ECU Chapter of the Society for Neuroscience Presents:

12th Annual Neuroscience Symposium
Catalyst for Collaboration

Featuring the Keynote Address by

Sandra Kelly, Ph.D.
Professor, Department of Psychology
University of South Carolina

"Alcohol exposure during development and the epigenome"

Additional Guest Lecturers:

Sonja Pyott, Ph.D.
Assistant Professor, Department of Biology
University of North Carolina Wilmington

"Novel roles for the BK channel in efferent synapses of the inner ear"

Tuesday, November 2nd, 2010
East Carolina Heart Institute
8:00 am - Registration
8:30 am - Program Begins

Registration and Abstract Submission information at
www.ecu.edu/neurochapter/

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12th Annual Neuroscience Symposium Program

8:00-8:30 **Registration**

8:30-8:40 **Opening Remarks: Vice Chancellor Mageean, Dean Alan White**

8:45-9:20 **Dr. Sonja Pyott**, University of North Carolina at Wilmington "*Novel roles for the BK channel in efferent synapses of the inner ear"*

9:20-9:45 **Dr. Ben Keeler**, Department of Physiology, ECU, "*Mechanisms of Activity-Dependent Plasticity After Spinal Cord Injury and the Role of Proprioceptive Input*"

9:45-10:00 **Dr. Irene Hamrick,** Department of Family Medicine, ECU, "The Use of Days of the Week in a Modified Mini-Mental State Exam (M-MMSE) for Detecting Dementia in Geriatric Patients"

10:00-10:15 **Kristal Mills,** Department of Communication Sciences and Disorders, ECU, "Vestibular and Ocular Motor Function Following Blast Injuries" (Student Presentation)

10:15-10:30 **Break/Vendor Show**

10:30-11:45 **Keynote Address: Dr. Sandra Kelly**, University of South Carolina, "*Alcohol exposure during development and the epigenome"*

11:45-12:45 **Lunch/Vendor Show**

12:45-2:15 **Posters/Vendor Show**

2:15-2:50 **Dr. Abdel Abdel-Rahman**, Department of Pharmacology, ECU, "*ERK1/2 phosphorylation in brainstem: Good or bad for blood pressure control"*

2:50-3:25 **Dr. Sonja Bareiss**, Department of Physical Therapy, ECU, "*A role for GSK-3β signaling in sensory dysfunction following spinal cord injury*"

3:25-3:40 **Dr. Mona McConnaughey,** Department of Pharmacology and Toxicology, ECU, Effects Observed After Four Months of Chronic Aspartame Consumption on Brain Ca^{++} ATPase Activities and Various Receptors in the Rat"

3:45 **Closing Remarks and Awards**

Oral Presentations

Novel roles for the BK channel in efferent synapses of the inner ear

Sonja Pyott, Ph.D.

Department of Biology, University of North Carolina at Wilmington, Wilmington NC

Outer hair cells are the specialized sensory cells that empower the mammalian hearing organ, the cochlea, with its remarkable sensitivity and frequency selectivity. Sound-evoked receptor potentials in outer hair cells are shaped by both voltage-gated K^+ channels that control the membrane potential and also ligand-gated K^+ channels involved in the cholinergic efferent modulation of the membrane potential. The objectives of this study were to investigate the tonotopic contribution of BK channels to voltage- and ligand-gated currents in mature outer hair cells from the rat cochlea. We used patch clamp electrophysiology and i***mmunofluorescence*** in tonotopically defined segments of the rat cochlea to determine the contribution of BK channels to voltage- and ligand-gated currents in outer hair cells. Although voltage and ligand-gated currents have been investigated previously in hair cells from the rat cochlea, little is known about their tonotopic distribution or potential contribution to efferent inhibition. We found that apical (low frequency) outer hair cells had no BK channel immunoreactivity and little or no BK current. In marked contrast, basal (high frequency) outer hair cells had abundant BK channel immunoreactivity and BK currents contributed significantly to both voltage-gated and ACh-evoked K^+ currents. Our findings suggest that basal (high frequency) outer hair cells may employ an alternative mechanism of efferent inhibition mediated by BK channels instead of SK2 channels. Thus, efferent synapses may use different mechanisms of action both developmentally and tonotopically to support high frequency audition. High frequency audition has required various functional specializations of the mammalian cochlea, and as shown in our work, may include the utilization of BK channels at efferent synapses.

