Eastern Carolina Chapter of the Society for Neuroscience Presents:

18th Annual Neuroscience Symposium
Catalyst for Collaboration

Featuring:
Sergi Ferré, Ph.D.
Senior Investigator
Integrative Neurobiology Section
National Institute on Drug Abuse

“Allosterism within G Protein-Coupled Receptor Oligomers: Back to Symmetry”

Monday, October 31st, 2016
East Carolina Heart Institute
www.ecu.edu/neurochapter

(Courtesy: Dr. Johanna Hannan, ECU)
The Eastern Carolina Chapter of the Society for Neuroscience would like to express our sincere gratitude to the following entities for their generous support of the 2016 Neuroscience Symposium:

The Society for Neuroscience
North Carolina Biotechnology Center
Charles River
Multidisciplinary Studies Program in Neuroscience, ECU
Neuroscience Student Association, ECU
The Harriet and John Wooten Laboratory for Alzheimer’s and Neurodegenerative Diseases Research

Officers:
President: Dr. Stefan Clemens
Past-President: Dr. Tuan Tran
President-Elect: Dr. Alexander Murashov
Treasurer: Dr. Brian McMillen
Interim Secretary: Dr. Kori Brewer

Council Members:
Dr. Kelly Harrell
Dr. Fadi Issa
Dr. Xiaoping Pan

Student Council Members:
Aenia Amin
Katie Clements
Kayla Thompson
Neuroscience Symposium Schedule

October 31st, 2016

9:00 – 10:00
Registration (atrium)
Coffee / juices with students and Dr. Sergi Ferré (conference room)

10:00 – 11:30
Poster Session 1 / Coffee / Vendor exposition (atrium)

11:30 – 11:45
Opening remarks (conference room)
Dr. Clemens, Chapter President
Dr. Paul Gemperline (Dean, ECU Graduate School)

11:45 – 12:45
Sergi Ferré, MD/PhD, Senior Investigator, Integrative Neurobiology Section, NIH, National Institute on Drug Abuse, Bethesda, MD
“Allosterism within G Protein-Coupled Receptor Oligomers: Back to Symmetry”

12:45 – 2:45
Poster Session 2 / Heavy hors d’oeuvres / Vendor exposition (atrium)

2:45 – 4:15
Faculty / student presentations (conference room)

2:45 – 3:10
Sungwoo Ahn, PhD, Department of Mathematics
“Dynamics of Neural Synchrony in Mesocorticolimbic System of Rodents Performing a Pavlovian Drinking Task”

3:10 – 3:25
Tessa Holland (Doctoral Candidate, Department of Pharmacology & Toxicology)
“The Phytocannabinoid THC Mimics Effects of Chronic Mild Stress to Reduce Dendritic Spine Density in the Vocal Learning-Essential Brain Region Area X of Zebra Finch Striatum”

3:25 – 3:50
Chris Mizelle, PhD, Department of Kinesiology, ECU
“Movement Neuroscience: Studies of Cognitive Motor Control and Sensorimotor Integration”

3:50 – 4:15
Karen Litwa, PhD, Anatomy and Cell Biology, ECU
“Peering into the Patient Brain: Neurodevelopment in a 3-D Autism Model”

4:15 – 4:30
Closing remarks and Awards (conference room)
Podium Presentations
(in order of presenter)
The Monod-Wyman-Changeux model provided the most influential interpretation of allosterism within the frame of a symmetric oligomeric structure of regulatory enzymes. The initial studies of allosteric properties of G protein-coupled receptors (GPCRs) departed from these classical concepts of allosterism, considering GPCR monomers as main functional units. However, the phenomenon of GPCR homo- and heteromerization is becoming widely accepted. A new concept is that the pentameric structure constituted by one GPCR homodimer and one heterotrimeric G protein provides a main functional symmetric unit and oligomeric entities can be viewed as multiples of dimers. GPCR heteromerization opens up the possibility of allosteric interactions between different orthosteric ligands. Furthermore, the same properties of allosteric ligands demonstrated when considering GPCR as putative monomeric entities, mainly saturability, ability to separately alter the affinity and efficacy of the orthosteric ligand, probe dependence and functional selectivity, are also demonstrable with interactions between orthosteric ligands within the GPCR heteromer. A GPCR heterotetramer constituted by two molecularly different homodimers coupled to their cognate G protein and to adenylyl cyclase seem to constitute a common structure of a GPCR heteromer. Recent studies indicate that the canonical Gs-Gi interaction at the adenylyl cyclase level is a specific property of the GPCR heterotetramer. The evidence for GPCR oligomerization and the elucidation of symmetrical minimal functional units of GPCR homomers and heteromers, brings back the classical concepts of allosterism and promotes oligomerization and allosterism within GPCR oligomers as necessary elements in the research of GPCR physiology and pharmacology.
Dynamics of Neural Synchrony in Mesocorticolimbic System of Rodents Performing a Pavlovian Drinking Task

S. Ahn, A.M. McCane, L. Rubchinsky, S.S. Janetsian, D.N. Linsenbardt, C. L. Czachowski, C. C. Lapish

East Carolina University, Indiana University-Purdue University Indianapolis

Identifying neural circuits that process stimuli predictive of drug availability and the experience of drug taking is critical for the development of novel approaches to treat alcohol dependence. The present study explored how neural synchrony was altered in the mesocorticolimbic system by simultaneously recording local field potentials (LFPs) from the prefrontal cortex (PFC), nucleus accumbens (NA), and ventral tegmental area (VTA) of Wistar and P rats engaged in a Pavlovian conditioning task, where presentation of a stimulus light (CS) predicted the availability of ethanol (US). While, overall, synchrony was significantly stronger between the NA and PFC than the PFC-VTA or VTA-NA in both strains, P rats showed reduced NA-PFC synchrony compared to Wistars. However, during drinking, robust increases in theta synchrony were observed in P rats, and to a lesser extent in Wistars. These data further implicate aberrant connectivity between the PFC and NA in cued alcohol seeking behaviors. Additionally, these data suggest a link between impaired PFC-NA connectivity and genetic vulnerability to excessively seek and consume alcohol.
The Phytocannabinoid THC Mimics Effects of Chronic Mild Stress to Reduce Dendritic Spine Density in the Vocal Learning-Essential Brain Region Area X of Zebra Finch Striatum

Tessa L. Holland and Ken Soderstrom

Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834

Zebra finches learn a complex song during a developmental sensitive period through a process of sensorimotor integration and auditory feedback, which shares features with language development in humans. Chronic treatment with synthetic cannabinoid full agonist WIN 55,212-2 during this developmental period persistently alters song quality, suggesting that exogenous cannabinoid agonist exposure alters normal late-postnatal brain development.

Presently, we are studying the persistent effects of psychological stress on vocal development and adult song patterns. An acute stressor activates the hypothalamic-pituitary-adrenal (HPA) axis, causing increased release of the stress hormone corticosterone. This stimulates endocannabinoid release as part of a mechanism of feedback inhibition on HPA activity. We hypothesize that enhanced endocannabinoid signaling following psychological stress may alter song learning in a manner similar to that caused by exogenous agonist exposure.

Developing (50 days old) and adult (>100 days old) male zebra finches (n=4) were administered vehicle or THC (3 mg/kg) treatments under concurrent no stress or stress conditions daily for 25 days. Injections occurred at 11 AM, and stress treatments were administered at 2-7 PM using a mild unpredictable stress paradigm. 2-3 stressors were randomly chosen per day, and possible stressors included restraint stress (30 min), white noise (1 h), rubber snake (1 h), bright light (1 h), or food and water deprivation (1 h). Following the 25 day treatment period, zebra finches received no treatment for >25 days, until developing animals had matured. Vocalizations were recorded for 24 hours, and brains were collected for Golgi-Cox staining.

Area X is a striatal song region necessary for vocal learning as part of a basal ganglia-thalamocortical circuit. Both THC + No stress and Vehicle + Stress groups had reduced dendritic spine densities relative to Vehicle + No stress controls (p<0.05, two-way ANOVA, Student-Newman-Keuls post-test). In contrast, spine densities following treatments in adult groups did not differ.

