with a Zeiss confocal microscope and resulting images were analyzed with ImageJ. We find that FMRP appears to be present in puncta throughout the cell, and that it prominently co-localizes with COX IV mitochondria-labeled sites in areas that appear adjacent to, or part of, dendritic spines. Future work will attempt to localize FMRP to a specific subcellular fraction in biochemical experiments and by electron microcopy.

Acknowledgements: (Funding and support): NSF-REU grant DBI-1359230 and NIH-NS045876.

Keywords: Fragile X Mental Retardation Protein (FMRP)

Abstract #56

THE ANTI-INFLAMMATORY ROLE OF 4R CEMBRANOID

W. I. Velez[1]; F. A. Morales-Vias[1]; L. A. Rojas-Colon[1]; M. Lebron-Davila[1]; V. Washington[2]; P. A.

1. Department of Biochemistry., Universidad Central Del Caribe, Bayamon, PR
2. Department of Biology, University of Puerto Rico-Rio Piedras Campus, San Juan, PR

The cembranoid (1S, 2E, 4R, 6R, 7E, 11E)-cembra-2,7,11-triene-4,6-diol (4R) is a natural compound found in tobacco leaves that has been shown to significantly decrease the infarct volume in a rodent model of ischemic stroke. There is evidence that this effect is mediated through alpha 7 nicotinic acetylcholine receptors (a7). The purpose of this study was to determine if 4R treatment decreases inflammatory cytokines and if a7 receptors are involved in this effect. This could explain 4R&8217;s beneficial role in reducing brain damage after stroke. C57BL/6J wild type and a7 knockout (a7ko) mice received 6mg/kg of 4R dissolved in DMSO subcutaneously 90 min before an intraperitoneal injection of 1mg/kg LPS. Two hours later blood was collected and cytokines measured by flow cytometry. An LPS time-curve performed in wild type mice showed that TNFalpha peaked at 1 hr, while IL-6, IL-10, and MCP-1 peaked at 2 hrs. LPS treatment significantly increased the levels of all 4 cytokines in both wt and a7ko mice compared to mice receiving saline. Wt mice receiving either vehicle + LPS or 4R + LPS had significantly lower TNFalpha, MCP-1, and IL-10 cytokine levels compared to those receiving LPS alone. However, in the a7ko mice only the vehicle + LPS group had significantly lower levels of IL-6, TNF, MCP-1, and IL-10 compared to the LPS group. Cytokine levels in the 4R + LPS group were not significantly different from those in the LPS group in these mice. T-test analyses showed significant differences between wt and a7ko mice with regard to IL-6 (3,605 ± 1340 versus 10,021 ± 5,100, respectively) and Il-10 (62 ± 36 versus 863 ± 200, respectively) cytokine levels. These data show that cembranoid 4R lowers LPS-induced TNF, MCP-1 and IL-10 levels by an a7-dependent mechanism.

Acknowledgements: GRANT: U54NS083924

Keywords: Inflammation; Acetylcholine receptor; Cytokine

Abstract #57

MECHANICAL RELIABILITY OF IMPLANTABLE POLYIMIDE-BASED MAGNETIC MICROACTUATORS FOR BIOFOULING REMOVAL

Christian G. Figueroa-Espada; Qi Yang; Hyowon Lee

1. Department of Mechanical Engineering, University of Puerto Rico-Mayagüez,
2. School of Electrical and Computer Engineering
3. Weldon School of Biomedical Engineering
4. Center for Implantable Devices, Birck Nanotechnology Center, Purdue University, USA

Hydrocephalus is a neurological disorder that typically requires a long-term implantation of a shunt system to manage its symptoms. These shunt systems are notorious for their extremely high failure rate. More than 40% of all implanted shunt systems fail within the first year of implantation. On average, 85% of all hydrocephalus patients with shunt systems undergo at least two shunt-revision surgeries within 10 years of implantation. A large portion of this high failure rate can be attributed to biofouling-related obstructions and infections. Previously, we developed flexible polyimide-based magnetic microactuators to remove obstructions formed on hydrocephalus shunts. To test the long-term reliability of these magnetic microactuators, here we evaluate the impact of actuation cycle on mechanical stability of these microdevices. Over 50, 500, and 5000 minutes, 8 devices were actuated at 100 Hz at 37 °C continuously in phosphate buffered solution. By measuring the primary resonant frequency of each device, we were able to quantify changes in the structural integrity of each actuator. On average, the devices showed a drop of 2.15% in resonant frequencies. Although additional evaluations are necessary to ascertain appropriate actuation duty cycles, preliminary results suggest that our polyimide-based devices have good mechanical reliability, which bodes well for our ultimate goal of improving quality of life and care for hydrocephalus using our MEMS-enabled self-clearing catheters.

Acknowledgements: We would like to thank the Summer Undergraduate Research Fellowship (SURF) Office, in special to Vicki Leavitt, SURF's Manager. This research project would not be possible without the support of the Weldon School of Biomedical Engineering and the Laboratory of Implantable Microsystems Research, and it's greatly appreciated. Special thanks to Prof. Jeff Rhoads for letting us use the laser Doppler vibrometer.

Keywords: Magnetic microactuators , hydrocephalus, MEMS, biofouling

Abstract #58

THE ROLE OF THE CANNABINOID RECEPTOR 2 IN REGENERATION OF HAIR CELLS IN THE LATERAL LINE OF ZEBRAFISH LARVAE

Juan Cantres-Vélez [1,2]; Agnes Acevedo-Canabal[1]; Luis R. Colon[1]; Gaurav Varshney[3]; Shawn Burgess[3]; Guillermo Yudowski[4]; Martine Behra[1]

1Department of Anatomy and Neurobiology, University of Puerto Rico, Medical Sciences Campus, San Juan, P.R., 2University of Puerto Rico, Río Piedras Campus, San Juan, P.R., 3National Human Genome Research Institute, NHGRI/NIH, Bethesda, MD, USA,4 Department of Anatomy and Neurobiology, University of Puerto Rico, Institute of Neurobiology, San Juan, P.R.