Mechanisms of Activity-Dependent Plasticity After Spinal Cord Injury and the Role of Proprioceptive Input

[1]Benjamin Emerson Keeler, Ph.D. and [2]John D. Houlé, Ph.D.

[1]Department of Physiology, ECU Brody School of Medicine, Greenville NC
[2]Department of Neurobiology and Anatomy, Drexel University College of Medicine, Philadelphia PA

Recovery following spinal cord injury (SCI) is attributable to the innate plasticity of the nervous system and retention of spinal circuits involved in locomotion and other behaviors. However, repetitive patterned activity in the form of exercise has been shown to improve this recovery by increasing plasticity of the spinal cord and minimizing possible adverse effects. To better understand the underlying features of activity-dependent plasticity, we analyzed molecular changes in the spinal cord resulting from a passive exercise regimen. In doing so, we paid special attention to the role of the sensory system in modulating the molecular response and reflex activity of neurons below the level of injury.

We have previously shown that sensory input was necessary for reflex normalization with exercise following SCI. We used pyridoxine to block proprioceptive stimulation, which is toxic to large, sensory neurons. Our results showed that hyper-active reflexes associated with SCI were not normalized in animals treated with pyridoxine, demonstrating a critical role of proprioceptive stimulation in reflex normalization.

To determine the molecular changes involved, we examined changes in mRNA expression following injury and exercise in cells of the motor system and proprioceptive sensory system, with emphasis on neurotrophic factors because of their ability to modify neuronal circuits. Exercise increased the expression of the neurotrophic factors, but only in cells of the motor system and not in proprioceptive neurons.

Next, we examined the role of sensory stimulation in the molecular changes observed with exercise. We analyzed gene expression in motoneurons after SCI and exercise, in animals lacking either proprioceptive sensory stimulation or all sensory stimulation caudal to the injury. Exercise failed to increase expression of neurotrophic factors in motoneurons in animals lacking either proprioceptive stimulation or more complete sensory loss. Overall, these results demonstrate possible molecular mechanisms of activity-dependent plasticity and the role of proprioceptive input.

*POST-DOCTORAL PRESENTATION

The Use of Days of the Week in a Modified Mini-Mental State Exam (M-MMSE) for Detecting Dementia in Geriatric Patients

R. Hafiz MD, I. Hamrick MD, S. Morrissey

Department of Family Medicine, East Carolina University Brody School of Medicine, Greenville, NC

The purpose of the study: The Folstein Mini Mental State Exam (MMSE) is highly dependent on education and literacy and thus may falsely identify patients of low literacy and education with dementia. Does the modified MMSE classify patients with dementia more accurately than MMSE?
Methods: In this is a prospective open label cohort study, we administered the Folstein MMSE to 222 patients with complaints of memory loss or diagnosis of dementia. In addition to spelling "WORLD" backwards and serial 7s, we asked participants to recite the days of the week backwards with a score of up to 5 points if correctly answered. The Mini-Cog, consistent of clock drawing test and 3 item recall, was used as control.
Results: All normal subjects correctly recited the days of the week backwards (score= 5 of possible 5) and scored higher on it than Serial 7's (score= 2.02) or spelling (score= 3.85). Compared with the Mini-Cog, sensitivity / specificity respectively are 0.89/053 for MMSE and 0.96/.049 for our Modified-MMSE. Among less than high school graduates (n=111), the modified-MMSE using a cut-off of 27/30 identified the same number of patients with dementia (n=86) as the Mini-Cog.
Conclusion: Taking the educational part (Spelling and Counting) out of the MMSE by using the days of the week backwards, and using a cut off 27/30 for all levels of education and literacy the modified MMSE detects dementia
with fewer false positives.

