The FSU Life Sciences Symposium

2016 Life Sciences Symposium

Seeing the Heart & Mind: Modern Imaging Techniques

February 25-26, 2016 | College of Medicine Auditorium |Florida State University

View Speaker Abstracts | View Poster Abstracts

Information for Attendees | Information for Poster Presenters

Schedule

Thursday, February 25, 2016			
12:30 PM	Welcome and Opening Remarks	Janet Kistner, Ph.D.	Interim Vice President for Faculty Development & Advancement
12:35 PM	Novel Techniques for fMRI Data Analysis	Mingzhou Ding, Ph.D.	Pruitt Family Professor, University of Florida Department of Biomedical Engineering
1:30 PM	Engineering Mini-Brain Construct from Human Induced Pluripotent Stem Cells	Yan Li, Ph.D.	Assistant Professor, FAMU-FSU College of Engineering

2:00 PM	High Field MRI/S in Stroke	Jens Rosenberg, Ph.D.	Research Faculty 1, FSU National High Magnetic Field Laboratory
2:30 PM	Category Learning Increases Discriminability of Relevant Object Dimensions in Visual Cortex	Jonathan Folstein, Ph.D.	Assistant Professor, FSU Department of Psychology
3:00 PM	Coffee and Networking		
3:30 PM	Investigating functional brain networks in animal models of drug use disorders	Marcelo Febo, Ph.D.	Assistant Professor and Program Director of Translational Research Imaging, Department of Psychiatry, McKnight Brain Institute, University of Florida
4:30 PM	Discerning the cellular pathologies underlying microcephaly and ciliopathy disorders using imaging technologies	Timothy Megraw, Ph.D.	Associate Professor, FSU Department of Biomedical Sciences
5:00 PM	Oxytocin in Social Brain Development	Elizabeth Hammock, Ph.D.	Assistant Professor, FSU Department of Psychology
5:30 PM	Reception		

Friday, February 26, 2016			
9:00 AM	Incorporating brain measures into assessment of health-related trait characteristics	Christopher J. Patrick, Ph.D.	Professor, FSU Department of Psychology
9:30 AM	Taming Tumors: Exploring the Role of Ultra-High Field MRI in Brain Cancer Care	Cathy W. Levenson, Ph.D.	Professor, FSU Department of Biomedical Sciences
10:00	Seeing the process of smelling	Wen Li,	Associate Professor, FSU

AM		Ph.D.	Department of Psychology
10:30 AM	Professional Development	Gary K. Ostrander, Ph.D.	Vice President for Research
11:30 AM	"Come to my Poster" Poster Preview Talks New this year! Graduate students and postdocs who are presenting posters will be able to give a 1-2 minute talk to get people interested in their posters.		
12:30 PM	Poster Session and Lunch Visit the posters you heard about at the preview and talk with the presenters. Then grab a boxed lunch and enjoy it with your colleagues from across campus. Lunch will be provided to registered attendees with tickets (register above).		
2:00 PM	Quantitative Techniques in Cardiovascular MRI	Thomas S. Denney Jr., Ph.D.	Director, Auburn University MRI Research Center
3:00 PM	Skeletal Muscle Index Derived from Computed Tomography Predicts Post-operative Recovery and Hospital Length of Stay after Transcatheter Aortic Valve Replacement: Insights Into a Novel Marker of Frailty	Wayne B. Batchelor, M.D.	Cardiologist, Southern Medical Group/Tallahassee Memorial Healthcare
3:30 PM	Coffee and Networking		
4:00 PM	Following the heart during aging and exercise training: Imaging Walls, Blood Flow, and Blood Vessels	Judy Delp, Ph.D.	Professor, FSU Department of Biomedical Sciences
4:30 PM	Unlocking the secrets of the striated muscle thick filament using cryoelectron microscopy	Kenneth A. Taylor, Ph.D.	Professor, FSU Institute of Molecular Biophysics
5:00 PM	Relationship between diastolic dysfunction, aging, and mood disorder as revealed by a mouse model of hypertrophic cardiomyopathy	Amanda Dossat	Graduate Student, FSU Department of Biomedical Sciences
5:30 PM	Awards Presentation and Closing Remarks		

Information for Attendees:

Registration Desk: The registration desk opens at 11:00 AM. Don't forget to stop by and pick up your packet. This includes your name badge and your lunch ticket. There will be a limited number of walk-up registrations available, so if you know of someone who missed the registration deadline, please let them know.

There will be a badge recycling box available at the registration table throughout the symposium. You are welcome to keep your name badge as a souvenir, but if you don't want it, we can recycle it for you.

Social Media: Follow us on Facebook (**https://www.facebook.com/FSUBMS**) and Twitter (@FSUBiomed) for late breaking announcements. Use #FSULSS to tweet along.

Food: Lunch will be served on Friday to those who have tickets, and to anyone else on a first-come, first-served basis. There will be coffee breaks both days to allow plenty of time to talk.

Thursday evening at 5:30, please join us for a Reception.

Poster Competition: This year, we are trying something new! On Friday at 11:30 AM, poster presenters will have the opportunity to give a 1-2 minute talk to convince people to visit their poster during the poster session. The poster session is on Friday from 12:30-2:00. Presenters will be available at that time to discuss their research.
Instead of having a small panel of judges, this year, YOU are the judge! Voting will be available online (link will be included in your registration packet). Voting closes promptly at 4:00 pm on Friday. Poster winners will be announced at Award Presentation on Friday evening.