We hypothesize that chronic THC treatment or stress during development, but not during adulthood, will persistently alter song quality. This is currently being tested via analyses of song recordings. Results may illuminate persistent effects of chronic THC or stress exposure on late postnatal brain development.
Movement Neuroscience: Studies of Cognitive Motor Control and Sensorimotor Integration

J.C. (Chris) Mizelle, Ph.D.
Department of Kinesiology, East Carolina University, Greenville, NC

Modern neuroimaging techniques give us an incredible ability to study the underlying neurobiology of complex phenomena, including many topics related to motor control. Along these lines, I will provide an overview of how neuroimaging can be applied to cognitive motor control and sensorimotor function. More specifically, my talk will focus on two aspects of motor control, beginning with how healthy young and older adults encode and understand complex, goal directed actions. Over recent years, we have worked to develop a conceptual model for how complex actions are encoded in the brain, and to then validate our model against brain activations recorded through functional MRI. Our findings shed new light into our understanding of how complex action representations are stored and recalled within the brain, and also may help to describe a basis for cognitive motor dysfunction commonly arising after neurological illness. I’ll then discuss some of my recent work in sensorimotor integration, which is concerned with how vision and somatosensation are used to help guide visuomotor behaviors, and what happens in the brain when these sensory signals are compromised. It is well known that both our visual and somatosensory feedback streams are important for accurate motor behavior, yet debate remains as to how the relative contribution of each is weighted in sensorimotor integration. I will describe a functional MRI study in which reliability of vision and somatosensation were reduced during a motor task, and how brain activations were altered in response to both.
Peering into the Patient Brain: Neurodevelopment in a 3-D Autism Model

Karen A. Litwa, Ph.D.
Department of Anatomy and Cell Biology, Brody School of Medicine, East Carolina University, Greenville, NC

A series of beautifully orchestrated and dynamic events drives the formation of trillions of synaptic connections within the brain, underlying cognitive function. The goal of our research is to use 3-dimensional brain models from patient samples to dissect out the molecular mechanisms of brain development and Autism pathology. My previous research demonstrated that regulation of the actomyosin cytoskeleton underlies synaptic development and plasticity. We are now translating these findings into a physiologically-relevant disease model, neurons derived from Autism patient induced pluripotent stem cells (iPSCs), to better understand how actomyosin regulation contributes to disease pathogenesis. While neurons from Autism patients initially form limited neurites as compared to controls, this defect can be rescued by inhibiting myosin II activity. Using 3-dimensional cerebral organoids (“mini-brains”), we observe disorganized neurite formation in Autism-derived samples together with an increase in the number of excitatory synapses, as has been previously described for post-mortem brain samples from Autism patients. We are now investigating how inhibition of myosin-II activity shapes neural networks in 3-D, and could serve to restore normal synaptic development in Autism. Thus, the use of patient samples together with a 3-D organoid culture system allowed us to create a model of idiopathic autism that captures the development of altered neural circuitry. This model provides a window into the molecular mechanisms that build neuronal connections as well as a platform to test how pharmacologic and genetic intervention alters brain development.
Poster Session 1
(in alphabetical order by presenting author)
The Effects of Prenatal Hormone Exposure on Associative Learning in a Rodent Model of Autism Spectrum Disorder

Aenia Amin¹,², Keith Dolman¹, Brooke Cantor³, Tuan D. Tran, PhD¹,²

¹Multidisciplinary Studies Program in Neuroscience, East Carolina University, Greenville, NC
²Department of Psychology, East Carolina University, Greenville, NC
³Department of Biology, East Carolina University, Greenville, NC

Purpose: Approximately 1 in 68 children are diagnosed with an autism spectrum disorder (ASD), and lifetime costs in the U.S. per child are estimated at $2.3 million. While the precise causes for abnormal brain development in ASD are not known, environmental contributions from endocrine disrupting chemicals (EDCs) – particularly androgens and estrogens - may play an adverse role. Prenatal hormone exposure (PHE) may affect hippocampal and cerebellar function, but the underlying mechanisms of disruption are not well-understood. In this study, we examined whether PHE affected two variations of eyeblink classical conditioning (ECC), each of which is mediated by the hippocampus or cerebellum. Because neuron number is an important indicator of behavioral function, the possible link between changes in neuron number and alterations in associative learning were correlated.

Methods: Pregnant Sprague-Dawley rats received daily injections of either dihydrotestosterone propionate (8 mg/kg), estradiol benzoate (50 µg/kg), or corn oil (vehicle) from embryonic days 15-19. Their offspring were tested as adults (postnatal day 90+) using trace or delay ECC. Neuron number was quantified using unbiased stereology within hippocampal cell layer CA1 (which supports trace ECC) and cerebellar regions that support delay ECC (interpositus nucleus and Purkinje cells).

Results: Preliminary results indicate altered learning in PHE rats. Learning was enhanced in animals that received trace ECC and impaired in those that received delay ECC, providing support for the idea that the hippocampus undergoes reorganization to mediate cellular activities that improve hippocampal function in humans with ASD (e.g., greater spatial reasoning) but at the cost of simple motor-related function served by the cerebellum.

Discussion: EDCs may produce organizational brain changes that underlie certain forms of ASD, as evidenced by neurobehavioral alterations in associative learning. Findings from this study may help elucidate the link between cellular changes and ASDs, so that treatments targeted at enhancing cellular function can be implemented.
The Effect of Social Stress on the Dopaminergic Pathway of the Escape Circuit in Zebrafish

Katie N. Clements, Thomas Miller, Eoon Hye Ji*, Fadi Issa

Department of Biology, East Carolina University
*Department of Physiology, David Geffen School of Medicine, UCLA

The occurrence of a chronic stressor causes an elongated state of physiological changes, affecting both peripheral and central body systems in a detrimental manner to the health of the organism. One approach to test the effects of a chronic stress is to induce a social hierarchy. Zebrafish form social hierarchies that consist of either socially dominant or subordinate fish. We have observed that once a social hierarchy has been established, behavior patterns between males reflect their social standing; social dominants display aggressive behaviors (attacks), while socially subordinates display submissive behaviors (retreats). The objective of this project is to determine the effects of chronic stress on dopaminergic neurotransmission and its effects on the highly characterized Mauthner escape circuit. When startled, zebrafish produce a stereotyped escape response that is mediated by auditory activation of the Mauthner command neurons which innervate spinal motor neurons. We tested the effects of social stress on the Mauthner escape response by recording the far-field potentials of fish escape to auditory pulses of increasing intensity (70-105 decibels). We observed that socially subordinate fish have a lower threshold for producing an escape when compared to both dominant and communal fish. These results suggest that a chronic state of stress on the subordinates influences the underlying neural signaling responsible for this escape behavior. To better understand the neural bases of social status-dependent change in Mauthner sensitivity, we tested whether social stress affects the dopaminergic system. Through application of the dopamine precursor, L-DOPA, we have found that the dopaminergic system is socially regulated. We have also applied dopamine receptor agonists and antagonists. We observed that chronic stress may be affecting the presence of dopamine 1 (D1) and dopamine 3 (D3) receptors in submissive animals, causing a sensitization in production of escape response. Gaining a better understanding of how chronic social stress influences the dopaminergic pathway will facilitate the advancement of new treatments of disorders that disrupt natural dopaminergic function.
Nicotine and THC Persistently Increase Cocaine Reinforcement in Zebra Finches Following Exposure During Periods of Vocal Learning but not During Adulthood

Julien Dodu*, Ahmed Aldhafiri, Ali Alalawi, Nariman Emadizadeh, Dr. Ken Soderstrom

*Multidisciplinary Studies Program in Neuroscience, East Carolina University, Greenville, NC
Department of Pharmacology and Toxicology, ECU Brody School of Medicine, Greenville, NC, 27834

Cocaine is effective in producing conditioned place preference (CPP) in rodents and many other species. CPP is useful for studying efficacy of manipulations to alter reinforcement. Because we have found that cannabinoid agonists alter vocal learning, we want to determine if this persistent behavioral change produced during development is associated with similarly persistently altered reinforcement. The current project was done to develop methods to study CPP in zebra finches. Modified two-compartment CPP chambers with environments distinguished by color and perch texture were constructed. Initial dose-response experiments indicated cocaine produces CPP in zebra finches with an EC50 = 2.5 mg/kg. Next we investigated effects of chronic developmental exposure to nicotine (0.4 mg/kg/day, given every morning for 25 days from 50–75 days of age, and then to adults for 25 days) and Δ-9-tetrahydocannabinol (THC, 3 mg/kg/day for the same 25 day regimen) on cocaine (2.5 mg/kg)-induced CPP during adulthood. The day following CPP, birds were randomly assigned to either cocaine- or vehicle-paired environments and placed within them for 15 min and brains prepared for anti-c-Fos staining to investigate brain regions activated by CPP. Dose-response experiments demonstrated increased c-Fos expression in song regions; Area X, lMAN, HVC and RA, and medial striatum following reinforcement with 5 and 10 mg/kg cocaine. Results of the nicotine/developing animals experiment demonstrated increased CPP scores (VEH=373+/-135 vs. NIC=702+/-70, p=0.03, n=7-8). Increased CPP was not seen following adult treatment (VEH=308+/-161 vs. NIC=336+/-157, p>0.05, n=7-8). Treatment during development with THC also increased CPP (VEH=90+/-226 vs. THC=660+/- 42, p=0.02, n=4). THC did not clearly increase CPP in adults (VEH=0+/-243, THC=135+/-258, p>0.05, n=4). Our findings demonstrate that: cocaine-induced CPP may be productively studied in zebra finches; involves brain regions also important to vocal learning and production; and is selectively increased following developmental exposure to both nicotine and THC.
Endoscopic Third Ventriculostomy Treatment of Hydrocephalus Secondary to Tectal Glioma in a 20 Year Old Collegiate Tennis Player: A Case Report