Vestibular and Ocular Motor Function Following Blast Injuries

Kristal N. Mills[1], Stephanie Cole[2], Andrew Stuart[1], Timothy A. Jones[1], Sherri M. Jones[1]

[1]Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC, [2]Department of Otolaryngology, Naval Hospital Camp Lejeune, Camp Lejeune, NC

The war on terror has produced over 30,000 wounded troops with approximately 68% of injuries attributed to blasts (Dept. of Defense, 2010; Chandler, 2006). Dizziness and imbalance are common complaints among blast injured (BI) military personnel. The purpose of the study was to characterize vestibular and oculomotor function in military personnel less than one year post blast (N = 33) and greater than one year post blast (N = 60). Overall, 33 of the 93 participants exhibited normal findings on all test results (less than one year, N = 10 and greater than one year, N = 23). Ocular motor abnormalities (OMA) were the most common finding with 51 of 93 participants demonstrating at least one abnormal finding. Saccadic latency was the most commonly observed OMA with mean latencies of 178 ms for those classified as normal and 345 ms for those with OMA. Less than 1 year post-blast, 7 out of 33 (21%) exhibited findings consistent with a unilateral weakness (UW). One participant in this group had findings consistent with a bilateral weakness (BW). Greater than 1 year post blast group, 4 participants (6%) showed a UW and 11 (18%) had findings suggestive for a BW. All participants were able to visually suppress the vestibular ocular reflex (VOR) and the vast majority (95%) could also visually enhance VOR gain. Vestibular evoked myogenic potentials were normal in 42 of the 47 participants tested suggesting that saccular function is not significantly affected by blast exposure. The Dizziness Handicap Inventory and the Activities-specific Balance Confidence scales were were poor indicators of peripheral vestibular status. Participants reported symptoms and case history did not predict test outcomes. Overall, the findings suggest that peripheral vestibulopathy is not prevalent following BI at least during the time frame tested here (4 months to 5 years post blast); however, ocular motor abnormalities are common in the BI population.

*STUDENT PRESENTATION

Alcohol Exposure during Development and the Epigenome

Sandra J. Kelly, Ph.D.

Carolina Trustee Professor, Department of Psychology, University of South Carolina, Columbia, SC

Fetal Alcohol Spectrum Disorders continues to be the leading known cause of mental retardation in the Western World and includes long-lasting changes in the central nervous system. Previous work examining the mechanism underlying the impact of alcohol on the central nervous system has focused on oxidative stress but it is clear that from these findings that there are likely going to be multiple mechanisms. Recently, it has been demonstrated that alcohol is capable of causing changes within the epigenome, resulting in long-lasting changes in gene expression without actually altering the genome. Our current research has focused on the impact of alcohol exposure during development on epigenetic markers within brain and has demonstrated long-lasting changes in the epigenome within brain after exposure to alcohol during development. These findings have implications for possible trans-generational effects of alcohol exposure during development and also treatment of Fetal Alcohol Spectrum Disorders.

ERK1/2 phosphorylation in brainstem: Good or bad for blood pressure control?

Abdel Abdel-Rahman, Ph.D., FAHA. Distinguished Professor, Department of Pharmacology and Toxicology, The Brody School of Medicine, East Carolina University, Greenville, NC

Our cell culture studies have identified ERK1/2 phosphorylation as a signaling product of activating the imidazoline I_1 receptor (I_1R). These findings lead to testing the provocative hypothesis that ERK1/2 phosphorylation within the brainstem underlies the sympathoinhibition and hypotension caused by central I_1R activation. We adopted a multidisciplinary approach that encompassed integrative, gene knockdown, and molecular studies to test our hypothesis. Our findings were the first to demonstrate correlative association between increased ERK1/2 phosphorylation in the rostral ventrolateral medulla (RVLM) and the hypotensive response elicited by I_1R activation by rilmenidine. Importantly, these findings contradicted the view that implicated the enhanced ERK1/2 phosphorylation in the RVLM in sympathoexcitation and pressor response. Therefore, further studies were conducted to: (i) confirm a causal role for the enhanced ERK1/2 phosphorylation in the RVLM in the hypotensive response; (ii) identify the molecular mechanism that links ERK1/2 phosphorylation to the hypotensive response. Collectively, our findings support the hypothesis that enhanced ERK1/2 phosphorylation enhances downstream NOS-NO signaling within the RVLM and ultimately causes sympathoinhibition and hypotension.

A Role for GSK-3β Signaling in Sensory Dysfunction Following Spinal Cord Injury

Sonja Bareiss, Ph.D.