Information for Poster Presenters:

We have space for 30 poster presentations. Posters will be accepted on a first come, first served basis. Please register using the link above (be prepared to enter your poster's abstract!). A $250 prize will be awarded for one outstanding poster on Friday evening.

Please bring your poster to the COM atrium beginning 11:00 a.m. on Thursday, February 24. Please have your poster up before 3:00 on Thursday. We will have 40" x 60" foam core poster boards, easels, push pins, and binder clips available for you to use to display your work.

If you plan to present a talk during the "Come to my Poster" session on Friday at 11:30 AM, please email your intent to Tiffany McNabb no later than 7:00 pm Thursday night. You may submit one slide to accompany your talk. Please have your slide to Tiffany no later than 7:00 pm on Thursday. We will not be able to accept any submissions after that. Remember that there is a prize for the best poster, so you want to convince people to vote for you!

Please remove your posters by 4:30 pm on Friday, February 26. Any posters left behind will be kept for 2 weeks for pickup in the Biomedical Sciences Office. After that, any posters remaining will be disposed of.

Speakers: Invited speakers will be registered automatically. If you have any special needs, please contact **Tiffany McNabb**. If you are traveling from outside Tallahassee, please contact **Jonquil Livingston** for assistance and reimbursement.

About the Symposium: Sponsored by the Department of Biomedical Sciences, College of Medicine, Florida State University, the Life Sciences Symposium is a dynamic forum that

brings together speakers doing innovative, cutting edge, and ground-breaking research from different departments at FSU. These presentations are augmented with talks from speakers outside of FSU who are leaders in their respective fields. While promoting the broad-spectrum of biomedical science research at FSU, the Life Sciences Symposium also provides a venue for faculty, postdocs and students to present their research, spark interdisciplinary and interdepartmental collaborations, and foster professional development.

Organizing Committee:

- Richard Nowakowski (Chair), Biomedical Sciences
- Judy Delp, Biomedical Sciences
- Sam Grant, Chemical & Biomedical Engineering and the National High Magnetic Field Laboratory
- Wen Li, Psychology

Administrative support

Tiffany McNabb, Biomedical Sciences

Jonquil Livingston, Biomedical Sciences

Department of Biomedical Sciences Staff

College of Medicine IT Services

For additional information about the symposium, please email **LSS@med.fsu.edu**.

Posters

Investigation of non-centrosomal microtubule organizing center in fat body cells of Drosophila

Presenter: Rebecca Buchwalter
Biomedical Sciences, Florida State University

While the centrosome is the most well-studied microtubule organizing center (MTOC), non-centrosomal MTOCs are present in many cells such as certain types of differentiated cells. However, much is not known about their molecular makeup and how they assembly. We have discovered a MTOC located at the nuclear periphery in the fat bodies of larval Drosophila. Similar to adipose and liver tissues in mammals, Drosophila fat bodies are tissue that have roles in metabolism and lipid storage that provide energy for the organism under starvation conditions. After a survey of centrosomal proteins, we found that pericentriolar material (PCM) proteins localize to the site, but the centriolar proteins do not appear to be present. The microtubules (MTs) are uniquely modified through certain posttranslational modifications (PTMs) and do not associate with the common microtubule-associate protein (MAP) Jupiter. The fat body cells also contains a multiprotein complex: Linker of Nucleoskeleton and Cytoskeleton (LINC), a candidate structural anchor of MTs from fat body MTOC to the nuclear periphery. In the future, we will study what proteins are required for initial assembly. Morphology and regrowth experiments between wild type and various protein mutants lines will be used for this analysis. We will also further characterize the MTs in order to better understand their dynamics and stability in the fat body.

Fractionation-Dependent Improvements in Proteome Resolution in the Mouse Hippocampus by Isoelectric Focusing

Presenter: Joseph Bundy B.S.
Biomedical Sciences, Florida State University College of Medicine

Mass spectrometry is a tool for investigating the abundance of small molecules including peptides. Mass spectrometry-based proteomics can identify and quantify simultaneously hundreds of proteins in a single biological sample. By pre-fractionating biological samples with isoelectric focusing (IEF), the number of proteins identified can be increased to the realm of thousands. However, fractionation also increases analysis time and is cost-prohibitive. Therefore, it is advantageous to ascertain and understand the benefits and drawbacks of IEF before designing large IEF-enabled experiments. To investigate the benefits of IEF-enabled analysis of hippocampal tissue, a systematic analysis of IEF-fractions and pooled fractions was conducted. This analysis focused on improvements in functionally relevant protein identifications, quantitative resolution, and statistical power.