Hannah Ellis

Department of Athletics-Athletic Training, East Carolina University

Tectal gliomas are a rare aycotic brainstem tumor that often goes undetected until complications associated with hydrocephalus present. Treatment options include surgical interventions such as ventricular-peritoneal shunt placement or endoscopic third ventriculostomy (ETV) to control the symptoms caused by the aqueductal stenosis. When a tectal glioma is the underlying cause to non-communicating hydrocephalus, treatment of the increased intracranial fluid should be first addressed. This case report of a 20 year old division 1 collegiate tennis player underwent two ETV procedures to address hydrocephalus symptoms secondary to an aqueductal stenosis from a tectal glioma. In addition to surgery, treatment included postural manual therapy exercises designed to promote spinal fluid movement. The patient presented with diffuse headache and intermittent dizziness associated with muscular spasms of the upper back and neck. Soft tissue manual therapy and trigger point release were unsuccessful in relieving the increasing symptoms. Differential diagnoses included: dehydration, migraines, nutritional unbalance, muscle tightness, and/or eye strain. Magnetic resonance Imaging (MRI) showed hydrocephalus and aqueductal stenosis and was addressed by ETV. Later, computed tomography (CT) was obtained after increase of pain, pressure, and vomiting after ETV, showing increased ventricular size and intraventricular hemorrhage. An external ventriculostomy drain was used for 3 days before removal and discharge. Following this procedure the patient was able to return to tennis type activities within 3 weeks, although still experiencing intermittent mild headaches without the associated nausea, dizziness, or other related symptoms. The patient was cleared without restrictions 2 months after the first ETV, but developed a slow progression of symptoms similar to the initial presentation over approximately 4 months. Repeat MRI showed that the first ETV failed and the fluid buildup caused a recurrence of symptoms. A second ETV procedure was scheduled, and a reserve was placed to monitor fluid movement and offers an ability to drain excess fluid. This case study allows for further discussion in the literature about the prevalence of hydrocephalus treatment in a young adult population via ETV and other possible treatment options, including manual therapy and postural longitudinal stretching techniques.

Key Words: Tectal glioma, Endoscopic third ventriculostomy, hydrocephalus, postural manual therapy
The Effect of Visual Queues on Social Status Formation and Mauthner Sensitivity in Zebrafish (*Danio Rerio*)

Keever, Jared, Issa, Fadi, Clements, Katie, Miller, Thomas

Department of Biology, East Carolina University, Greenville, NC, United States

Zebrafish are freshwater fish with vibrant body coloration consisting of alternating strips of blue and white. It is thought that during social interactions zebrafish use these visual cues to communicate with their conspecifics their aggressive levels to facilitate the formation of stable social hierarchies that consist of either dominant (aggressive) and submissive (passive) animals. Our lab demonstrated that social status affects the activation pattern of the neural network underlying the startle avoidance response mediated by the Mauthner circuit. Submissive animals are more sensitive to auditory stimuli compared to dominant animals. However, the importance of body coloration in dominance formation and its effects on the sensitivity of the Mauthner circuit is poorly understood. The first objective of this project is to determine the importance of visual cues in the formation and stability of social relationships between male zebrafish that completely lack skin pigmentation by using the mutant zebrafish line Tüpfel Long-fin Nacre (TLN). Our second objective is to determine whether improper visual social cues in the TLN mutant line affects the activation pattern of the Mauthner neural circuit. We show that the social interactions in the TLN mutants are characterized by elevated levels of aggression that persisted over time, which led to unstable social relationships. Consequently, the activation pattern of the Mauthner neural circuit did not differ between dominant and submissive animals, contrary to the result in wild-type fish. These results indicate that proper visual cues play an important role in regulating social interactions and fostering the formation of stable social structure, the lack of which significantly affects the activation pattern of the Mauthner neural circuit.
Metabolic Control By Medial Basal Hypothalamic Glutamatergic Neurons

Laing, Brenton1,2,3 Prete, Amber3, Bunner, Wyatt1,2, Huang, Hu1,2,4

1 Department of Kinesiology, East Carolina University, Greenville, NC, United States
2 Diabetes and Obesity Institute, East Carolina Heart Institute, Greenville, NC, United States
3 Multidisciplinary Studies, East Carolina University, Greenville, NC, United States
4 Department of Physiology, East Carolina University, Greenville, NC, United States

Introduction: Obesity arises from improper balance of energy intake and expenditure. The medial basal hypothalamus (MBH) in the brain coordinates energy balance. The MBH contains neuron populations including anorexigenic pro-opiomelanocortin (POMC) neurons and orexigenic Agouti-Related Peptide (AgRP)/Neuropeptide Y (NPY) neurons. Another population of glutamatergicMBH neurons exists, but their role to control metabolism via post-synaptic targets remains unclear. We investigated gain/loss of function in glutamatergicMBH neurons on energy balance in mice.

Methods: By using a Vglut2-Cre knock-in model that expresses Cre-recombinase in glutamatergic neurons combined with the Designer Receptor Exclusively Activated by Designer Drug (DREADD) technique, we investigated the effect of gain/loss of glutamatergicMBH neuron function on whole body metabolism. We assessed energy intake under fed/fasted status by tracking food consumption and energy expenditure by Comprehensive Lab Animal Monitoring System (CLAMS). We measured metabolic parameters after intraperitoneal saline or designer drug clozapine-N-oxide in the same animal. Furthermore, we crossed a Vglut2-Cre mouse with a NPY-GFP reporter mouse for double transgenic Vglut2-Cre x NPY-GFP. We are using this model for Channelrhodopsin2 Assisted Circuit Mapping (CRACM) to find post-synaptic NPY targets of glutamatergicMBH neurons.

Results: Loss of function (n = 5) of glutamatergicMBH neurons results in decreased night time (p < .05) and fasting induced food intake (p < .05), while gain of function (n = 8) increases fasting induced food intake (p < .05) but not night time food intake. After inhibition of glutamatergicMBH neurons VO2 (p = .08) is reduced, but there is no effect on VCO2 (p = .79), resulting in increased respiratory exchange ratio (RER) (p = .07). After activation of glutamatergicMBH neurons, VO2 (p = .14) and VCO2 (p = .09) both decreased, notably resulting in decreased RER (p = .03). We demonstrated feasibility of glutamatergicMBH neuron expression of channelrhodopsin2 (ChR2), and optical activation of ChR2 expressing glutamatergic neurons to excite targets for forthcoming studies.

Discussion: Our data demonstrate that glutamatergicMBH neurons contribute to energy balance by driving energy consumption and conservation. We are searching for a mechanistic switch for controlling energy balance as an intervention for obesity and disorders of glutamatergic synaptic integration.
A Genetically-Encoded N-Cadherin Tension Sensor Reveals Localized Tension Associated with Synapse Maturation

Karen Litwa, Aarti Urs, Rick Horwitz, and Brenton Hoffman

East Carolina University, Duke University, University of Virginia

Dendritic spines are actin-enriched protrusions that serve as the major site of excitatory neurotransmission in the central nervous system. NMDA receptor excitatory stimulation activates the actin-associated motor protein, non-muscle myosin II, driving spine maturation and synapse formation. In non-neuronal cells, the contractile activity of non-muscle myosin II exerts forces on actin-associated adhesions. Using a genetically encoded N-Cadherin FRET tension sensor, we tested whether non-muscle myosin II activity similarly generates force across the synapse. Our results demonstrate that non-muscle myosin II drives localized forces specifically across mature synapses. Myosin II inhibition disrupted tension across N-Cadherin coincident with a reversion to immature spine morphologies. Furthermore, synaptic tension increased in response to NMDA receptor activation. Finally, using an SH2 biosensor, we observe a corresponding decrease in Src signaling and Src-mediated phosphorylation of NMDA receptors in response to myosin II inhibition. We thus demonstrate the existence of synaptic forces that underlie localized signaling events, leading us to propose that the synapse functions as a mechanosensory unit of the brain.
Restless legs syndrome (RLS) is a chronic sensorimotor disorder characterized by “creepy-crawling” discomfort in the limbs and the urge to move the legs. Symptoms occur in the evening and can severely disrupt sleep, and currently, the first line of drug therapy for RLS are dopamine (DA) compounds that target the inhibitory D3 receptor subtype. Two animal models have been proposed for RLS: dopamine D3 receptor knockout (D3KO) and MEIS1 knockout mice (MEIS1KO). D3KO mice express hypersensitivity, increased locomotor activities, and hypertension. The MEIS1KO model was developed after genome-wide association studies suggested a SNP mutation around MEIS1 in RLS patients, but its behavioral phenotype has not yet been tested. Here, we tested and compared thermal pain withdrawal reflexes as a model of acute pain sensitivity in MEIS1KO and D3KO animals and their respective controls. Animals were subjected to levodopa (10 mg/kg), pramipexole (D3R agonist, 0.5 mg/kg), SKF 38393 (D1R agonist, 1 mg/kg), SCH 39166 (D1R antagonist, 0.1 mg/kg), or morphine (2 mg/kg). At baseline conditions, MEIS1KO were not significantly different from controls, while D3KO showed a significant decrease in reflex latencies. L-dopa induced an increase in latencies in control and MEIS1KO but not D3KO, while SCH 39166 led to a significant increase in both D3KO and MEIS1KO. In contrast, control and WT and MEIS1KO mice showed significant increases in latencies after treatment with morphine, while D3KO mice were morphine-tolerant. Together, these data indicate that D3KO animals exhibit sensorimotor features associated with RLS in the clinic, while MEIS1KO animals expressed a differential effect over controls predominantly after treatment with D1R-targeting compounds. As the D1R plays an important role in locomotor function, we hypothesize that D3KO may represent the sensory features of RLS, while MEIS1KO might become a model for the periodic leg movements observed during sleep (PLMS) that are regularly observed in RLS patients.
GMCSF-MOG Abrogates Experimental Autoimmune Encephalomyelitis (EAE) Through the Induction of MOG-Specific Regulatory T Cells

Cody D. Moorman, Alan D. Curtis, II, Mark D. Mannie Ph.D.