Department of Physical Therapy, ECU School of Allied Health Sciences, Greenville NC

Chronic neuropathic pain and sensory abnormalities are an important secondary consequence of spinal cord injury (SCI). Structural changes associated with neuroplasticity, in the form of aberrant axonal sprouting, is an integral feature that contributes to SCI sensory dysfunction. A potential mechanism controlling aberrant axonal sprouting involves the enzyme glycogen synthase kinase-3β (GSK-3β), a key intracellular mediator of neuronal growth and survival. *In vitro*, inhibition of GSK-3β leads to robust axonal outgrowth, however the role of GSK-3β signaling in neuropathic pain has not been established. We hypothesized that post-SCI pain may result from aberrant sprouting of primary afferent fibers in response to inhibition of GSK-3β activity and that modulation of GSK-3β activity can prevent the sprouting and onset of pain and sensory dysfunction. In a series of studies we evaluated if SCI altered GSK-3β activity in the spinal cord and dorsal root ganglia (DRG) to determine if peripheral neuronal sprouting contributes to the development of sensory abnormalities. The excitotoxic model of SCI was used to produce pathological and behavioral changes in a rodent model consistent with those observed after human SCI including pain and sensory dysfunctions**.** Western blot analysis of SC injured animals showed significant inhibition of GSK-3β activity compared to (non-injured) controls. SCI was also associated with robust DRG neuronal sprouting and increased neuronal length compared to (non-injured) controls. In a second *in vivo* study, pharmaceutical inhibition of GSK-3β resulted in DRG sprouting similar to that of injured animals. Based on these data, we suggest that injury to the spinal cord results in GSK-3β inactivation and enhanced peripheral neuronal sprouting which is correlated with pain behaviors. These studies point to a role for GSK-3β as a mediator of post-SCI pain and sensory dysfunction. Future studies that will manipulate the activity of GSK-3β can elucidate the impact of this enzyme to reduce aberrant sprouting and pain related behaviors following SCI. Funding provided in part by the Wooten Laboratory for Alzheimer's and Neurodegenerative Disease Fund.

Effects Observed After Four Months of Chronic Aspartame Consumption on Brain Ca^{++}-ATPase Activities and Various Receptors in the Rat

M. M. McConnaughey and K. L. McConnaughey

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

The specific aim of this study was to determine if long-term aspartame administration to rats would cause a change in brain receptor densities or Ca^{++}-ATPase activities, as a possible biochemical explanation for our previous results showing memory impairment following chronic aspartame consumption. Male Sprague Dawley rats (225 g) received either plain tap water or aspartame in the drinking water (250 mg/kg/day) for 4 months. They were then sacrificed, brains quickly removed, made into homogenate preparations and frozen. Radioligand binding assays were used to determine total apparent numbers of receptors in brain preparations. Ca^{++}-ATPase activities were determined by measuring Pi from hydrolyzed ATP. Whole brain preparations from aspartame-treated rats showed a statistically significant increase in total apparent opioid receptor numbers when compared to controls ($P<0.05$). No significant differences were seen between controls and treated animals for total apparent numbers of dopamine receptors, serotonin receptors or GABA receptors. Ca^{++}-ATPase activities were slightly elevated in the midbrain area from aspartame-treated animals. More animals and various brain areas need to be studied to further define potential differences. Studies such as these are needed to identify possible long-term biochemical changes due to aspartame.

Poster Presentations

Contralateral Suppression of Transient Otoacoustic Emissions and Speech Recognition in Noise in Normal-hearing Young Adults

Alyson K. Butler & Andrew Stuart

Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC

In animals, it has been demonstrated that, activation of the medial olivocochlear (MOC) efferents improves the detection and encoding of signals in noise. Poorer speech recognition in noise among individuals with vestibular neurectomy supports this notion, as well. Subsequent studies with normal hearing listeners have been equivocal. Herein, speech recognition in noise and efferent activity was further examined. Specifically, sentence recognition in noise was examined in 18 young adult females with normal middle ear function and normal pure tone thresholds. The functioning of the MOC efferents was evaluated noninvasively through contralateral suppression of otoacoustic emissions (OAEs). Contralateral suppression of OAEs refers to a reduction in amplitude of OAEs with stimulation of the contralateral ear.