A splice variant of Centrosomin converts mitochondria to MTOCs to facilitate sperm morphogenesis in Drosophila

Presenter: Jieyan Chen Ph.D.
Biomedical Sciences, Florida State University

Mitochondria are energy centers in cells. In Drosophila (fruit fly) spermatids, they also play an important role in sperm morphogenesis by providing a structural platform for microtubule (MT) organization to support the elongating tail. Centrosomin (Cnn) is a core centrosomal protein whose gene expresses several variants falling into two major forms: the centrosomal form (CnnC) and a non-centrosomal form in testes (CnnT). CnnC is essential for functional centrosomes, the major Microtubule-Organizing Centers (MTOCs) in animal cells. We found that CnnT is expressed exclusively in testes, and unlike CnnC that resides at centrosomes, it localizes to giant mitochondria in spermatids. In cell culture, CnnT targets to the mitochondria and recruits the MT-nucleating complex ?-TuRC to assemble MTs on mitochondria, converting mitochondria to MTOCs. We mapped two separate domains on CnnT that are necessary and

sufficient to target it to mitochondria, and to recruit the ?-TuRC and nucleate MTs, respectively. Disrupting the conserved CM1 domain (also shared with CnnC) in CnnT abolishes MT-nucleating function but does not block ?-TuRC recruitment, indicating that CM1 is essential for the activation but not recruitment of ?-TuRC. In vivo, CnnT forms speckles on the surface of nebenkerns in spermatids, where it is required to recruit ?-Tubulin. We propose that CnnT assembles unique MTOCs on nebenkerns to facilitate the morphogenesis of the extremely long sperm that are found in Drosophila.

Rootletin organizes the ciliary rootlet to achieve neuron sensory function in Drosophila

Presenter: Jieyan Chen Ph.D.
Biomedical Sciences, Florida State University

Cilia are important for cell signaling and sensory perception. In many cell types, a cytoskeletal structure called the ciliary rootlet links the cilium to the cell body. Previous studies indicated that rootlets support the long-term stability of some cilia. Here we report that Drosophila melanogaster Rootletin (Root), the sole orthologue of the mammalian paralogs Rootletin and C-Nap1, assembles into rootlets of diverse lengths among sensory neuron subtypes. Root mutant neurons lack rootlets and have dramatically impaired sensory function, resulting in behavior defects associated with mechanosensation and chemosensation. Root is required for cohesion of basal bodies, but the cilium structure appears normal in Root mutant neurons. We show, however, that normal rootlet assembly requires centrioles. The N terminus of Root contains a conserved domain and is essential for Root function in vivo. Ectopically expressed Root resides at the base of mother centrioles in spermatocytes and localizes asymmetrically to mother centrosomes in neuroblasts, both requiring Bld10, a basal body protein with varied functions.

A tunable artificial circadian clock in clock-defective mice

Presenter: Matthew D'Alessandro
Biomedical Sciences, Florida State University

Self-sustaining oscillations are essential for diverse physiological functions such as the cell cycle, insulin secretion and circadian rhythms. Synthetic oscillators using biochemical feedback circuits have been generated in cell culture. These synthetic systems provide important insight into design principles for biological oscillators, but have limited similarity to physiological pathways. Here we report the generation of an artificial, mammalian circadian clock in vivo, capable of generating robust, tunable circadian rhythms. In mice deficient in Per1and Per2 genes (thus lacking circadian rhythms), we artificially generate PER2 rhythms and restore circadian sleep/wake cycles with an inducible Per2 transgene. Our artificial clock is tunable as the period and phase of the rhythms can be modulated predictably. This feature, and other design principles of our work, might enhance the study and treatment of circadian dysfunction and broader aspects of physiology involving biological oscillators.

Tyrosine Phosphorylation of the Transcription Factor Yin Yang 1

Presenter: Susan Daraiseh
Biomedical Sciences, Florida State University

Tyrosine phosphorylation controls multiple aspects of cell and organism growth, differentiation and function by modulating cellular signaling pathways, and if deregulated can result in various types of cancer and disease. Yin Yang 1 (YY1) is a multifunctional zinc finger transcription factor that can activate or suppress gene expression depending on both the promoter and co-factors it recruits. In addition to the transcriptional functions of YY1, it has been found to regulate a broad spectrum of biological processes such as development, apoptosis, DNA repair, autophagy, oncogenesis and X-chromosome inactivation. Several of the functions carried out by YY1 have been found to be regulated by post translation modifications. Our previous work has shown the modulation of the function of YY1 through phosphorylation at amino acid residues serine and threonine. However, little is known about the molecular mechanisms by which tyrosine phosphorylation regulates YY1's function. We have developed

a rabbit poly-clonal phosphospecific antibody against tyrosine residue 251 (anti-pY251). We have utilized high-throughput proteomic and computational data in combination with biochemical assays and our phosphospecific antibody as well as the general phosphotyrosine antibody to identify several protein tyrosine kinases that phosphorylate YY1. Our findings will have great importance for understanding cellular signaling pathways that regulate this multifunctional protein.

A transcriptomic analysis of the estrous cycle in 4 regions of the mouse brain

Presenter: Lisa DiCarlo
Biomedical Sciences, Florida State University College of Medicine

For many years biomedical research, and in particular neuroscience research, has often focused on male subjects. Female subjects have frequently been excluded due to the perceived complications of the hormonal changes across the estrous cycle and the potential need to include the appropriate control groups. We utilized transcriptomic analysis of the hypothalamus, hippocampus, neocortex, and cerebellum of female C57BL/6J (B6) mice to examine changes in gene expression. Not surprisingly, there are ~10,000 differentially expressed genes (DEGs) between the hypothalamus, hippocampus, neocortex, and cerebellum at a false discovery rate less than 0.05. The hippocampus vs cerebellum (n=10,610) and neocortex vs cerebellum (n=10,464) comparisons have the most DEGs and the hippocampus vs neocortex (n=9,166) comparison has the least. In contrast to the ~10,000 DEGs between brain regions, within each brain region there are fewer than 70 stage-specific DEGs as a result of the estrous cycle. The hippocampus has the most DEGs (n=67), followed by the neocortex (n=55), hypothalamus (n=53), and cerebellum (n=20). This dataset demonstrates that the differences between brain regions overwhelm changes in gene expression as a result of the estrous cycle. We expect that our results will be a useful guide for researchers in the field of neuroscience in incorporating females in future experiments as well as shedding light on the interactions of hormones and gene expression in different brain regions.