Hearing relies on the transduction of sound waves by mechanosensory cells called hair cells (HCs), located in the inner ear. This organ is highly conserved among vertebrates and so are HCs. However, higher vertebrates lack the ability to regenerate HCs, while lower vertebrates like birds and fish regenerate them actively during their entire life span. We use the zebrafish (Danio rerio) to identify the genetic players influencing HC regeneration in a superficial sensory organ found in fish and amphibians which is called the lateral line (LL). This organ is composed of discrete sensory patches called neuromasts that are formed by HCs and supporting cells (SCs) and which are highly similar in structure and function to sensory tissues of the inner ear. Previous studies have linked the cannabinoid receptor 2 (CB2) to immunomodulation. In particular, it was demonstrated that activation of CB2 suppresses leukocyte inflammatory migration. Inflammatory responses are an important part of the wound healing and the regeneration process. Therefore, CB2 involvement in regeneration has been repeatedly postulated but remains to be demonstrated. We have generated stable KO zebrafish using the CRSIPR-Cas9 genome editing technology of the cnr2 gene which encodes CB2. Homozygous cnr2-/- animals are viable and completely lacking CB2 receptors. We propose to test the following hypothesis in wild type (wt) and in cnr2-/- animals. Suppression of CB2 signaling increases inflammation and thereby reduces regeneration of HCs. We will specifically kill HCs with a copper treatment in 5 days post fertilization (dpf) wild type and cnr2-/- larvae. We will count the newly generated HCs at 24, 48 and 72 hours post treatment (hpt). Copper treated animals will be subsequently treated with CB2-specific agonist and antagonist to identify the impact of the CB2 activity in regeneration. Establishing the involvement of the endocannabinoid signaling in regeneration of HCs in a sensory tissue will be of tremendous value to the hearing field and move us closer to effective treatments of hearing diseases linked to HC deficiencies. Furthermore, it will better our understanding of regeneration of neural tissues at large with significant implications for neurodegenerative diseases.

Acknowledgements: National Institute of Drug Abuse (R01_DA037924) NIH- Intramural Research Program of the National Human Genome Research Institute (NHGRI) NIH-K99/R00 grant #4 R00 DC009443-02 from the National Institute of Deafness and other communication disorders (NIDCD), Puerto Rico Science technology and Research Trust Fund, NIH- National Center for Research Resources (2G12 -RR003051) and the National Institute on Minority Health and Health Disparities (8G12-MD007600) NSF - HRD-1137725 CREST Puerto Rico Center for Environmental Neuroscience, MBRS-RISE (R25.GM061838)

Keywords: lateral line; regeneration; cannabinoid receptor 2, zebrafish

Abstract #59

TAMOXIFEN EFFECTS ON THE REACTIVE OXYGEN SPECIES SCAVENGING MACHINERY AFTER A SPINAL CORD INJURY

Carrillo, L.G.; Maldonado, W.I.; Colón, J.; Rodríguez, L.A.; Millán, D.N.; Arroyo, Y; Colón, J.M.; Torrado, A.I.; Salgado

1. Department of Natural Sciences, UPR, Carolina Campus, PR 00984
2. Department of Cellular Molecular Biology, UPR, Rio-Piedras Campus, PR 00931,
3. Department of Physiology and Biophysics, UPR, School of Medicine, San Juan, PR 00936

Spinal cord injury (SCI) is a devastating condition that affects 282,000 people in the U.S. SCI triggers a variety of events at the cellular and molecular level, such as apoptosis, demyelination, inflammation and gliosis, generating a non-permissive environment for axonal regeneration and cell survival. Tamoxifen (TAM) is an FDA approved drug that selectively modulates estrogen receptors. Our recent studies had shown that TAM administration after SCI can confer neuroprotection. However, the mechanism by which TAM may be exerting its neuroprotective effect has not been fully elucidated yet. Our study aims to determine if TAM administration after SCI may be having a beneficial effect upon inducing the reduction of reactive oxygen species (ROS) generation after injury. Adult female Sprague-Dawley rats received a moderate contusion at the thoracic vertebrae (T10) with the NYU impactor device. This project consisted of four groups of animals; 1) Sham animals treated with placebo (placed subcutaneously, in the mid-scapular region), 2) Sham animals treated with TAM, 3) Injury animals treated with placebo, 4) Injury animals treated with TAM. To evaluate the effects of TAM at the ROS level, changes in protein expression associated to ROS scavenger system such as, Superoxide dismutase -1 (SOD1), Superoxide dismutase-2 (SOD2), and Catalase were analyzed. Our results showed that TAM administration after SCI does not changes the level of expression of the proteins studied associated to the reduction of reactive oxygen species (ROS) in a significant way. Taken together these results we can conclude that TAM may be exerting its neuroprotective effects after SCI through a mechanism different than the modulation of the ROS scavenging machinery. Making future studies of TAM after an injury of paramount importance in order to establish a specific mechanism of action by which TAM is conferring neuroprotection.

Acknowledgements: COBRE (P20-GM103642), MBRS-RISE (R25-GM061838), RCMI Program (5G12MD007600) and Title-V cooperative (P031S130068)

Keywords: Spinal cord injury, ROS, Tamoxifen, Western Blot

Abstract #60

DEEP BRAIN STIMULATOR

Alondra Jimenez

University of Puerto Rico - Río Piedras Campus

Parkinson's disease (PD) is a disorder of the central nervous system caused by a lack of dopamine, a brain chemical used to send messages to the muscles to make them move properly. The loss of dopamine may cause a variety of movement problems, including: Shaking (tremor), difficulty walking, muscle stiffness or aching slowness of movement, small handwriting and decreased facial expression. People with Parkinson's disease may lose up to 80% of dopamine in their bodies before symptoms appear. In addition, special imaging tests of the brain show that dopamine may decline as much as 10% per year in people with Parkinson's disease. We have taken a neurostimulator and programmed it with artificial intelligence, and adapted it to measure the electrical activity in the biological brain and the tremor will be predicted and a current signal will be applied to stop the tremors before they even start. Instead of stimulating the brain all the time the device will predict when stimulation is needed. As we tested it on the biological brain the most of the tremors were predicted and stopped before they even started, so as a result my project was a success by lowering and almost eliminating all the tremors. Our aim is to permanently or almost eliminate tremors from PD patients. One of our main results was a 96% decreasement in tremors on our biological brain. And as time and technology keeps advancing we can accomplish curing Parkinson's disease permanently.