An adaptive technique was employed to determine reception thresholds for sentences (RTSs) monaurally and binaurally in quiet and in a fixed level of background of competing continuous and interrupted noises. The noises differed only in their temporal continuity. "Release from masking" was computed by subtracting RTS S/Ns in interrupted noise from continuous noise. Transient evoked otoacoustic emissions (TEOAEs) were obtained bilaterally with 80 dB peSPL non-linear 0.1-ms-duration click stimuli. To examine contralateral suppression, TEOAEs were evaluated with 60 dB peSPL linear 0.1-ms-duration click stimuli with and without a contralateral 65 dB SPL white noise suppressor.

A binaural advantage was observed for RTSs in quiet ($p < .0001$). In noise, performance was superior in the interrupted noise (i.e., RTSs were lower vs. continuous noise; $p < .0001$) while there was no ear advantage ($p = .061$). TEOAEs were larger with the 80 dB peSPL ($p < .0001$) evoking stimuli and there was no significant difference between ears ($p = .77$). There was no significant difference in the amount of suppression between ears ($p = .55$). There was no significant correlation or a predictive linear relation between the amount of TEOAE suppression and any indices of sentence recognition in noise (i.e., RTSs and release from masking; $p > .05$). We suggest that these results are not consistent with the notion that increased MOC efferent feedback, as assessed via TEOAE suppression, is associated with improved speech perception in noise.

*STUDENT PRESENTATION

Aging Modifies the Balance of Dopamine D1 and D2 Receptor Expression in the Spinal Cord

Stefan Clemens, Ph.D.

Department of Physiology, Brody SOM, East Carolina University, Greenville NC

Aging processes have a strong impact on all physiological functions of an organism, and with age there is an increase in autonomic dysfunctions (e.g. cardiovascular disease, hypertension). Spinal cord neurons in the intermediolateral nucleus (IML) of the thoracic spinal cord regulate autonomic output, and they receive strong modulatory inputs from descending Dopamine (DA) sources. With age there is a significant decline in the number of DA-related nerve terminals in the spinal cord, at a time when overall autonomic output increases. DA acts via excitatory (D1-like) or inhibitory (D2-like) pathways, and DA drugs targeting D2 receptors can restore normal blood pressure in conditional hypertensive animals. These data suggest that a decrease of DA inhibitory actions might be involved in the up-regulation of autonomic output seen in the elderly. To better understand the impact of aging on the different DA receptor subtype populations in the spinal cord, we analyzed the receptor levels of D1 and D2 receptors in the thoracic and lumbar segments of young (6-8 weeks) and aged (20-24 months) mice (C57BL/6) with standard Western blot techniques. Spinal cords were harvested and divided into thoracic and lumbar segments, and subsequently processed with the antibodies against D1 and D2 receptors (ABcam). Protein expression for each receptor subtype was compared in thoracic and lumbar tissues within the same age group, and between young and old animals. For the D1 receptor, protein expression was similar in the thoracic and lumbar cord of the young animals. In contrast, in the aged animals, expression was ~3 fold higher in the lumbar than in the thoracic cord ($p=0.003$). Comparing thoracic and lumbar cords of young and old animals, we found no significant change in the thoracic cord, but again a ~3-fold increase of D1 expression in the lumbar cord ($p=0.016$). Regarding the D2 receptor, in young animals, signal intensities were ~2-fold higher in the thoracic than in the lumbar cord. Additionally, with age, D2 receptor expression strongly declined in both thoracic (to ~45%, $p<0.001$) and lumbar cords (to ~25%, $p=0.019$), when compared to the young controls. These data suggest that during aging DA-mediated signaling in the spinal cord undergoes important changes. The relative increase in D1 receptor levels in the lumbar cord and the parallel decrease in D2 receptor levels in both thoracic and lumbar cord support the hypothesis that a shift in the balance of these receptors towards overall excitation could play a role in the overall increase of autonomic tone observed with age.

Chronic MOG-Induced Atypical EAE in the Lewis Rat: A New Model for Neurodegenerative Disease

Alan D. Curtis, II, Derek J. Abbott, Ashton E. Thomasson, and Mark D. Mannie.