East Carolina University

FoxP3+ CD25high regulatory T-cells (Tregs) play a crucial role in maintaining peripheral tolerance by suppressing auto-reactive T-cells. Developing a safe therapeutic approach to induce autoantigen-specific Tregs could provide an effective treatment for Multiple Sclerosis (MS), as well as for other autoimmune diseases. Previous studies have shown that the fusion protein GMCSF-MOG has tolerogenic activity that inhibits experimental autoimmune encephalomyelitis (EAE) in mice. In an effort to elucidate the mechanism by which GMCSF-MOG is inhibiting EAE, we investigated the ability of GMCSF-MOG to induce Tregs in vivo. In this study, we provide evidence that GMCSF-MOG can induce MOG-specific FOXP3+ Tregs. Treatment of 2D2-FIG mice with GMCSF-MOG resulted in an increase of FOXP3+ Tregs in the blood, with an additional increase observed after multiple immunizations. Furthermore, the depletion of GMCSF-MOG-induced Tregs by use of the anti-CD25 mAb PC61 restores susceptibility to EAE in C57BL/6 mice. In conclusion, subcutaneous immunization with GMCSF-MOG induces antigen-specific Tregs, which play a role in the inhibition of EAE in mice.
Obesity is a world-wide health problem. While both genetics and environment contribute to obesity, genetic variants cannot explain neither the obesity epidemic nor a relatively high heritability of obesity. Therefore, there is an urgent need to identify other forms of variation, such as epigenetics marks. We have previously shown that long-term exercise programs mouse offspring for lower energy expenditure and increased risk for obesity. The purpose of this experiment was to determine if similar heritable changes occur for metabolic phenotype in Drosophila melanogaster when they are either placed on exercise or a high-sugar diet and if these changes could be tracked to alterations in miRNA content. Based on our previously published data in a mouse model, we hypothesized that chronic exercise in flies will program offspring for “thrifty phenotype” which will be transmitted to F1, F2 and F3. In this experiment, flies were separated into groups of 15 males and exercised or subjected to control or high caloric diet and subsequently bred with virgin control females to generate F1. Metabolic changes were monitored by measuring weight and motor activity, triglycerides, glucose and trehalose for the flies. Statistical analyses were conducted using One-Way ANOVA (Analysis of Variance) and the Tukey Multiple Comparisons test to check for significant differences. We found that our data fell in line with previously collected data, in that the exercise father offspring were programed for lower energy expenditure and were therefore at a higher risk for obesity. Our next steps include conducting PCR and Western blot assays in order to determine the effects of diet and exercise on miRNAs and metabolic genes expression between the different groups of F1, F2 and F3 flies. If differences exist, they could help to delineate epigenetic biomarkers of metabolic phenotype. Eventually, this data could help create an epigenetic test for humans which would predict the likelihood of someone developing metabolic disorders such as Type 2 diabetes or obesity.
Changes in Protein Levels During the Development and Decay of Heterologous Tolerance to Morphine at Varying Lengths of Treatment

Schmitt, Mark¹, Masterson, Jackie², Thompson, Benjamin², Taylor, David A.²

¹Multidisciplinary Studies Program in Neuroscience, East Carolina University, Greenville, NC
²Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC

Our hypothesis is that heterologous tolerance brought on by chronic morphine treatment is an adaptive process caused by alterations in cell signaling. Heterologous tolerance onset and decay is well documented, and previous studies defined a time table for the onset and decay of tolerance to the analgesic effects of morphine following subcutaneous (sc) injection using paw pressure testing. In addition, tolerance to the inhibitory response to morphine and 2-chloroadenosine was measured in the longitudinal muscle/myenteric plexus (LM/MP) obtained from guinea pigs. Treatment for 1, 2, 4, 7, and 10 days was assessed. Changes in cellular signaling proteins, were evaluated using, western blot method and protein densitometry to analyze different known signaling proteins. Western blot data on LM/MP homogenates has shown no change in the number of µ, A₁, or A₂a, receptors at any time. It was also found that the A₂b receptor and the β1 receptor as well as PKCε in the LM/MP showed no significant changes in protein abundancy. Recent studies have shown that the α₃ subunit of Na+/K+ ATPase decreased as tolerance developed then returned to the baseline after tolerance decayed. The same was found for PKCγ in the brainstem, that upon the induction of heterologous tolerance a decrease in this protein was found, and reversed back to baseline as tolerance decayed. PKCγ is found in the brainstem which is involved in the analgesic response to morphine, but is not found in the LM/MP. The decrease in concentration of the α₃ subunit of the Na+/K+ ATPase in the LM/MP and PKCγ of the brainstem has led us to suggest that the cellular mechanisms of tolerance development and decay in the LM/MP and brainstem may differ.
Can’t Sleep? Individual Differences and Resting Frontal Alpha Asymmetry Are Related to Insomnia and Sleep Quality Components

Alexandra J. Stephenson¹, Andrea R. Winters¹, Ansley T. Corson, Eric M. Watson¹, & D. Erik Everhart¹,²

¹Department of Psychology, East Carolina University, Greenville, NC
²Vidant Sleep Center, Vidant Medical Center, Greenville, NC

Poor sleep is related to chronic health conditions, neurocognitive dysfunction, and impaired daily functioning. Reinforcement Sensitivity Theory (RST) has been employed to investigate individual differences associated with sleep disorders. RST consists of Behavioral Activation (BAS) and Behavioral Inhibition (BIS) Systems. BAS is associated with left frontal activity and approach, while BIS is associated with right frontal activity and withdrawal. In this study 75 college students were utilized to examine the relationships between BIS, BAS, sleep quality using the Pittsburgh Sleep Quality Index (PSQI), self-reported symptoms of insomnia using the Insomnia Severity Index (ISI), and baseline alpha (8-13 Hz) frontal asymmetry scores (log(right alpha)−log(left alpha)) for five frontal scalp site pairs. Higher asymmetry scores indicate relatively greater left frontal activity. It was hypothesized that BIS, and greater right than left frontal activity would be related to greater symptoms of insomnia on the ISI, and to components of the PSQI. This was partially supported. BIS was associated with the ISI, \( r(75) = -.479, p < .01 \), and with daytime dysfunction of the PSQI, \( r(75) = .337, p < .01 \). Further, greater right activity was weakly associated with sleep duration for F4-F3, \( r(52) = -.279, p < .05 \), while greater left activity was associated with latency for F4-F3, \( r(52) = .333, p < .05 \), and FC4-FC3, \( r(50) = .310, p < .05 \), and efficiency for F8-F7, \( r(53) = .453, p < .01 \), F4-F3, \( r(52) = .308, p < .05 \), FT8-FT7, \( r(52) = .354, p < .01 \), and FC4-FC3, \( r(50) = .300, p < .05 \). These results suggest that individual differences and frontal asymmetry are related to insomnia and components of sleep quality. Implications for these findings are discussed.
Changes in Cognitive Demand, Impact Forces and Knee Joint Loading to Reduce Risk Factors Associated with Tibial Stress Fractures in Response to In-field Gait Retraining

Dr. Nick Murray¹, Dr. Richard Willy², Tyler Whittier¹, Caitlin Melton¹

¹East Carolina University Department of Kinesiology
²East Carolina University Department of Physical Therapy