Acknowledgements: no funding nor support

Keywords: Parkinson, oxydopamine, artificial intelligence, neurostimulator

Abstract #61

HUMAN RABIES IN PUERTO RICO: A CASE REPORT AND REVIEW OF LITERATURE

Noelia Morales Cardona; Dario A. Sanabria Nuñez; Juan L. Perez Berenguer; Dario E. Sanabria Bellassai

University of Puerto Rico - Río Piedras Campus

Rabies is a viral disease that infects the central nervous system. There are two classifications depending on the clinical picture: furious rabies or numb rabies. Both can have hyper salivation, hyperventilation, convulsion and paresthesia. In Puerto Rico, the last case reported of human rabies was in 2003. We report a confirmed case of rabies who died in Puerto Rico in 2015, after a mongoose bite.

Acknowledgements: Recinto de Ciencias Médicas, Academia de Patología de PR, Instituto de Ciencias Forenses de PR

Keywords: Rabies; case report; Central Nervous System

Abstract #62

EFFECT OF A NICOTINIC RECEPTOR BLOCKER IN MURINE ASTROCYTE PRIMARY CULTURE AFTER INDUCED OXYGEN-GLUCOSE DEPRIVATION

Gabriel Sanchez; Vesna Eterovic; Yancy Ferrer

Department of Neuroscience, Universidad Central del Caribe

Stroke is the third leading cause of death in the United States. The most common type, ischemic stroke, is characterized by a reduction or blockage of blood flow to the brain, causing neuronal death. Astrocytes are key players in the multicellular response to brain injury such as stroke. After a stroke, astrocytes around the affected area change to a reactive state and proliferate. This neuroglia subtype isolates the perimeter of inflammation forming a barrier around the affected area called the glial scar, thus inhibiting neuronal growth. This study aims to decrease astrocytic reactivity upon stroke-like conditions, promoting an environment for neurite growth. Among the diterpenoids, the NR1 cembranoid has been shown to decrease astrocyte reactivity in vivo upon neurotoxic insults and has neuroprotective effects against ischemic stroke. Evidence suggests that this neuroprotective effect is mediated by nicotinic acetylcholine receptors (nAChRs). The goal of this study is to determine whether NR1 exerts a direct effect on the reactivity of astrocytes after exposure of these cells to ischemic-like conditions in vitro. We hypothesize that NR1 induces a neuroprotective effect in the central nervous system (CNS) by reducing reactive astrogliosis. In this investigation, we examine astrocyte proliferation and reactivity after an induced astrogliosis, using an in vitro model for cerebral ischemia-like conditions in the presence and absence of NR1. To ascertain the effects of NR1, primary astrocytes were isolated from mouse cortices and grown to confluency. They were then exposed to oxygen-glucose deprivation (OGD) for 6 hours. After this insult, cells were treated for 24 hours with NR1 or vehicle. Glial fibrillary acidic protein (GFAP) and proliferating cell nuclear antigen (PCNA) expression were used as astrocyte markers for reactivity and proliferation, respectively, and detected by immunofluorescence. Preliminary results by immunocytochemical analysis suggests that treatment with NR1 reduces the ratio of GFAP ($p<0.05$) and PCNA ($p<0.005$) expressing cells, which correlates to a decrease in astrogliosis. This study aims to add important information to the current understanding on astrocyte biology and their nAChRs under normal and ischemic stroke-like conditions. Most importantly, it will help us elucidate the mechanisms of action of a new candidate molecule that affords neuroprotection upon ischemic stroke in animal models. The knowledge added from this research will introduce to the panorama of ischemic stroke treatments, a small molecule candidate to afford neuroprotection upon the devastating effects after this injury.

Acknowledgements: Specialized Neuroscience Research Program (SNRP)

Keywords: Ischemia Stroke Astrocytes nAChRs

Abstract #63

HAIR CELL REGENERATION IN THE LATERAL LINE IN ZEBRAFISH IS IMPAIRED BY CRUDE ROOT EXTRACTS OF VALERIANA OFFICINALIS

Santana-Cruz, Alexis E.; Rodríguez-Morales, Roberto E.; Behra, Martine

1. University of Puerto Rico, Bayamón Campus, Bayamón, PR
2. University of Puerto Rico, Medical Sciences Campus, San Juan, PR

Hair cells (HCs) are mechanosensory receptors of both the auditory system and the vestibular system in the inner ear of vertebrates. When the apical cilia of HCs get mechanically deflected by sound waves, these cells are activated, transducing an electric signal via classical synapses with the central nervous system (CNS). This signal transduction is at the heart of the hearing process in vertebrates. HCs are fragile and sensitive to several ototoxic agents (noise, aminoglycosides and metals) and their loss is the leading cause of deafness in humans. HC regeneration does not take place in mammals, but does in lower vertebrates, such as the zebrafish, in the sensory epithelium of their inner ear but also in a superficial sensory organ called the lateral line (LL). Because this structure is superficial, HCs are exposed directly to the surrounding waters and offer an ideal way to assess regeneration. We are interested in finding new waterborne agents that can hamper or promote HC regeneration. Our hypothesis is that the effect of such an agent will be visible if present as a single molecule or as a component of a complex mixture. To test this, we assessed the effects of a crude root extract of Valeriana officinalis (Val) and a single component Valproic acid (VPA) which is a synthetic derivate from one of its main compounds, valeric acid (VA). We exposed five days post-fertilization (dpf) larvae to copper to specifically eliminate HCs and monitored their regeneration for 3 days post-copper treatment (dpt) in the presence or absence of Val or VPA. We found that both treatments were hampering HC regeneration. Next, we asked if this decrease in HC number was due to apoptosis of HCs and/or HCs progenitors. For this, we performed immunohisto chemistry with an antibody against cleaved-caspase 3, which is an important enzyme specifically activated in apoptotic cells. We found an increased number of apoptotic HCs at 48 and 72 hpt only in regenerating NMs of Val-treated larvae. This would suggest that Val is harming specifically nascent regenerated HCs. VPA alone did not have such an effect, suggesting that our components of the crude extract were involved. Identifying such components will not be trivial but our study proves that the effect on HC regeneration can be detected even when applying complex mixtures. This approach will be useful in fast screening of potential new agents interfering with HC regeneration and speed up discovery of new molecules with important roles in this process, providing new long-term tools for tackling deafness.