Estrous cycle surpasses sex differences' regulation of the medial prefrontal cortex transcriptome in rats and reveals an important underlying role of early growth response 1 (Egr1).

Presenter: Florian Duclot PhD
Biomedical Sciences, Florida State University

Males and females differ in cognitive functions and emotional processing, which in part have been associated with baseline sex differences in gene expression in the medial prefrontal cortex. Nevertheless, a growing body of evidence suggests that sex differences in medial prefrontal cortex (mPFC)-dependent cognitive functions are attenuated by hormonal fluctuations within the menstrual cycle. Despite known genomic effects of ovarian hormones, the interaction of the estrous cycle with sex differences in gene expression in the mPFC remains unclear and warrants further investigations. We undertake a large-scale characterization of sex differences and their interaction with the estrous cycle in the adult mPFC transcriptome and report that females with high and low ovarian hormone levels exhibit a partly opposed sexually biased transcriptome. The extent of regulation within females vastly exceeds sex differences, and supports a multi-level reorganization of synaptic function across the estrous cycle. Genome-wide analysis of the transcription factor early growth response 1 (Egr1) binding highlights its role in controlling the synapse-related genes varying within females. In addition to illustrating the importance of accounting for the estrous cycle in females, our data set the ground for a better understanding of the female specificities in cognition and emotional processing.

Acute prenatal exposure to valproic acid (VPA) alters social and anxiety-like behaviors in prairie voles

Presenter: Lindsay Elvir
Biomedical Sciences, Florida State University

Previous studies have shown that rats and mice prenatally treated with sodium valproate (valproic acid, VPA) exhibit deficits in social behaviors that resemble some aspects of autism spectrum disorders. In this

study, the socially monogamous prairie vole (Microtus ochrogaster) was used as a novel animal model to examine social behaviors following prenatal VPA exposure. Male and female control and VPA-exposed subjects were assessed on a battery of social tests to evaluate the VPA-induced social deficits and anxiety-like behavior. VPA-pretreated voles engaged in fewer play behaviors and had reduced social interaction with novel conspecifics of the same age, compared to control animals. VPA-pretreated male, but not female, subjects showed enhanced anxiety-like behavior and did not develop partner preference when they became adults. We are now in the process of examining whether some of these VPA-induced social deficits in male subjects can be ameliorated by histone deacetylase (HDAC) inhibitors administered at different developmental periods.

Simultaneous Measurement of Force and Lattice Spacing in Skinned Cardiac Fibers

Presenter: David Gonzalez-Martinez
Biomedical Sciences, Florida State University

This study explores the relationship between Ca2+-activated force and myofilament lattice spacing (LS) in skinned cardiac muscle fibers. LS measurements were obtained using synchrotron x-ray diffraction while isometric force was simultaneously monitored with a force transducer. Fibers were isolated from left-ventricle of wild-type (WT) or troponin C knock-in mice bearing the homozygous mutation Ala8Val, which causes hypertrophic cardiomyopathy (KI-cTnC-A8V+/+). Cardiac fibers from KI-TnC-A8V+/+ have been reported to have 2.5x greater myofilament Ca2+-sensitivity compared to WT. Since WT and KI-cTnC-A8V+/+ fibers generate different levels of force at the same [Ca2+], they provide a powerful tool for investigating whether LS is tightly coupled to force-development. Compared to WT, KI-TnC-A8V+/+ fibers tended toward smaller LS at all [Ca2+] tested. However, at pCa6.2 and 5.8, both WT and KI-TnC-A8V+/+ fibers maintained LS similar to that recorded at pCa8 in each muscle. Interestingly, WT fibers developed ~15% of the maximal force at pCa5.8 while KI-TnC-A8V+/+ fibers developed ~70%. Because the lattice spacing vs pCa curves are approximately parallel, these data suggest that at submaximal [Ca2+], skinned fiber force does not strictly determine the distance between thin and thick filaments and that force levels can reach up to 70% of the maximal force without shortening of LS.

Investigating psychiatric disorders using novel mouse models

Presenter: Kourtney Graham BS
Authors: Yuying Wu PhD, Yi Zhou PhD
Neuroscience, Florida State University

Neurodevelopmental defects play a particularly important role in the pathophysiology of schizophrenia, a complex mental disorder affecting approximately one percent of the US population. Recently, we have reported the successful creation of a new mouse model that addresses the role of the 14-3-3 family of proteins. These transgenic mice express a 14-3-3 peptide inhibitor that antagonizes 14-3-3 bindings to its endogenous partners and is thus considered a 14-3-3 functional knockout (FKO). We have shown that these 14-3-3 FKO mice exhibit a variety of behavioral and morphological deficits reminiscent of the core endophenotypes of established schizophrenia animal models. In this study, we created adeno-associated viruses (AAVs) expressing either the 14-3-3 peptide inhibitor or a siRNA against GFP. The 14-3-3 peptide inhibitor AAV will be used to induce the schizophrenic-like phenotypes in wildtype mice in a temporal- and region-specific manner. The AAV expressing the siRNA will be used to knockdown expression of the 14-3-3 peptide inhibitor in 14-3-3 FKO mice in a temporal-and region-specific manner in order to attenuate the schizophrenic-like phenotypes. Together, these new mouse models will shed some light on the role that 14-3-3 plays in the development of psychiatric disorders.