High impact forces and knee joint loading during running have been associated with both anterior knee pain and tibial stress fractures. Previous research during a single evaluation session has demonstrated that small increases in a running step rate (steps per minute) will decrease knee joint loading and impact forces during running. However, no research has been done to determine if runners can maintain the new running pattern especially outside the laboratory. Thus, it is unknown if runners are able to maintain a retrained running gait pattern in the field. In addition, it is unclear the increased cognitive demand of gait retraining. The primary purpose is to determine, if field-based gait retraining is effective in reducing cognitive demand as measured through EEG and promotes positive transfer of learning. Our hypothesis is that in-field gait retraining effectively reduces the faulty mechanics associated with tibial stress fractures without increasing cognitive workload following learning. Runners with a history of tibial injury completed a gait retraining protocol which included a baseline run on an instrumented treadmill to establish original gait pattern followed by a retraining feedback phase in which participants increased their step rate by 10%. EEG was collected in both phases. Results demonstrated an increase in cognitive demand based on an increase power in the beta bandwidth, increased theta activity at frontal sites and suppression of alpha activity during initial retraining with a corresponding decrease in EEG indicators of cognitive demand following learning (p < .01). Furthermore, increasing step rate reduced high impact forces and knee joint loading during running following retraining. Overall, the results demonstrated the use of EEG as an effective tool to measure cognitive demand during running and the effectiveness of in-field retraining to reduce high impact forces and knee joint loading.
The Therapeutic Effect of Rho Kinase Inhibitor Y-27632 on Protection from Chemotherapy-Induced Peripheral Neuropathy in a Tumor-Bearing Mouse Model

Yi Zhu¹,³, George A Howard IV², Keith Pittman², Christi Boykin¹, Kathryn Verbanac², Qun Lu¹,³

¹Department of Anatomy and Cell Biology; ²Department of Surgery, ³The Harriet and John Wooten laboratory for Alzheimer’s and Neurodegenerative Diseases Research, The Brody School of Medicine at East Carolina University, Greenville, North Carolina 27834

Cisplatin often causes loss of touch sensitivity in the hands and feet of cancer patients as well as tingling, numbness, and a shooting or burning pain; these clinical symptoms are referred to as chemotherapy-induced peripheral neuropathy (CIPN). CIPN frequently results in a reduction or cessation of chemotherapy, and there is currently no effective intervention or prevention for CIPN. Therefore, it is important to understand the mechanism of CIPN pathogenesis and determine associated signaling pathways to identify potential therapeutic targets. Previously, we created a CIPN mouse model in 3 month old C57/BL6 mice by injections of 6µg/g cisplatin every 14 days. Our data indicated that the RhoA signaling pathway was responsible for attenuating CIPN since the preserved touch sensitivity was achieved in cisplatin-treated mice by injecting LM11A-31, a p75 neurotrophin receptor ligand mimetic that serves as an upstream inhibitor of RhoA signaling pathway. In order to fully capture the clinical situation, we extend this approach to CIPN in a syngeneic murine Lewis Lung Carcinoma (LLCab) model in which mice were concurrently treated with weekly injections of cisplatin and a RhoA pathway inhibitor. In this tumor-bearing CIPN mouse model, we determined the therapeutic effectiveness of Y-27632 that selectively inhibits ROCK, a downstream effector of the RhoA signaling pathway, in peripheral neuroprotection. Von Frey filament analysis of hind paw touch sensitivity indicated that Y-27632 treatment protected tumor-bearing mice from cisplatin-induced reduction of touch sensation. Furthermore, immunohistochemical analysis of cutaneous nerve fibers in foot pad tissue, which was acquired from the corresponding hind paw, demonstrated that the cisplatin-induced decrease in touch sensory associated-cutaneous nerve fibers could be alleviated by the concurrent treatment with Y-27632. Therefore, Rho kinase inhibitor Y-27632 can potentially protect peripheral nerve function in the tumor-bearing CIPN mouse model. Supported by grants from NIH CA111891 and NIH CA165202 as well as the Harriet and John Wooten Laboratory for Alzheimer’s and Neurodegenerative Diseases Research.
Poster Session 2
(in alphabetical order by presenting author)
Alzheimer’s disease (AD) is the leading cause of dementia. AD is characterized by many abnormal changes in the brain including two hallmark morphological changes, amyloid-β plaques and neurofibrillary tangles. Amyloid-β accumulation is linked to increased expression of Dickkopf-1 (Dkk-1), which may play a role in synaptic loss observed in AD. Kremen1 is a receptor for Dkk-1 and can be silenced by siRNA. This research study focuses on preventing synaptic loss and memory deficits, both of which are associated with AD, in the triple transgenic (3xTg) mouse model. It is hypothesized that application of siRNA in vivo will prevent synaptic loss and memory deficits in the 3xTg mouse model by downregulating Kremen1. Tail vein injections of RVG-9R/siRNA complexes and control injections were administered to groups of 3xTg and wild-type (WT) mice. In order to measure memory function, the Barnes Maze was used. The Barnes maze is designed to test spatial learning and memory in rodents. Primary latency, primary errors, total errors, and trial time were measured and recorded for analysis. Following completion of the Barnes Maze mice were sacrificed and brains were collected. The brains were analyzed for Kremen1 downregulation at the protein and mRNA levels. Immunohistochemistry (IHH) was performed on coronal brain sections. Kremen1 and TUJ1 antibodies as well as DAPI were used for IHH. These experiments were performed on mice at three different ages: 4 months, 6 months, and 12 months of age. Within each age cohort there were three different groups: 3xTg mice injected with RVG/siRNA, 3xTg mice injected with control peptide/siRNA, and WT mice injected with saline. Each group of mice was approximately half male and half female. The results of this study show increased primary errors and total errors in the Barnes Maze for 3xTg mice compared to WT mice. Results also show a lower percentage of 3xTg mice than WT mice were able to find the target in the Barnes Maze. The results from the study thus far show trends indicating that AD mice have behavioral deficits. This study illustrates potential for testing memory function when Kremen1 downregulation is targeted.
Pictures of Entoptic/Phosphene Visual Experiences: A Normative Study by Trained Artists

Cynthia Bickley-Green

School of Art and Design, East Carolina University, Greenville, NC, United States

Physicists, psychologists, art educators, medical doctors, space scientists, and anthropologists have written about entoptic/phosphene visual experiences. Hermann von Helmholtz, (1925) described entoptic experience as visual experience caused by biological structures. Tyler (1976) defined several entoptic phenomena and extended this category of visual experience to include “a range of conditions from free-viewing with closed lids to deep pressure on the eyeballs.” The representation of entoptic phenomena has been associated with artistic forms since at least 1965 when R. Kellogg, an art educator working with M. Knoll, noted form similarities between the description of phosphenes experienced by adults and young children’s scribbles. Kellogg used these basic visual experiences as represented in child art to catalog development in drawing. Hugo Ruberti (1991) used the same phosphene patterns as one source of imagery in the paintings of European abstract expressionists. Building upon the work Knoll and Kellogg, Vitz and Kamorina (2014) propose a hierarchical model of image construction that could be used both to identify drawing development in youth and deterioration of drawing abilities in adults experiencing mental pathologies. Interestingly, light flashes and phosphene perception is an area investigated by space scientists. Given the range of interdisciplinary curiosity about entoptic/phosphene experiences, it was motivating to explore this imagery in a typical art studio with highly trained artists drawing their visual experiences of entoptic/phosphene forms.

For the research project in the School of Art and Design, I asked accomplished artists to draw the appearance of entoptic experiences in different ambient conditions such as changing levels of sound and illumination and in performing different tasks such as humming and lifting twenty-ounce weights. This poster presentation shows some of the visual results of this normative art study and compares the images to those collected by other researchers and their participants.
The 2016 WHO classification of glial neoplasms of the central nervous system (CNS) now incorporates molecular data within diagnostic classification schemes, supplementing the previous classification schemes based on subjective data including phenotypic, histologic, and immunohistochemical findings (e.g. expression or lack of expression of glial or neural antigens) (1). Critical molecular data points for the diagnostic classification of glial neoplasms include the presence or absence of mutations in the IDH1 and ATRX genes and concomitant deletions of chromosomes 1p and 19q. Using this data, the controversial and subjective category oligoastrocytoma has been essentially eliminated. Molecular changes found to be present in high frequency in specific pediatric-predominant neoplasms (e.g. BRAF mutation in pleomorphic xanthoastrocytoma and BRAF-KIAA fusion in pilocytic astrocytomas) are now incorporated into the diagnosis of some of the childhood-predominant neoplasms. Using illustrative cases, this study summarizes the key changes and describes their application to the diagnostic process at an academic medical center. Incorporation of molecular data (1) recognizes early changes that take place in most adult astrocytomas and oligodendrogliomas, (2) acknowledges the limitations of phenotypic classification by histology and immunohistochemistry alone, and (3) reduces some of the subjective elements in the diagnosis of CNS neoplasms. The proposed changes help to optimally distinguish “primary” glioblastomas, which follow an aggressive course, from low grade astrocytoma, “secondary” glioblastoma, and oligodendroglioma entities. Clinical implications of this more rigorous classification scheme include more precise categorization of patients within specific treatment protocols. Highlighting the need for additional studies and periodic updates to the diagnostic workup, a significant subset of neoplasms will remain unclassified by the proposed scheme including, (1) among mostly adult-presenting neoplasms, a proportion of low grade astrocytomas and, (2) among mostly childhood-presenting neoplasms, most astrocytomas and oligodendrogliomas.