Acknowledgements: NIH- Intramural Research Program of the National Human Genome Research Institute (NHGRI), NIH-K99/R00 grant #4 R00 DC009443-02 from the National Institute of Deafness and other communication disorders (NIDCD), Puerto Rico Science technology and Research Trust Fund, NIH- National Center for Research Resources (2G12-RR003051) and the National Institute on Minority Health and Health Disparities (8G12-MD007600), NSF - HRD-1137725 CREST Puerto Rico Center for Environmental Neuroscience

Keywords: regeneration, hair cell, zebrafish, valeriana officinalis

Abstract #64

MODEL OF TRAUMATIC BRAIN INJURY USING IMAGING, PHYSIOLOGICAL AND PSYCHOSOCIAL PARAMETERS: THE VA CARIBBEAN HEALTHCARE SYSTEM EXPERIENCE -PILOT STUDY

Molina-Vicenty, Irma L., MD 1, 2,3; Santiago, Michelaldemar., MD 1; Matos, Amílcar 1; Vélez-Miró, Iván, MD 1,3; Motta-Valencia, Keryl, MD 1,3; Borrás, Isabel, MD 1,3; Figueroa Jeanette, MD 1,3; Jones, Gerty, MD 1; Freytes, Magaly, Ph.D 1; Méndez-Villarubia, José, Ph.D 1; Jusino-Suárez, Kathia 1,2; Faris-DeJesús, Verónica 1 & Quijano, Carlos Y. 1

1. VA Nuclear Medicine, VA Caribbean Healthcare System, San Juan PR.
2. University of Puerto Rico, Río Piedras Campus, San Juan, PR
3. University of Puerto Rico, Medical Sciences Campus, San Juan, PR"

The purpose of this study was to measure the size and location of TBI lesions using Tc99m-ECD-SPECT/CT and F-18-FDG-PET/CT in subjects with TBI, while assessing neurophysiologic parameters using Somatosensory-Evoked-Potentials (SSEP). The magnitude of the association between brain perfusion/metabolic impairments and electrical disturbances was correlated with quality of life measures. In addition, the study aimed to describe the psychosocial experiences of Puerto Rican veterans with TBI. Methods: This was a prospective, pilot study to characterize the brain injuries of OIF/OEF veterans. The study population included males and females returning soldiers who were diagnosed with TBI. The participants underwent SPECT/CT, PET/CT, neurological exam and SSEP within 2 weeks of TBI diagnosis confirmation by a Polytrauma expert. Quantitative data on functional status, activities of daily living and depression was obtained using Functional Independence Measure (FIM), Barthel Index and Beck Depression Inventory-II (BDI-II). Qualitative data on the daily activities and experiences were obtained using a semi-structured interview methodology. Results: Six-patients were enrolled in the pilot. The correlation between FIM and the imaging data showed a 0.87 Spearman-coefficient in both SPECT and PET. The correlation coefficient between SPECT and BDI-II and PET and BDI-II was 0.74 and 0.63, respectively. An increased severity and number of perfusion defects compared to metabolic defects were observed. The most common site of perfusion abnormalities was the frontal lobe and of metabolic abnormalities was the temporal lobe. Perfusion and metabolic findings were detected in the presence of negative CT. SSEP showed an abnormally increased Central Time from cervical to cortical response. Implications: The data showed that higher trauma severity is accompanied by greater rates of depression and low level of independence. Damage to the Basal-Ganglia correlated with the presence of severe depression (.89 -Spearman). The mismatch between perfusion and metabolic defects suggested up-regulation of cerebral glucose-transporters/receptors to compensate for diminished perfusion. The etiology of TBI may be related to impaired vasomotor response or endothelial dysfunction. Impacts: SPECT/CT and PET/CT have an add-value in the diagnosis of patients with TBI. A larger clinical trial is required in order to develop new predictive TBI model-systems and proposing algorithms to target rehabilitation interventions in post-deployment population.

Acknowledgements: This material is based upon work supported by SODEINC Inc. with resources and the use of facilities at the VA Caribbean Healthcare System. The contents of this presentation do not represent the views of the Department of Veterans Affairs or the United States Government.

Abstract #65

THE UTILITY OF SPECT-CT AND PET-CT IN THE DIAGNOSIS OF TRAUMATIC BRAIN INJURY AT THE VA CARIBBEAN HEALTHCARE SYSTEM (VACHS): RETROSPECTIVE DESCRIPTIVE STUDY

Frontera-Rodríguez, María, 1,2; Molina-Vicenty, Irma, MD 1,2; Motta-Valencia, Keryl, MD 1; Matos, Amílcar; Quijano, Carlos 1,2; Alonso-Quiñones, Héctor 1,2; Marrero-Barrera, Frances 1,2; Valdés, Sharyl 1; Reyes-Rosario, Coral 1,2; García-Ferré, Alejandra 1,2; and Bezares, Nicolás 1,3

1 VA Nuclear Medicine, VA Caribbean Healthcare System, San Juan PR,
2 University of Puerto Rico, Río Piedras Campus, San Juan PR and
3 University of Puerto Rico, Cayey Campus, San Juan PR