High Accuracy Calibration Model Transfer between NIR Spectrometers for Prediction of Single Seed Nutrient and Shape Traits

Presenter: Gokhan Hacisalihoglu PhD
Biological Sciences, Florida A&M University

Seeds usually have substantial variation in their shape, size, volume, color, as well as chemical components. Rapid and reliable prediction of seed traits is important for phenotyping and seed quality assessment. However, wet analytical lab techniques are often labor intensive and require complex sample preparation. A single kernel near infrared (SKNIR) spectrometer was used to build calibration models for rapid and non-destructive prediction of seed composition, weight, size, and density traits in single soybean seeds. A total of 270 samples of globally diverse soybean seed samples were collected, scanned, and analyzed for relating spectral data (988 to 1688 nm) to analytical reference data. NIRS results were validated with independent soybean seed samples. Principal component analysis (PCA) and partial least squares (PLS) were used to develop the calibration models. PCA was able to discriminate between black and non-black seed color. PLS regression resulted stable predicted models for seed oil, weight, volume, protein, area, width, and length. Furthermore, this study compared PLS regression models from Florida and Kansas devices and showed successful and high accuracy calibration model transfer. These results are relevant to broader application of calibration model transfer between single seed NIR spectrometers without the need of individual NIRS calibration in seed nutrient and shape trait phenotyping.

K63-linked Ubiquitination and the Clearance of Misfolded Proteins

Presenter: Ryan Higgins
Biomedical Sciences, Florida State University

Misfolded proteins that evade chaperone-mediated refolding and proteasome-dependent degradation can form aggregates in cells. Protein aggregates are cytotoxic and they are linked to several neurodegenerative diseases including Alzheimer's and Huntington's diseases. In both mammalian and yeast cells, small protein aggregates can be transported to a centrosome-localized, cytoprotective structure termed an aggresome. Studies in higher eukaryotes have identified K63-linked ubiquitination as a signal to promote aggresome formation of specific substrates. Here, we demonstrate in yeast using a K63R ubiquitin mutant that K63-linked ubiquitination is required for aggresome formation of Huntington's disease-linked polyQ protein (Htt103QP). Furthermore, we have identified Ubc4 ubiquitin-conjugating and Rsp5 E3 ubiquitin ligase (Nedd4 homolog in yeast) enzymes necessary for Htt103QP aggresome formation. Mutated Huntingtin protein has previously been shown to be degraded via a proteasome-dependent mechanism and via autophagy. We expand upon these findings and show that prior to aggresome formation Htt103QP is cleared primarily through the proteasome, while after aggresome formation both proteasome-dependent and independent pathways are necessary.

The Structure of the Relaxed Thick Filaments from Lethocerus Asynchronous Flight Muscle

Presenter: Zhongjun Hu Graduate Student
Institute of Molecular Biophysics, Florida State University

The structure of relaxed muscle thick filaments is a key element for understanding muscle physiology. Relaxed thick filament structures by 3D-EM revealed an interacting head motif (IHM) first identified in smooth muscle myosin. A later model based on X-ray fiber diffraction of Lethocerus flight muscle showed an intermolecular interaction between myosin heads. The structure of the long alpha-helical coiled-coil rod of myosin in thick filaments is comparatively poorly understood. Here we show the relaxed structure of thick filaments from the flight muscle of the waterbug (Lethocerus) obtained using cryoEM to reveal not only the relative placement of individual myosin heads, but also the myosin rod alpha-helices. We find that flight muscle IHM is similar as others, but different orientation. The myosin rod, which can be traced from the head-rod junction to C-terminus at a resolution of 5.5 angstrom, follows a tortuous path from the outside of the thick filament backbone to the inside. Surprisingly,

threading their way among the forest of myosin rods embedded are four polypeptide chains. Their presence in this location could modulate the mechanical properties of the myosin backbone. Our results demonstrate the ubiquity of the myosin interacting head motif (IHM) in relaxed myosin filaments but with a variation that might shed light on the mechanism of stretch activation and shortening deactivation. Supported by NIH and AHA.

Nanoparticle analysis of cancer cell vesicles sheds budding insights into exosome and microvesicle biogenesis

Presenter: Stephanie Hurwitz
Authors: David Meckes Jr.
Biomedical Sciences, Florida State University

Extracellular vesicles (EVs) are important mediators of cell-to-cell communication in healthy and pathological environments. Because EVs are present in many biological fluids and contain molecular signatures of their origin, they have great diagnostic and prognostic value. The ability of EVs to deliver biologically active proteins, RNAs, and lipids to cells has generated interest in therapeutic utility. Despite their potential medical use, many of the mechanisms underlying EV biogenesis and secretion remain unknown. Here, we characterized vesicle secretion across the NCI-60 panel of cancer cells. The quantity of EVs secreted by each cell line was compared to reference transcriptomics data to generate a set of gene products associated with vesicle secretion. Gene products positively associated with the quantity of exosomal-sized vesicles included vesicular trafficking classes of proteins with Rab GTPase function and sphingolipid metabolism. Positive correlates of larger microvesicle-sized vesicles were enriched in gene products involved in cytoskeletal dynamics and exocytosis, as well as Rab GTPase activation. One of the identified targets, CD63, was further evaluated for its role in vesicle secretion. CRISPR/Cas9 knockout of the CD63 gene in HEK293 cells resulted in a decrease in small vesicle secretion, suggesting the importance of CD63 in exosome biogenesis. This study offers a foundation for further exploration of targets involved in EV biogenesis and secretion.