The Effects of Acute Exercise on Neuron Activity Levels in the Arcuate Nucleus of the Hypothalamus

Wyatt Bunner\textsuperscript{1,2}, Brenton Thomas Laing\textsuperscript{1,2,3}, Hu Huang\textsuperscript{1,2,3,4}

1. Department of Kinesiology, East Carolina University, Greenville, North Carolina, USA
2. East Carolina Diabetes and Obesity Institute, East Carolina University, Greenville, North Carolina, USA
3. Human Performance Laboratory, College of Human Performance and Health, East Carolina University, Greenville, North Carolina, USA
4. Department of Physiology of East Carolina University, Greenville, North Carolina, USA

Background: While much is known about pro-opiomelanocortin (POMC) and neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons control of energy balance, less is known about how forced energy expenditure manipulates these neurons and relates to energy intake. We investigated the effects of acute exercise on neuron activity in the arcuate nucleus of the hypothalamus. By improving understanding of hypothalamic response to exercise, we pave the way to mimic the effects of exercise on energy balance.

Methods: To investigate the effect neuron activation immediately post-exercise, we randomly assigned cohort 1 mice to a treadmill exercise group or sedentary group. We introduced the exercise group to a treadmill with a speed between 12-13 M/min and an 8.75% grade for a duration of an hour while the control group was on at speed of 0 M/min. Immediately after exercise, brains harvested. Next, in cohort 2 we assessed food consumption at 1, 3, 6, 9, and 24 hours post exercise. To determine the effects of exercise training to drive energy intake, in cohort 3 we conducted a delayed sacrifice experiment whereby mice received an hour of sedentary or exercise conditions and were sacrificed after a 2-hour daylight fasting period. cFOS immunofluorescence was conducted to reflect recently depolarized neurons. Visualization of POMC neurons was obtained by POMC immunofluorescence while NPY visualization was enabled by NPY-GFP reporter mice. c-FOS fluorescent images were used to identify activation of specific neuron populations.

Results: Immediately after exercise, c-FOS in the arcuate nucleus POMC are significantly increased compared to the control group (p = .001) while we observed no difference in c-FOS in NPY/AgRP neurons (p = .24). Moreover, based on our food intake study, the food intake discrepancy was greatest at 1-3 hours post-exercise. A two hour fast after exercise results in a trend towards greater NPY and cFOS co-localized neurons compared to sedentary with a two hour fast(p=.1).

Conclusion: We observed significantly reduced POMC neuron activation immediately after exercise. On the other hand, 2 hours fasting with an hour exercise training results in NPY neuron activation. We have demonstrated dynamics of POMC and NPY/AgRP neurons in response to exercise to energy expenditure.
Clinical, Radiologic, Surgical, and Pathological Evaluation of a Patient with Recurrent Symptoms Status-Post Percutaneous Intervertebral Disc Decompression: Case Report and Review of the Literature

Caldwell, Christopher1, Lee, Stuart2, Martin, Eric3, Boyer, Philip4

1Brody School of Medicine, East Carolina University, Greenville, NC, United States
2Vidant Neurosurgery, East Carolina University and Vidant Medical Center, Greenville, NC, United States
3Eastern Radiology, Greenville, NC, United States
4Department of Pathology and Laboratory Medicine, East Carolina University and Vidant Medical Center, Greenville, NC, United States

Various pathologic processes affect intervertebral discs including both degenerative disease and traumatic disruption which can manifest with symptoms associated with impingement on nerve roots or on the spinal cord. Treatment options include (1) conservative measures and (2) surgical removal of the disc with decompression of areas of impingement. Percutaneous disc decompression offers a “minimally invasive treatment” which employs various modalities (cannulation and extraction of disc material versus laser or plasma disruption without extraction) producing reduced intra-disc pressure and, ideally, reduction in symptoms. In this study we report clinical, imaging, intraoperative, and surgical pathology findings for a patient who underwent (1) Stryker “Disc Dekompressor” cannulation and partial disc extraction and (2) subsequent surgical excision of the affected disc. A detailed description of findings, including histopathologic changes, in a percutaneous procedure has not been reported to date. A 51-year-old man presented with a multiple-year history of both (1) low back pain and (2) subsequent buttock and left leg pain. Imaging studies identified a left L4-L5 paracentral and subarticular disc herniation abutting the left L5 nerve root. Conservative therapy was not successful and he underwent percutaneous decompression. While he experienced pain relief for approximately two weeks, the pain was subsequently recurrent and he ultimately underwent surgical disc excision nearly 7 months after the percutaneous procedure. Evaluation of pre- and post-procedure imaging studies did not reveal discernable differences. Intraoperatively, upon incision of the annulus fibrosis, soft and paste-like disk material exuded under pressure. Histologically, the disc showed moderate to severe degenerative and regenerative changes, similar to those seen in a consecutive group of 20 control cases. However, unlike in the controls, the cannulated specimen was markedly disrupted and fragmented and showed multifocal aggregates of variably fine, granular material. No evidence of thermal damage to the tissue was identified. In contrast, in the control discs, the disc material was largely intact and only very focal granular degenerative areas were noted. There was no evidence of an inflammatory infiltrate in either the cannulated or the non-cannulated specimens. Reporting of this data is important for quality control purposes with respect to the percutaneous procedure.
P300 Latency for Positively-Valenced Sleep Images is Reduced in Poor Sleepers

Ansley Taylor Corson\textsuperscript{1}, Alexandra J. Stephenson\textsuperscript{1}, Eric Watson\textsuperscript{1}, D. Erik Evehart\textsuperscript{1,2}

\textsuperscript{1}Department of Psychology, East Carolina University, Greenville, NC
\textsuperscript{2}Vidant Sleep Center, Vidant Medical Center, Greenville, NC

Event-related Potential (ERP) studies are limited when studying sleep due to the inability to behaviorally respond to stimuli. Using the oddball paradigm task to measure cortical arousal/excitability, ERP investigations attempt to assess information processing upon sleep onset or upon wakening as a means to examine daytime consequences. The current literature shows inconsistent results, with several studies identifying a significantly higher level of cortical activation (increased P300 amplitudes) for people with disordered sleep. Enlisting the participation of 37 undergraduate students, the present study examined subjective sleep quality in relation to recorded P300 amplitudes and latencies at electrode sites Fz, Cz, and Pz. ERP data were recorded within an oddball paradigm in which participants examined a series of valenced sleep-related images. Analyses were employed to examine group differences for P300 amplitude and latency. There were no significant findings associated with P300 amplitude. Analysis of P300 latency showed self-reported poor sleepers ($M = 339.91, SD = 51.86$) as having an earlier P300 onset than good sleepers ($M = 384.33, SD = 21.70$) at the Fz electrode site for positively-valenced sleep images, $t(18.41) = 3.50, p = .003, d = .94$. As compared to good sleepers ($M = 377.67, SD = 21.59$), the poor sleepers ($M = 339.80, SD = 49.49$) also demonstrated this significantly early P300 latency at the Cz electrode site, $t(17.35) = 3.02, p = .008, d = .84$. Results suggest poor sleep may have a paradoxical effect on information processing for visually appealing images.
Long-Term Treatment with Dopamine D3 Receptor Agonists Induces Augmentation-Like Phenotype that can be Rescued by Blocking the D1 Receptor

Dinkins, Mai-Lynne\(^1\), Lallemand, Perrine\(^2\), and Clemens, Stefan\(^3\)

\(^1\) Brody School of Medicine Department of Physiology, East Carolina University, Greenville, NC, United States

Restless legs syndrome (RLS) is a sensorimotor neurological disorder that affects between 15-30 million Americans. RLS is characterized by the uncontrollable urge to move one’s legs especially in the evenings, thereby perturbing falling asleep. RLS is primarily treated with dopaminergic drugs that target dopamine D3 receptors (D3Rs), however; after chronic use these drugs usually result in augmentation, a worsening of symptoms while on the treatment. We undertook this study to develop an animal model for augmentation, and to establish a timeline of the development of augmentation. Based on previous data, we hypothesized that D1R might play a critical role in augmentation, and we wanted to test if modifying D1R function could resolve augmentation. A total of 31 mice were used for this study, which assessed pain reflex responses before and after drug exposure; mice were randomly split into 5 separate groups with each group receiving a different drug. We tested the effects of the D3R agonists pramipexole and rotigotine (0.5 mg/kg/day), the D1R antagonist SCH 39166 (0.1 mg/kg/day), a combination of both rotigotine and SCH 39166, and 0.9% NaCl (sham). Animals were treated daily for 4 weeks. Thermal pain reflexes were tested a week prior to receiving their first injection, during the first week of their injections, and during the last week of their injections. We found that treatment with the D3R agonist led to opposite effects (early: analgesic; late: hyperalgesic) similar to augmentation in humans. We were able to reverse with effect by blocking D1R function, which, on its own, did not induce a switch in behavior like the D3R agonists. Our data suggest that continued D3R exposure leads to a switch in behavioral outcome that can be reversed by block of D1R function.
Downregulating Kremen1 in Corticohippocampal Neurons with a siRNA-RVG Complex in Triple-Transgenic Alzheimer Mouse Model