The main objective was to characterize brain injuries among OEF/OIF/OND VACHS veterans using functional Nuclear Medicine neuro-imaging. Aims: (1) Describe size and location of TBI lesions using Tc99m-ethyl-cysteinate-dimer-SPECT/CT and F-18-Fluorodeoxyglucose-PET/CT; (2) Assess symptomatology; (3) Estimate association between brain perfusion and metabolic impairment versus symptomatology; (4) Create a socio-demographic and health-characteristics profile of Puerto Rican Hispanic veterans diagnosed with TBI. Methods: This was a retrospective-descriptive study. CPRS records of all subjects that underwent a SPECT/CT and/or PET/CT for TBI during 01/01/2007-03/30/2012 were reviewed. Objectives 1, 2 and 3 variables included: results of SPECT/CT, PET/CT, CT and MRI, trauma mechanism, symptoms, motor FIM score, Barthel Index, Cognistat Assessment and Mental Status Exam. Objective 4 variables included: age, gender, marital status, race/ethnicity, income, education, TBI severity, co-morbidities, hospital admission, bed days of care, Occupational/Physical/Speech Therapy use, clinic nurse/doctor visits and prosthetic device use. Results: 150 records were eligible for the study. Preliminary results on the first 100 records analyzed (94%-males, average age-40 years/old, 73%-mild TBI, 80%-White/Hispanics, 9%-Black/Hispanics and 11%-no reported race/ethnicity) showed that the most common physical, cognitive and psychological symptoms were headaches, forgetfulness and irritability, respectively. 96 subjects had only SPECT/CT, 4 had only PET/CT and 3 had both. 39% of the SPECT/CT studies were abnormal. Most common location for TBI lesions was the frontal lobe. 51% did not have MRI or CT and 49% had CT and MRI all with normal results or showing minor abnormalities. Socio-demographic trends showed that 76% of veterans received some level of college education (51% did not graduate), 76% were married, and the average annual-income was $27,949. Service utilization during the first year after TBI diagnosis confirmation showed: hospital/admission-31%, average bed days of care-15, Occupational/Physical/Speech Therapy usage-88%, 85% and 46% respectively, average visits/year to providers-53, and prosthetics usage: eyeglasses -73%, dressing equipment-45% and bath equipment-51%. Implications: SPECT/CT and PET/CT have an add-value in the diagnosis of TBI. The study provided a profile of Post-deployment population of Puerto Rican veterans with TBI. Impacts: This line of research provides the basis to develop new predictive TBI model-systems and proposing algorithms to target rehabilitation interventions.

Acknowledgements: This material is the result of work supported with resources and the use of facilities at the VA Caribbean Healthcare System. The contents of this presentation do not represent the views of the Department of Veterans Affairs or the United States Government.

Abstract #66

LOSARTAN REDUCES INFARCT DAMAGE AND DECREASES BLOOD BRAIN BARRIER PERMEABILITY AFTER TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION

Martínez, S; Gonzalez, M; De-Mello, W; Martins, AH

Universidad Central del Caribe

Stroke is the leading cause disability the US and estimated to cost around 34 billion annually. Ischemic stroke is caused by blockade of a blood vessel in the brain with a blood clot and this type accounts for 87% of all strokes. Loss of oxygen and energy failure in neurons causes necrosis that gives rise to inflammatory conditions. There is only one drug currently approved by the FDA to treat ischemic stroke, new treatments are desperately needed. Blockade of angiotensin 2 type I receptor (AT1R) can be one of them. Discovery of AT1R located in neurons confirmed the existence of an endogenous brain angiotensin II system responding to angiotensin II (AT2) peptide. AT2 has been shown to increase inflammation and decrease blood brain barrier (BBB) integrity after stroke, while blockade of AT1R has been shown to have anti-inflammatory properties. As inflammation is responsible for secondary injury in the brain, we hypothesize that blocking AT1R may reduce of ischemic damage in stroke and that this effect is partially mediated through preservation of BBB integrity and decrease of inflammation after ischemic stroke. To verify this hypothesis we used in-vitro and in-vivo models. For in-vitro studies we performed immunofluorescence and western blot analysis to verify the expression of AT1R in mice macrophages (RAW 264.7) and human cerebral endothelial cells (hCMEC/D3) exposed to LPS and angiotensin II for 12 hours. AT1R was detected in macrophages but not in hCMEK/D3. AT1R expression on macrophages was increased two-fold when exposed to AT2 and three-fold when exposed to lipopolysaccharide, confirming overexpression of the receptor and its involvement in macrophage inflammatory response. Although no expression was observed in the conditions used, losartan decreased the size of hCMEK/D3 cells, showing off target effects. For in-vivo studies, male rats were submitted to middle cerebral artery occlusion (MCAO), an ischemic model of stroke and divided in control, Evan's Blue and TTC groups. Losartan or vehicle was injected intravenously through tail vein 5 min. before stroke induction. Evan's Blue 2% was injected intra peritoneal immediately after the surgery. After 24 hr, Evan's Blue and infarct volume size were analyzed. Results show a significant reduction of Evan's Blue dye infiltration and infarct volume between brain slices of rats injected with losartan compared to those injected with vehicle. These results suggest AT1R blockade reduces the blood brain leakage and reduces the size of brain damage after stroke. Further experiments will be performed to elucidate this alternative pathway and to ascertain if effects of losartan continues if injected after the stroke induction.