A high-throughput screening strategy for identifying therapeutic compounds to override uORF inhibition of gene expression

Presenter: Lataisia Jones MS
Biomedical Sciences, Florida State University

Approximately 50% of human gene transcripts contain upstream open reading frames (uORFs) that normally work with canonical ORFs (cORFs) to regulate gene expression. Single nucleotide polymorphisms (SNPs) can eliminate, modify or introduce upstream start codons (uAUGs) that induce post-transcriptional control of gene expression. Multiple disorders including cardiomyopathy and Alzheimer's disease are associated with SNP-induced uORFs. Previously, we showed that a -22C>T SNP induced uORF downregulates GTP Cyclohydrolase 1 (GCH1) expression in patients with levodopa responsive dystonia (DRD) by inhibiting translational initiation at the canonical start codon (cAUG). We utilized the -22C>T GCH1 model to generate a stable cell line to identify compounds that induce translational machinery to override the uAUG and increase the recruitment of ribosomes to the cAUG thus restoring GCH1 expression. Using bidirectional vectors with GCH1 (-22C/T) and Trk promoters driving luciferase or Renilla expression in opposite directions, we discovered a compound that improves translational efficiency of the cORF when the uAUG is present. Our results illustrate the significance of understanding the molecular mechanisms of cORF regulation to devise novel methods to identify treatments that repair gene expression and have the potential to mitigate disease phenotypes.

A conserved mechanism for coordinating replication fork helicase assembly with phosphorylation of the helicase

Presenter: Daniel Kaplan PhD
Biomedical Sciences, FSU

DDK phosphorylates Mcm2 during S phase in yeast, and Sld3 recruits Cdc45 to Mcm2-7. We show here DDK-phosphoryled-Mcm2 preferentially interacts with Cdc45 in vivo, and that Sld3 stimulates DDK phosphorylation of Mcm2 by 11-fold. We identified a mutation of Sld3, Sld3-m16, that is specifically defective in stimulating DDK phosphorylation of Mcm2. Wild-type expression levels of sld3-m16 result in severe growth and DNA replication defects. Cells expressing sld3-m16 exhibit no detectable Mcm2 phosphorylation in vivo, reduced RPA-ChIP signal at an origin, and diminished GINS association with Mcm2-7. Treslin, the human homolog of Sld3, stimulates human DDK phosphorylation of human Mcm2 by 15-fold. DDK phosphorylation of human Mcm2 decreases the affinity of Mcm5 for Mcm2, suggesting a potential mechanism for helicase ring opening. These data suggest a conserved mechanism for replication initiation: Sld3/Treslin coordinates Cdc45 recruitment to Mcm2-7 with DDK phosphorylation of Mcm2 during S phase.

A Paternal Nicotine Exposure Mouse Model and Consequential ADHD-Like Symptoms in their Offspring

Presenter: Sarah Lowe
Authors: Thomas Morgan Jr., Pradeep Bhide, Deirdre McCarthy
Biomedical Sciences, Florida State University

The consequences of tobacco use by pregnant women for the developing fetus are well documented. Clinical reports show that prenatal nicotine exposure increases the risk for attention deficit hyperactivity disorder (ADHD). However, smoking is more prevalent among men (21.6%) than women (16.5%) in the US [CDC 2013]. Yet the impact of a father's use of tobacco on an offspring's cognitive development remains unknown. We developed a paternal nicotine exposure model in which adult male mice were exposed to nicotine in drinking water and mated with drug naïve female mice. We analyzed working memory, attention, and locomotor activity in the offspring because these phenotypes are associated with ADHD. The offspring sired by the nicotine exposed fathers showed significant increases in locomotor activity and significant deficits in attention compared to the offspring from male mice that were not exposed to nicotine. Since the fathers were directly exposed to nicotine, a plausible mechanism of the phenotypic heritability is via nicotine-induced epigenetic modifications of the father's germ cell DNA, such as DNA methylation, which is known to control gene expression. We examined global DNA methylation in the father's spermatozoa and found that the nicotine-exposed fathers had increased global DNA methylation. Based on these data, germ cell DNA epigenetic modification could be a potential heritability mechanism of nicotine-induced behavioral phenotypes via the paternal line of descent.

Dopamine D1 receptor activation and neuronal migration

Presenter: Melissa Martin MS
Biomedical Sciences, Florida State University

Neurotransmitters play unique growth-promoting roles in the developing nervous system. A common theme underlying the etiology of psychiatric disorders with developmental onset such as schizophrenia, ADHD, autism and early onset dystonia, is perturbation of the dopamine (DA) and GABA neurotransmitter systems. Interestingly, it has been shown using animal models that prenatal exposure to drugs of abuse lead to changes in DA receptor signaling and GABA neuron migration. Yet, little is known about the mechanisms that underlie these changes in the DA and GABA systems. Here, we use micro-dissected MGE explants and a cortical substrate from E15 mice to examine the migratory behavior of GABA neurons following application of the nonselective DA type 1 receptor (D1R) agonist, SKF 81297. Using co-culture and live cell imaging techniques, we demonstrate that D1R activation significantly reduces the speed of migrating GABA neurons. GABA neurons typically migrate via a saltatory or jump-

like behavior followed by a resting phase. We found that D1R agonist application increases the percentage of time that GABA neurons spent in resting phase compared to controls, whereas, the percentage of medium and large jumps is reduced after D1R agonist treatment. Collectively, these results demonstrate that changes in D1R activation alter the migratory behavior of GABA neurons during early brain development and that these changes may have implications in the development of psychiatric disorders.