Fisher, Amanda¹, Hoff, Lee², Murashov, Alexander³, Baker, Kelly⁴

¹Department of Biology, East Carolina University, Greenville NC, United States
²Department of Math, East Carolina University, Greenville NC, United States
³Brody School of Medicine Department of Physiology, East Carolina University, Greenville NC, United States
⁴East Carolina University, Department of Neuroscience, East Carolina University, Greenville NC, United States

This research deals with Alzheimer’s Disease (AD) which aims to determine if the presence of a siRNA-RVG complex can be associated with a decrease of the integral protein Kremen1 within corticohippocampal cells of a triple-transgenic (3xTg) AD mouse model. To do this, short interfering RNA (siRNA) strand was complexed with rabies virus glycoprotein (RVG). Corticohippocampal cells obtained from 3xTg-AD mice were cultured for two weeks, transfected with the siRNA-RVG complex for 72 hours, and then photographed. The staining intensities of Kremen1 were then measured. A one-way ANOVA and Tukey's post hoc test were conducted to test the differences between the intensities of each group. Significant differences were found between the intensity of the experimental group and all other groups (p<0.0001). These results suggest that the presence of the siRNA-RVG complex does result in the down-regulation of Kremen1.
Effect of Spinal Cord Injury on Dopamine Receptor Expression and Morphine Responsiveness

Nzita M. Lutete, Jacob Yow, Kori L. Brewer Ph. D

Department of Emergency Medicine at East Carolina University, Greenville, NC.

Spinal Cord injury often results in chronic neuropathic pain that is frequently treated with opioids such as morphine. Morphine’s effect on SCI pain decreases over time. Interactions between μ-opioid (MOR) receptor and the Dopamine (DA) receptors may play a role in this loss of effect. The objective of this study was to determine if SCI alters the expression of DA receptors in the spinal cord in a manner that might contribute to the loss of morphine responsiveness after injury. Prior to surgery baseline testing was conducted on 8 rats in which thermal pain threshold and mechanical pain threshold were measured through the Hargreaves and Von Frey tests respectively. The rats were then divided into 2 groups, QUIS-injected (SCI) and Sham surgery (control). The animals were tested for sensory thresholds again 21 days post surgery. Animals were then tested again over a 3-day period after administration of morphine, a D1 receptor antagonist, or a mixture of the two drugs. Spinal cords were then collected and protein extracted for Western Blot analysis of DA receptor expression levels. Both morphine and the combination of morphine + D1 antagonist increased mechanical thresholds compared to the 21-day post injury levels (p=0.04 & 0.02, respectively). Thermal thresholds were significantly increased with the combination treatment (p=0.03) but not with morphine alone (p=0.10). Western blot results showed that there were no differences in DRD1, DRD2 or DRD3 expression levels in QUIS-injected vs. sham rats 25 days post injury. The results were not what was expected, which may have been due to the small group sizes or the severity of the lesion of the QUIS rats. Analysis of the spinal cord lesion showed only mild injury which may not have been severe enough to induce changes in protein expression. Future studies will need to decrease variability by increasing the group sizes and also confirm sufficient lesion in the injured animals.
The Brain Hemodynamic Responses during Gait Speed Changes in Younger Adults-
Functional Brain Imaging Study

MacCreery, A, Lin, CC, Williams, B

Department of Physical Therapy, East Carolina University, Greenville, NC, United States

Functional Infrared Spectrometry (fNIRS) can be utilized to study brain hemodynamic changes during various gait-related tasks. It is not fully understood how certain gait tasks affect activation in the prefrontal and vestibular cortex in the human brain. The purpose of this study is to investigate brain activities in young healthy adults during three different gait tasks using fNIRS. Twelve healthy young adults (Age 24 ± 1 y.o., right hand domain) participated in this study. Participants were excluded if they had unstable medical conditions, neurologic or orthopedic disorders, received a score less than 21 on the Functional Gait Index, less than a 19 on the Dynamic Gait Index, and below 67% on the Activities-specific Balance Confidence scale (ABC). A functional Near-Infrared Spectrometry (fNIRS) device (NIRSport, NIRx, Germany) was used on each subject to sense hemodynamic changes in the left side of the brain, over the dorsal-lateral prefrontal cortex (regions of interest: ROI-P) and temporoparietal (vestibular) cortex (ROI-V). An A-B-A block design (baseline-test-baseline) was used. Each subject performs each block four times. Subjects performed three gait tasks that included: 1) Standing - Normal Walking - Standing; 2) Normal Walking – Fast Walking – Normal Walking; 3) Normal Walking – Slow Walking – Normal Walking. fNIRS data were analyzed based on subject level and Group-level using a random-effects model of brain activity. There was no significant brain activity in the ROI-P and ROI-V during the first walking condition when contrasting the walking blocks to the standing blocks. Significant brain activity was found in the ROI-P and ROI-V (p < 0.05) during the second and third walking conditions when contrasting the fast walking blocks and the slow walking blocks to the normal walking blocks. The ROI-P and ROI-V showed increased activity during the slow walking conditions. The dorsal-lateral prefrontal cortex and vestibular cortex are involved with the change of walking speed. The young subjects demonstrated significant hemodynamic changes in the prefrontal cortex and vestibular cortex during fast and slow walking. Further research should investigate the effect of aging during gait speed changes.
Social Status-Dependent Molecular Regulation of Dopaminergic Pathways in the Brain of Zebrafish (Danio rerio)

Thomas Miller¹; Katie Clements¹; Eoon Hye Ji²; Fadi A. Issa¹

1. East Carolina University, Department of Biology
2. UCLA Brain Research Institute

In zebrafish (Danio rerio), social interactions between adult males consist of a series of aggressive encounters that ultimately lead to the formation of stable hierarchies of either socially dominant or subordinate animals. Although it has been shown that social status leads to neurophysiological changes in brain structure and function, our understanding of how identified brain circuits are modulated by social status in vertebrate model systems is limited. Preliminary results have shown that there is a social-status dependent effect on the sensitivity of the C-start escape response. We hypothesize that the activation pattern of the Mauthner neural circuit, that mediates the C-start escape response in zebrafish, is likely affected by social experience through the regulation of the dopaminergic system. The focus of this study is to determine how social experience affects the regulation of the expression on genes in the dopaminergic pathway in the zebrafish brain, and how this may relate to the Mauthner neural circuit. Our results indicate that the brain-wide dopaminergic system is modulated on a transcriptional level, with social status-dependent regulation of dopamine supply and receptor expression. We show that although there were no significant differences in the expression of tyrosine hydroxylase (th), dopa decarboxylase (ddc) and vesicular monoamine transporter (vmat) in dominant and subordinate animals, we found that whole brain expression of dopamine active transporter (dat) was significantly up-regulated in dominant animals compared to subordinates. In addition, drd1b receptor expression was down-regulated in dominants compared to subordinates. Finally, the hypothalamic and hindbrain sub-regions also display social status-dependent transcriptional modulation of the dopaminergic system. To fine tune our examination of the dopaminergic system relative to the Mauthner Neural circuit, we plan to examine expression of dopaminergic genes in the Mauthner Command neurons and specific regions of the hypothalamic nuclei known to project to the spinal cord. Our findings suggest that there is a social-status dependent regulation of the dopaminergic system via modulation of pre- and post- dopaminergic synaptic pathways.
One in seven men are diagnosed with prostate cancer and more than a third will undergo radiation therapy (RT) which frequently results in erectile dysfunction (ED). Prostatic radiation causes damage to the vasculature and the nerves supplying the penis. There are currently no treatment strategies to prevent or recover radiation-induced ED which significantly impacts the quality of life of prostate cancer survivors. This study examined the impact of low and high radiation on neurite growth and survival of sympathetic and nitrergic neurons and apoptosis in primary cultured neurons from major pelvic ganglion (MPG). MPGs were collected from male Sprague-Dawley rats (n=3) and neurons were dissociated and plated on laminin coated coverslips. After 24 hours, neurons were radiated at 200cGy or 800cGy and grown for an additional 48 hours and compared to time-matched control neurons. Neurons were fixed and stained with immunofluorescence for neuron-specific class III beta-tubulin to measure axon length and branching, neuronal nitric oxide synthase (nNOS, nitrergic), and tyrosine hydroxylase (TH; sympathetic) and TUNEL assay for apoptosis. Images of all neurons were taken at 100x of each coverslip for analysis. Neurite length was unchanged with radiation, however, neurite branching significantly decreased with both doses of radiation (p<0.005). There was a 3-4 fold increase in the percentage of apoptotic TUNEL positive neurons in both groups following radiation (p<0.0001). There was no change in the relative number of sympathetic TH positive neurons with radiation. In contrast, there was a marked decrease in nNOS positive neurons that increased with the dose of radiation (Control: 45±4.4%, 200cGy: 20±3.1%, 800cGy: 5±0.7%;p<0.0001). These data demonstrate that radiation therapy stimulates an increase in neuronal cell death and a substantial decrease in the number of erectile promoting nitrergic neurons. Although there is no change in neurite length, neurite branching which is essential for the establishment of appropriate neuronal connections and regeneration was impaired. Future studies will examine the impact of prostatic radiation in an animal model to determine if these neuronal impairments contribute to radiation induced ED in order to elucidate the pathological mechanisms to lead to new therapeutic strategies for prostate cancer survivors.
Inhibition of Microglia Activity Inhibits Nicotine-Induced Induction of ΔFosB Expression, but not Sensitization to Cocaine in the Periadolescent Rat