Acknowledgements: SNRP

Keywords: losartan; stroke;

Abstract #67

PRESERVATION OF NEUROLOGICAL FUNCTION BY 4R-CEMBRANOID FOLLOWING AN ISCHEMIC STROKE

V. Eterovic; W. Castro; H. Martins; B. Cuadrado; Y. Ferrer; G. Grudziak; P. Ferchmin

Department of Neuroscience, Universidad Central del Caribe, Bayamón

4R-cembranoid (4R) is a non-toxic, brain permeable compound that decreases the infarct volume caused by middle cerebral artery occlusion (MCAO) in rats and mice (Martins et al, 2015). This work addresses two questions: 1) Does 4R preserve neurological function after an ischemic stroke? 2) Can somatosensory evoked potentials (SSEP's), measured during occlusion and re-perfusion, predict the infarct volume observed 24 hours after the MCAO? To answer these questions, rats were subjected to 1 hour of MCAO and 1 hour of re-perfusion, followed by a subcutaneous injection of vehicle or 4R (6mg/kg). To answer the first question, SSEP's were evaluated throughout the experiment, and behavior was measured using the Neurological Severity Score (NNS) 24 hours after occlusion. Rats treated with 4R, but not with the vehicle, exhibited reemergence of SSEP's 15 to 30 min after administration ($p<0.01$) and showed an increased neurological function, measured 24 hours after the initial occlusion ($p<0.05$). Rats treated with 4R also exhibited decreased astrocyte reactivity around the infarction area, as measured by GFAP staining ($p<0.05$). These results suggest that one of the mechanisms by which 4R helps preserve neurological function is by decreasing astrocyte reactivity. To address the second question, a scoring system for the SSEP's amplitude measured during the occlusion and re-perfusion was created. These scores were then evaluated and compared to the resulting infarct volume measured by TTC. A negative correlation was obtained when comparing the SSEP score and the infarction volume ($r2$, 0.85; $p<0.001$). These results confirm that 4R not only decreased the tissue damage caused by ischemic stroke, but also promoted a fast re-emergence of neurophysiological function that translated in an increase in neurological function. Our results also confirm that the SSEP score system we developed, used during occlusion and re-perfusion, offers a novel tool to predict tissue damage outcome 24 hours after the initial occlusion.

Acknowledgements: NINDS Specialized Center Cooperative Agreements, award # U54NS083924, NIMHHD AWARD # G12MD007583, Bromedicon, Inc.

Keywords: Stroke, Neuroprotection,

Abstract #68

PF-562271, A PYK2 INHIBITOR, REDUCES GLIOMA TUMOR GROWTH AND INVASION

J. Ortiz-Rivera; K. Rolón-Reyes; A. Méndez; A. Albors; L.A. Cubano; L.Y. Kucheryavykh

1. Universidad Central del Caribe, Bayamón, P.R
2. University of Puerto Rico, Río Piedras Campus, San Juan, PR
3. University of Medicine and Health Sciences, St. Kitts

Glioblastoma is an extraordinarily aggressive type of brain cancer due to its invasive and proliferative nature. The tumor microenvironment, with microglia as a critical player, has an important role in tumor progression. Microglia infiltrate majority of gliomas and release factors, which favor tumor growth and invasion. Previously we demonstrated that microglia residing within the tumor stimulate glioma cell invasion through the proline rich tyrosine kinase 2 (Pyk2) signaling cascade (Rolon-Reyes et al. 2015). We hypothesize that the use of pharmacological inhibitors of Pyk2 together with currently applied glioblastoma chemotherapy can significantly reduce invasiveness of tumor and improve the outcome of treatment. Using C57Bl/6-Gl261 mouse glioma implantation model we investigated the effect of the combined treatment for glioblastoma with temozolomide (TMZ, 50 mg/kg, once/day, orally) together with a Pyk2 inhibitor PF-562271 (twice/daily, 25 mg/kg, orally) vs. TMZ monotherapy. Treatment was provided during 14 days, beginning the 5th day after glioma implantation. For the assessment of the effectiveness of treatment animal survival, tumor size, and invasion area were evaluated. Western blot were used for the evaluation of the level of Pyk2 phosphorylation in tumor cells. For the purification of glioma cells from total tumor tissue Percol gradients were used. The study revealed that treatment with PF-562271 reduced invasion of glioma cells at the tumor edge, while TMZ reduced the tumor growth. Combined treatment showed significantly more prominent effect on reduction of tumors growth compared to TMZ monotherapy and additionally reduced invasiveness of tumors similar to PF-562271 monotherapy. In both PF-562271 monotherapy and combined treatments the downregulation of Pyk2 phosphorylation in glioma cells has been recorded. Survival analysis demonstrated a significance increase of survival of animals received combined treatment compare to TMZ monotherapy. In conclusion, we can state that combined treatment with TMZ together with PF-562271 reduced Pyk2-related tumor growth and invasiveness and increased animal survival.

Acknowledgements: PRSTRT2016-00157, NIH Grant Numbers 1SC2GM102040-01A1, 8G12MD007583-27, R25GM110513, Title V grant number P031S130068, US dept. of Education

Keywords: Gliomablastoma, Microglia, Invasion

Abstract #69

CHARACTERIZING CATHEPSIN B/SERUM AMYLOID P COMPLEX-INDUCED NEURONAL DYSFUNCTION IN A MOUSE MODEL OF HIV-ASSOCIATED ENCEPHALITIS

Alexandra Ogando[1]; Yisel M. Cantres-Rosario[2]; Marines Plaud[2]; Santhi Gorantla[2]; Loyda M. Melendez[2]

1 Department of Biology, University of Puerto Rico - Río Piedras Campus
2 Department of Microbiology and Medical oology, UPR-Medical Sciences Campus