A role of the IgG family proteins, Dprs/DIPs, in fru P1-expressing neurons in Drosophila to control sexually dimorphic behaviors

Presenter: Nicole Newell
Biomedical Sciences, Florida State University

D. melanogaster adults display sex differences in reproductive behaviors that are specified by the sex determination hierarchy. This hierarchy consists of an alternative pre-mRNA splicing cascade that culminates in the production of sex-specific transcription factors doublesex (dsx) and fruitless (fru). Both dsx and fru are required for these reproductive behaviors by directing sex-specific differences in neuronal arborization patterns, connectivity, neuron number and physiology. Our previous genomic studies showed the immunoglobulin extracellular receptor superfamily (IgSF) is enriched under the regulation of sex-specific Fru isoforms. Additionally, functional analyses showed that one of these IgSF members, Dpr, is important for male courtship behaviors. However, it is still unknown when and where these IgSFs are localized within fru-expressing neurons or if they show sex differences in their spatiotemporal expression patterns. Using an in vivo live staining assay we show that certain Dpr/DIPs are co-localized with fru P1 neurons in the subesophageal ganglion and that the number of cells that show co-localization are sexually dimorphic but that Fru is not required for Dpr/DIP binding. We further show that the Dprs/DIPs are expressed in unique patterns in the brain and have shared and distinct co-localization with fru P1 neurons. These results have encouraged us to continue to explore the underlying mechanism of the role of IgSF proteins in fru- expressing neurons.

Transmembrane Domains Mediate Intra- and Extra-cellular Trafficking of Epstein-Barr Virus LMP1

Presenter: Dingani Nkosi MBBS
Authors: David Meckes Jr
Biomedical Sciences, Florida state university

Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is released from latently infected tumor cells in small membrane-enclosed vesicles called exosomes. LMP1-modified extracellular vesicles can be taken-up by cells resulting in the activation of cellular signal transduction pathways through paracrine or autocrine mechanisms. LMP1 is a major driver of exosome content and functions. LMP1-modified exosomes can influence cell growth, migration, differentiation and immune cell function. Despite the importance of exosomal LMP1 on the infected microenvironment, little is known about how this viral protein enters or manipulates the host exosome pathway. In this study, we analyzed deletion mutants of LMP1 for the ability to traffic to exosomes. The results revealed that specific domains within the trans-membrane (TM) regions of LMP1 are required for efficient exosomal sorting. One of the mutants lacking TM domains 1 through 4 (TM5-6) had higher co-localization with endoplasmic reticulum and early endosome markers when compared to the wild-type. Other mutations resulted in enhanced levels of secretion, alluding to potential positive and negative regulation mechanisms for LMP1 exosome sorting. Surprisingly, TM5-6 maintained the ability to co-localize and form a complex with CD63, an abundant exosome protein that is important for the incorporation of LMP1 into exosomes. These data suggest new functions of TM domains in LMP1 exosome trafficking.

BSIR Sample Preparation Services

Presenter: Georgia Platt
Biological Sciences, FSU

The FSU Biological Science Imaging Resource offers sample processing for transmission electron microscopy (TEM). Users may purchase sample preparation services (plastic embedding, sectioning, and staining). We also offer full-service packages which will provide images of the user''s sample for use in research or publications.

The interactome of the Epstein-Barr virus oncoprotein evaluated by the proximity-based BioID approach

Presenter: Mark Rider PhD
Authors: David Meckes Jr.
Biomedical Sciences, Florida State University

The Epstein-Barr virus latent membrane protein 1 (LMP1) is an oncogene required for immortalizing resting B lymphocytes. The discovery and characterization of LMP1 protein-protein interactions will likely generate new targets to treat EBV-associated cancers. Unfortunately, classical molecular tools for identifying protein associations are restrictive. Immunoaffinity purification techniques, for example, rely on harvesting stable protein complexes that are frequently difficult to isolate, and often overlook proteins with transient or weak interactions. It is especially difficult to identify interaction partners of hydrophobic, multi-pass membrane proteins that interact with the cytoskeleton, such as LMP1. In this study, we defined a broader LMP1 interactome using the BioID method. We fused a bacterial biotin protein ligase to LMP1 and harvested biotinylated target proteins; the biotin ''tag'' indicated proteins with vicinal, transient, or stable associations with LMP1. Complementary proteomics approaches allowed us to identify over 1,000 proteins with direct or indirect relationships to LMP1. Pathway analysis suggested that many are involved in signal transduction and endosome trafficking. It is likely that LMP1, which signals from endosomal membranes and is secreted from cells in exosomes, modifies protein trafficking throughout the cell. By way of manipulating the endosomal pathway, LMP1 may exert its oncogenic effects on the surrounding microenvironment.