Partha Nagchowdhuri, Kristen Lane, Helen Williams, Brian McMillen

Brody School of Medicine

Nicotine exposure during adolescence is believed to enhance the brain’s sensitization to illicit drugs in the future. Nicotine induces the transcription factor ΔFosB that may facilitate this process. A once daily, 10-day administration of 0.4 mg/kg nicotine intraperitoneally (i.p.) that bracketed the onset of puberty in rats (PD 35-44), induced ΔFosB expression in important memory and reward areas of the rat brain. This change persisted into adulthood (PD 80), especially in the nucleus accumbens (NAc) and dentate gyrus of the hippocampus (DG) (Soderstrom et al. Psychopharm 191:891, 2007). This periadolescent nicotine exposure sensitized the rat to cocaine at adulthood determined by a conditioned place preference (CPP) paradigm (McMillen et al. Eur J Pharm 509:161, 2005). Here we determine whether a link exists between ΔFosB induction and microglia activation. Minocycline, a brain active tetracycline antibiotic that inactivates microglia, was injected i.p. at 30 mg/kg into male Sprague Dawley (PD 35-44) rats 30 min. prior to nicotine administration to assess effects of microglia inactivation on ΔFosB induction by nicotine. As expected, 0.4 mg/kg i.p. of nicotine during PD 35-44 increased the density of ΔFosB labeled nuclei in the DG from the vehicle control by 52.4 % (n=7-8, p<0.05). Similar increases, but not significant, were observed in the NAc and the medial prefrontal cortex (mPFC). Minocycline-prior-to-nicotine reduced the density of ΔFosB labeled nuclei in the DG by 34% (n=7-8, p<0.05), 10% (n=7-8, ns) in the NAc, and 28% (n=7-8, ns) in the mPFC. The number of activated microglia was reduced by 50% (n=5, p<0.05) in the NAc, and 32% in mPFC (n=5, p<0.05) in the minocycline pretreatment group when compared to nicotine alone. The effect on the sensitization to cocaine at PD 80 was determined using CPP. The difference in time (seconds) between pre- and post-conditioning for the nicotine-only group was increased by 80% over vehicle. Minocycline pretreatment did not attenuate the time the rats spent in the cocaine-paired chamber over the preferred chamber. These data suggest that microglia activation may not be responsible for the nicotine-induced sensitization to cocaine.
3-D Cerebral Organoids Model the Development of Autism Pathology

Pranaya Pakala, Colin Johnson, Storm Davis, Haroon Dar, Adrienne Orbita, and Karen Litwa

East Carolina University

Autism is a genetically complex neurodevelopmental disorder in which patients exhibit social deficits in both verbal and non-verbal forms of communication and display restricted and repetitive behaviors. Emerging evidence suggests that altered neural connectivity, particularly at the level of synaptic connections, contributes to disease pathology. In idiopathic autism cases, post-mortem patient brain samples exhibit increased numbers of excitatory synaptic connections in cortical brain regions that govern social behavior (PMID: 21346746). However, the use of post-mortem brain samples prevents researchers from capturing the development of this altered brain circuitry. Thus, we set out to develop a physiologically relevant model of idiopathic Autism that recapitulates defective neuronal circuitry at the level of both neurite and synapse formation. We began by reprogramming Autism patient fibroblasts into human induced pluripotent stem cells (hIPSCs), which we subsequently differentiated into 3-D cerebral organoids (‘mini-brains’) using a low-attachment protocol (PMID: 26005811). Similar to the in vivo cerebral cortex, these ‘mini-brains’ contain diverse brain cells, including neural progenitor cells, both excitatory and inhibitory neurons, and supporting glial cells. Additionally, brain ventricles develop. However, in Autism hIPSC-derived mini-brains, we observe dramatic differences in ‘mini-brain’ organization. In neurotypic controls, neurons develop around brain ventricles and their neurites associate with one another to form a patterned organization within the subventricular zone. By contrast, Autism-derived ‘mini-brains’ have negligible ventricle formation and their neurites form a disorganized meshwork throughout the organoid. Furthermore, Autism-derived ‘mini-brains’ exhibit increased levels of excitatory synapse formation. Thus, we describe a model that recapitulates the development of altered brain circuitry associated with idiopathic Autism. Importantly, this model will enable us and other researchers to dissect out the molecular mechanisms contributing to Autism pathology and to test whether specific pharmacologic interventions can rescue altered neurite and synapse formation associated with Autism.
Pharmacological Regulation of Neural Circuit Formation in hIPSC-Derived Neurons and ‘Mini-Brains’

Taylor Rudisill, Jordan Tweedy, Brandon Phillips, and Karen Litwa

East Carolina University

Autism is a genetically complex neurodevelopmental disorder in which patients exhibit social deficits in both verbal and non-verbal forms of communication and display restricted and repetitive behaviors. Emerging evidence suggests that altered neural connectivity, particularly at the level of synaptic connections, contributes to disease pathology. Post-mortem patient brain samples exhibit increased numbers of excitatory to inhibitory synaptic connections, referred to as an E/I imbalance (PMID: 21346746). In order to understand the mechanisms that underlie the formation of these synaptic circuits, we develop 3-D cortical organoids (‘mini-brains’) from human induced pluripotent stem cells (hIPSCs). Previous research demonstrates that dynamic rearrangements of the actomyosin cytoskeleton drive neural circuit formation, in particular the development and maturation of actin-enriched spines at excitatory synapses. We are currently investigating how pharmaceutical regulation of actomyosin activity affects neuronal connectivity during neurite formation in 2-D and excitatory synapse formation in 3-D ‘mini-brains’. The ROCK inhibitor, Y-27632, both inhibits non-muscle myosin II and leads to a corresponding increase in Rac-driven actin polymerization. In 2-D, Y-27632 increases neurite formation, and even rescues defective neurite formation in Autism-derived neurons. However, in 3-D, acute Y-27632 treatment increases excitatory synapse area, consistent with an increase in Rac-driven excitatory synapse area (PMID: 26169356). Thus, while Y-27632 rescues defective neurite formation in Autism, it elevates excitatory synapse area. This study demonstrates the need for physiologically relevant brain models, such as 3-D cortical organoids, to assess the impact of drug therapies on developing neural circuits.
Alzheimer's disease (AD) is the only disease among top ten US diseases that cannot be cured, prevented or slowed down. At molecular level both Aβ peptides and Ca2+ have been implicated in AD progression. For example, mechanism of AD onset has been closely associated with misfolding of Aβ40 and Aβ42, which is also well supported by the genetic data for AD. On the other hand extensive research efforts have revealed metal ions could manipulate Aβ equilibrium, especially Ca2+ ions. Using biophysical methods like SEC, filtration methods, fluorescence, EM and CD we observed Ca2+ ions at physiological concentrations (1 or 2 mM Ca2+) accelerate the rate of aggregation of Aβ42 to form intermediate soluble associated species and fibrils similar to those found in AD patients. CD spectra confirmed predominantly β-sheet conformation in the presence of Ca2+. These concentrations of Ca2+ significantly decreased the lag time of Aβ42 fibril formation (ThT fluorescence).

Surprisingly, Aβ40 did not appear to be as significantly affected by Ca2+ addition as Aβ42. In an effort to understand the distinctive behavior of Aβ40, we monitored changes of Aβ40 aggregation by fluorescence and CD but took different approaches for data processing. We were able to characterize a subtle effect of 2mM Ca2+ resulting in an increase in the rate of transformation from monomer to β-sheet rich fibrilar or intermediate species formation in Aβ40. Interestingly, kinetics observed by intrinsic fluorescence, ThT fluorescence, SEC or EM studies were not able to unravel the existence of this effect in Aβ40.

Our studies thus address two areas; 1) that Ca2+ as a amyloid inducer may contribute to early onset AD and thus could be a good target for chelation therapy proposed for AD. 2) There is a need of differential but scientific data treatment of the data sets to unravel the subtler effects for better mechanistic elucidation of the complex molecular events taking place during the onset of AD.