HIV-infected macrophages infiltrate the blood brain barrier to the brain, where they secrete viral and cellular proteins that trigger neuronal dysfunction and death. Despite the efficacy of combined antiretroviral therapy, mild forms of HIV-associated neurocognitive disorders (HAND) remain prevalent. One of the proteins secreted is cathepsin B, a lysosomal protease, which interacts with serum amyloid p component (SAPC) at the extracellular level. Cathepsin B and SAPC secreted from HIV-infected macrophages induce apoptosis of primary rat neurons in vitro, which is decreased by the pre-treatment of macrophage-conditioned media (MCM) with anti-cathepsin B and SAPC antibodies. The pre-treatment of HIV-infected MCM with these antibodies also reduce amyloid peptides in neurons. These two proteins also co-localize with β-amyloid peptides in tissues from HIV-associated dementia (HAD) patients and Alzheimer's disease patients, compared to healthy patients, suggesting a role of the complex in neurodegeneration. We have demonstrated that recombinant active cathepsin B added in MCM is internalized by neurons. Moreover, the levels of cathepsin B internalization are proportional to the levels of HIV infection. Therefore, we hypothesize that targeting cathepsin B/SAPC complex in MCM represents a viable approach to elucidate the mechanism of cathepsin B-induced neuronal dysfunction and test its potential as a candidate for drug development against HAND.To test this hypothesis, we exposed SK-N-SH neuroblastoma cells to histidine-tagged cathepsin B in neuronal culture media alone or in presence of anti-cathepsin B antibody, and localized the histidine tag in neurons by intracellular flow cytometry. Histidine-tagged cathepsin B was internalized by neurons (52.0%)in vitro, a mechanism that was partially reduced by the pre-treatment of the histidine-cathepsin B media with anti-cathepsin B antibody (34.9%). The neuroprotective potential of cathepsin B antibody was confirmed by immunofluorescence. We also sought to determine the presence of cathepsin B and SAPC in the brain of a mouse model of HIV-encephalitis (HIVE), generated by intracranial inoculation of control and HIV-infected MDM. Cathepsin B and SAPC were identified in the striatum of the mice inoculated with HIV-infected MDM by western blot and immunohistochemistry, along with increased expression of cleaved caspase-3 , compared to control animals inoculated with uninfected MDM. Our results reveal a novel mechanism by which cathepsin B triggers neuronal dysfunction, and validate the use of HIVE mice as an in vivo model to test the effectiveness of anti-cathepsin B and SAPC inhibitors against HAND.

Acknowledgements: NeuroID, R24 OD 018546-0, NIGMS SC1GM113691, NIMH G12MD007600

Keywords: cathepsin B, HAND, neurodegeneration

Abstract #70

EARLY EFFECTS OF SIV AT THE PREFRONTAL CORTEX OF RHESUS MACAQUES SHOW A DIMINISHED CONCENTRATION OF POSTSYNAPTIC PROTEINS

Abdías Díaz[1]; Ricardo Torres[2]; Leonardo González[3]; Annabell Segarra[3]

1 Department of Biology, University of Puerto Rico - Río Piedras Campus
2. Universidad Metropolitana, SUAGM, San Juan, Puerto Rico
3. Department of Physiology, UPR-Medical Sciences Campus

Human Immunodeficiency Virus is a lentivirus that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections that may lead to cancer and dead. Although the development of antiviral therapy to treat this disease has increased the rate of survival of these patients, HIV still has dreadful consequences, particularly among neural systems such as HIV Associated Neurocognitive Disorder (HAND). However the mechanisms involved are largely unknown. To address this issue, we studied the brains of females Rhesus Macaques infected with the Simian Immunodeficiency Virus 251 (SIVmac251). Brains were obtained from female macaques sacrificed at early stages post-infection (40 days) and donated to us by Drs. Montaner and Kraiselburd. We hypothesized that brains infected with SIV would show a decrease in synaptic contacts, and an increase in reactive astrocytes and in estrogen receptors in early stages of infection. The Prefrontal Cortex (PFC), an area associated with higher cognition and abstract thinking, was dissected from macaques with a high SIV viral load, a low viral load and SIV free (n=3/group). Using Western Blot quantification, we measured the following proteins: (1) Post-synaptic density 95 (PSD-95) (2) Synaptophysin (a presynaptic protein) (3) Glial fibrillary acidic protein (GFAP) as a marker for astrocytosis and (4) Estrogen receptors (ER)-as a marker for the neuroprotective hormone estradiol- . Interestingly, we observed a decrease in PSD-95 in animals with a high viral load (HVL) compared to SIV free macaques. Macaques with a low viral load (LVL) showed levels between high and SIV free macaques. No changes were observed in the other proteins measured. Our data demonstrates that early stages of SIV infection are associated with synaptic damage, with post-synaptic proteins being more susceptible. These changes antecede changes in behavior or cognition. The lack of change in GFAP supports previous findings indicating that gliosis is present during late stages of HIV infection, those associated with encephalitis. These data partially support our hypothesis, that neural damage can be detected in early stages of SIV infection, and suggests that these changes may be a prequel to the appearance of HAND in more advanced stages of SIV infection.

Acknowledgements: (Funding and support): BP-Endure, Neuro ID, Dr. Annabell Segarra

Keywords: SIV, HIV, Pre and Post-synaptic proteins, Prefrontal Cortex

Abstract #71

THE ROLE OF STB-1 OF MICROGLIA/MACROPHAGE PROLIFERATION AND PHAGOCYTOSIS AFTER AN ISCHEMIC STROKE INDUCED INFLAMMATION

Maxine N Gonzalez-Vega; Solianne Martinez; Dr. Pedro Ferchmin; Dr. Antonio H Martins Baccin

Universidad Central del Caribe

Post-ischemic inflammation plays a critical role in neuronal injury, death, and secondary brain damage. The inflammatory response in the CNS not only activates microglia, but also compromises the blood brain barrier, increasing its permeability, and recruit macrophages from the PNS to the injury site. In murine stroke models these cells change from an inactive form (M0), initially opting an anti-inflammatory (M2) state, and gradually shifting to a pro-inflammatory (M1) state. Not only is the increase of these immune cells responsible for excessive inflammation, but also their phagocytic response. Modulation of these cells towards the M2 state could lead to neuroprotection in ischemic stroke patients by preventing an excessive inflammatory response and reduced phagocytic function after secondary brain damage. Recently, a natural cyclic diterpenoid named Stb-1, was shown to have neuroprotective properties in a rodent temporary ischemic stroke model. This compound reduced the infarct volume produced by the induced occlusion, and improved movement recovery in rats when compared to animals treated with vehicle only. These outcomes after an ischemic stroke could be associated to a decrease in the inflammatory response, which led to the hypothesis that Stb-1 neuroprotection could be associated to an anti-inflammatory effect by reducing proliferation and phagocytosis of these cells. So far, the specific molecular mechanisms of Stb-1 in the CNS inflammatory response after ischemic stroke are yet to be studied. To begin elucidating some of the Stb-1 pathways promoting neuroprotection, in vitro models of inflammation will be used. Proliferation marker PCNA increased when treated with lipopolysaccharide (LPS), inflammation model, compared to the control group. In contrast, Stb-1 did not affect proliferation in the presence or absence of LPS. When measuring phagocytosis in macrophages, Stb-1 did not affect the uptake of the fluorescent particles in cells treated with LPS. We can conclude that Stb-1 does not alter the cells capacity for proliferation and it does not affect their principle inflammatory response of phagocytosis. Future studies will determine Stb-1's role in promoting a polarization in states of these cells using specific markers that determine their functional states (M1 or M2). Addressing the molecular mechanisms of Stb-1 in these cells, which will help us demonstrate the anti-neuro inflammatory potential of this compound, extending its use to other neurodegenerative diseases.