Rapid sex- and hormone-dependent changes in signaling pathway activation and protein levels in the hippocampus following low-dose ketamine administration: a phosphoproteomics approach

Presenter: Samantha Saland MS
Biomedical Sciences, Florida State University

We recently reported a greater sensitivity of female rats to the fast-acting antidepressant ketamine when compared to males, and that gonadal estrogen and progesterone are required for this heightened response. However, the mechanisms underlying this sex-dependent sensitivity remain unknown. Therefore, a phosphoproteomics approach was used to identify ketamine-induced changes in signaling pathway activation and protein abundance within the dHPC of intact adult male rats and female rats in either diestrus or proestrus. Tissue was collected 30m following saline or an acute low dose (2.5 mg/kg) of ketamine, after which total and TiO2-enriched phosphopeptide lysates were prepared and processed via high-resolution LC-MS/MS. Changes in protein phosphorylation patterns were evaluated by gene ontology and pathway analyses to identify biological processes selectively altered by ketamine in female versus male rats. Results revealed striking dissimilarities in the dHPC proteome and phosphoproteome of male and female rats both at baseline, and following low-dose ketamine treatment. Notably, these differences were heavily influenced by hormonal status in female rats. Together, these data suggest that both biological sex and the hormonal milieu are critical modulators of ketamine’s rapid actions within this brain region, and provide greater insight into potential post-translational processes underlying sex- and hormone-dependent modulation of ketamine’s therapeutic effects.

Sex differences in the abuse potential of low-dose ketamine

Presenter: Kristin Schoepfer
Authors: Samantha Saland M.S., Frank Johnson Ph.D., Mohamed Kabbaj Ph.D.
Biomedical Sciences, FSU College of Medicine

In clinical studies, low subanesthetic doses of the NMDAR antagonist ketamine (KET) have been shown to elicit rapid (~2 hours) and long-lasting (=2 weeks) antidepressant effects in treatment-resistant patients. Further, recent clinical findings have demonstrated a powerful extended antidepressant response if the drug is administered repeatedly. However, KET''s documented history of recreational abuse at higher doses may pose a challenge for long-term depression treatment. We have shown that female rats are more sensitive than males to KET's antidepressant-like effects, responding to 2.5 and 5 mg/kg i.p., respectively. Thus, we aimed to determine whether there are also sex differences in response to repeated low-dose KET treatment in adult rats. We found that neither sex formed a conditioned place preference to 2.5 or 5 mg/kg KET, suggesting that repeated therapeutically relevant doses of KET are not rewarding, but a higher dose (10 mg/kg) elicited a sex-dependent effect on reward state. Also, we found a dose- and sex-dependent behavioral sensitization to repeated KET treatment which was correlated with increased markers of synaptic plasticity in the nucleus accumbens. Taken together, this data suggests that repeated low-dose KET can elicit behavioral and physiological changes like those induced by other drugs of abuse. More studies like this are needed to establish the safety of repeated low-dose KET treatment in both sexes of patients with treatment-resistant depression.

Simple Robotic Imager for Multi-well plates

Presenter: Thayumanasamy Somasundaram Ph.D
Institute of Molecular Biophysics, Florida State University

We have built a simple and low-cost imager that is suitable for capturing images of multi-well plates (96-, 192-, and 288- wells) used for crystallization screening and cell culture. The imager is assembled from commercially available variable zoom USB microscope and a home-built 2-dimensional stage. The software that controls the movement and captures the images is a modified open source version and the microprocessor control is built on Arduino platform. Stage movement is driven by small stepper motors controlled by an Arduino microprocessor running the GRBL firmware commonly found in 3D printers. Instructions are sent through the USB serial connection to the Arduino by a python program developed in-house. The same program utilizes the python bindings for the OpenCV to perform the image acquisition. The program was developed under both Mac OS X and Linux. The imager reduces the time and fatigue involved in manual inspection while providing image history of the multi-well plates during the course of an experiment. Since the design is flexible further modifications to suit other multi-well plates and experiments are possible.

Centrosome proteins regulate autophagy at phagophore assembly and autophagosome closure

Presenter: Yiming Zheng MS
Biomedical Sciences, Florida State University

The centrosome is the major microtubule-organizing center in animals. Mutations in centrosomal genes cause autosomal recessive primary microcephaly (MCPH), a neurological disorder characterized by smaller brain size due to insufficient neural progenitors and neurons, highlighting the importance of centrosome proteins in neuronal health. The etiological basis for MCPH pathogenesis, however, remains unclear. Autophagy is emerging as a neuroprotective mechanism of pathophysiological processes in various neurodegenerative disorders. Autophagy is a highly conserved process through which damaged proteins or organelles are engulfed by a double-membrane vesicle structure called the autophagosome that delivers cargos to the lysosome for degradation, thereby contributing to quality control and homeostasis. In this study, we show that two of the nine known MCPH centrosomal proteins, Centrosomin (Cnn) and spindle assembly defective-4 (Sas-4) are required for starvation-induced

autophagy. These centrosome proteins regulate an early stage of autophagosome assembly. Our results implicate a new etiological basis of MCPH, from being a mitotic division defect in neural progenitors, to a metabolic disorder. A role for MCPH centrosomal proteins in autophagy regulation is a major paradigm shift for the basis of MCPH syndrome, and also a previously unexplored role for centrosomal proteins in differentiated cells.