Acknowledgements: A.H.M. is grateful for the grants U54NS083924, 8G12MD007583, and 8G12MD007600. We thank NIH-NIMHD-G12-MD007583 grant for the facility support.

Keywords: Ischemic Stroke; Inflammation; Microglia/Macrophage; Polarization

Theme: Techniques

Abstract #72

DETERMINATION OF PAIR-WISE PROTEIN INTERACTIONS BETWEEN SPT7 AND DROSOPHILA MELANOGASTER SAGA SUBUNITS

Edwin E. Colón Acosta[1]; Rachel Stegeman[2]; Arryn Harris[2]; Vikki M. Weake[2]

1Department of Biology, University of Puerto Rico, Río Piedras, Puerto Rico
2 Department of Biochemistry, Purdue University, Indiana

The association between spliceosomal components and the transcriptional machinery offers an interesting interconnection between the mechanisms of pre-mRNA splicing and genetic transcription. Recent findings demonstrate the existence of two novel protein subunits in the D. melanogaster SAGA transcriptional co-activator protein complex, SF3B3 and SF3B5, which are known to be part of the SF3B complex in the U2snRNP. Previous work has demonstrated that SF3B3 and SF3B5 interact in a yeast two-hybrid system with both Spt7 and Sgf29 subunits in SAGA, thus Spt7 and Sgf29 provide the potential candidates to incorporate SF3B3 and SF3B5 into the SAGA complex. Recent findings show that SF3B5 is necessary for the expression of a subset of SAGA-regulated genes independent of splicing, although not necessary for SAGA's histone-modifying activities (Stegeman et al, 2016). However, the functions of both SF3B3 and SF3B5 within the SAGA complex are not completely characterized. In response to this, an Spt7 mutant allele library was developed to screen for mutations that disrupt protein-protein interactions with SF3B3 and SF3B5 but retain the interactions that wild type Spt7 has with the SAGA complex. Finding specific mutants will potentially allow us to obtain a SAGA-specific phenotype that determines the functions of SF3B3 and SF3B5 within the SAGA complex. To screen this Spt7 mutant allele library it is necessary to determine, beforehand, the protein subunits that wild type Spt7 interacts with in SAGA. In a yeast two-hybrid system, we tested the Spt7 subunit for protein-protein interactions with most of the SAGA subunits. Here, we report the protein subunits that wild type Spt7 is known to interact with in the SAGA complex, including a previously undetermined interaction with the TAF-10B subunit. These findings will allow us to screen for Spt7 mutants that do not interact with SF3B components, but still interact with the SAGA complex.

Acknowledgements: NSF-REU, Purdue Biochemistry and Weake laboratory

Keywords: SAGA, Splicing Factors, Yeast-Two Hybrid Assay, Gene Regulation

Abstract #73

"ANXIETY" MEASUREMENTS IN ADULT ZEBRAFISH (DANIO RERIO)

J. Lugo-Cuevas; D. Joubert-Miranda; H. Rosa-Lopez; D. Consuegra-Garcia; MdR Reyes; J.G. Ortiz

1. School of Science and Technology URGREAT-MBRS-RISE, Universidad del Este, Carolina PR.
2. Department of Pharmacology and Toxicology, University of Puerto Rico School of Medicine, San Juan, Puerto Rico

Danio rerio most commonly known as zebrafish, is recognized as an animal model among various scientific fields. Their inner systems (nervous, endocrine, and digestive) have a similar genetic structure to humans. They share 70 % of genes with us, also 84% of genes known to be associated with human disease have a zebrafish counterpart. The main objective of the investigation is to compare individual vs group "anxiety" behavior in open field and light-dark chambers. 2. Adult male and female wild-type short-fin Danio rerio (zebrafish), 3–6 months old and weighing 0.25 ± 0.04 g (mean ± SEM) were obtained from Caribe Fisheries Inc. (Lajas, Puerto Rico). Zebrafish were maintained in an aquarium with an automatic filtration system and covered with blue contact paper to reduce stress. Zebrafish were tested either as groups (3) or as individual in open field or light-dark chambers. Our results showed that in groups, zebrafish behavior follow a normal distribution. When the same fish are tested individually, there is marked dispersion of their behavior. This effect is readily reversible. There is a good correlation between open field (green background) and light-dark group measurements. Open field and light-dark preference are used to measure generalized anxiety in rodents. These methods can be used in zebrafish provided that they are tested in groups. Zebrafish "anxiety" can be tested provided they are tested in groups.

Acknowledgements: UNE Institutional Funds and URGREAT-MBRS-RISE @ UNIVERSIDAD DEL ESTE – NIH GRANT: 5R25GM066250

Keywords: Anxiety, Zebrafish, Open Field Test, Light-dark Test

Iniciativas de Investigación y
Actividad Creativa Subgraduadas

Neuro-ID Program
University of Puerto Rico - Río Piedras Campus

ENDURE
NIH Blueprint for Neuroscience Research

une
UNIVERSIDAD DEL ESTE