Department of Microbiology and Immunology, East Carolina University Brody School of Medicine, Greenville, NC

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system that is characterized by focal inflammatory lesions together with demyelinating plaques in periventricular and perivascular regions of CNS white matter (1). The chronic inflammatory insult against CNS myelin results in progressive neurodegeneration with extensive neuronal loss and gross brain atrophy. It is thought that the antecedent inflammatory damage facilitates the subsequent neurodegenerative process. Experimental autoimmune encephalomyelitis (EAE) is an extensively studied rodent model of MS(2). EAE can be readily induced in Lewis rats by a single immunization with myelin basic protein (MBP) or encephalitogenic deternimants of MBP in Complete Freund's Adjuvant (CFA) (3). This form of EAE is characterized by a monophasic disease course marked by 3-5 days of tail and hind-limb paralysis followed by complete spontaneous remission and functional motor recovery. The critical adjuvant component of CFA is heat-killed *Mycobacterium tuberculosis*, which is essential for induction of EAE. In contrast, MBP emulsified in Incomplete Freund's Adjuvant (IFA) does not cause EAE. Myelin oligodendrocyte glycoprotein (MOG) is also a constitutive myelin protein, and causes EAE in rodents when emulsified in CFA. Immunization of Lewis rats with MOG in CFA causes a mild monophasic form of EAE (4). The monophasic course is characterized by a complete spontaneous recovery and is similar to an isolated attack of relapsing-remitting MS. Recently, we have discovered a novel means to induce chronic atypical EAE in Lewis rats. The protocol involved immunizing Lewis rats with the major encephalitogenic determinant of MBP (amino acid residues 69-88) and the recombinant extracellular domain of rat myelin oligodendrocyte glycoprotein (MOG) in CFA. After recovery from monophasic EAE, the rats were then boosted with MOG in IFA. This booster resulted in the induction of chronic atypical EAE that persisted in several rats without recovery. Atypical EAE is marked by unusual clinical signs such as forelimb weakness without hindlimb involvement, ataxia without flaccid paralysis of the tail, dystonia, rigid asymmetric extension of a hindlimb or forelimb (as opposed to flaccid paralysis), and/ or vertigo/ disequilibrium and torticollis. Here we provide evidence that MOG is a sufficient antigen to induce a new model of chronic, atypical EAE in Lewis rats. These data also show that GP69-88 was insufficient for induction of chronic atypical EAE and that the secondary boost with MOG was essential for induction of chronic atypical EAE.

References:

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*STUDENT PRESENTATION

A Combination Therapy Promotes Quipazine-induced Stepping in Adult Spinalized Rats

Elizabeth Dugan, Ph.D.

Department of Emergency Medicine, BSOM, East Carolina University, Greenville NC

Our original hypothesis was that the combination of cellular transplants of neural and glial restricted precursor cells, 2) passive cycling, and 3) chronic 5-HT2 receptor agonist treatment with quipazine would result in increased locomotor recovery compared to the individual treatment elements alone. We found that the combination therapy promoted weight-supported stepping in the open field without body weight support or tail stimulation under acute quipazine administration. None of the other treatment groups were capable of supporting their own weight or performing weight-supported steps even when placed on the motorized treadmill. This is the first report of quipazine-induced stepping without the use of a body weight-support system or trainer- provided stimulation. Next we investigated anatomical connections within the lesion of the combination therapy animals and did not find evidence of reconnection of the caudal spinal cord. Examination of the NRP/GRP cell transplants revealed that the combination therapy promoted an increase in AP+ cell survival and caudal migration. The vast majority of the NRP/GRP cell transplants did not differentiate into mature cell types. Within the lumbar spinal cord molecular investigation of neurotrophin factor expression revealed that the combination therapy animals had increased expression of the neurotrophin, BDNF compared to the individual treatment elements and injury alone. This increase in BDNF mRNA expression was found to be increased in the lumbar motoneurons of the combination therapy animals as well suggesting that they may contribute to the increased BDNF mRNA expression observed in the whole lumbar spinal cord. The dendritic arborizations of motoneurons associated with hind limb muscle that resisted atrophy through passive cycling showed preservation of the dendritic branching in the combination therapy animals and those receiving passive cycling only. Furthermore, the mRNA expression of the 5-HT_{2A} and 5-HT_{2C} receptors of the lumbar motoneurons were also increased in the combination therapy animal compared to injury only, although no differences were observed within the intermediate grey area. Taken together these data suggest that the combination therapy led to an increase in spinal plasticity through stimulation of the lumbar spinal cord that resulted in the production of weight-supported stepping without supraspinal input.

*POST-DOCTORAL PRESENTATION

Morphological Changes in the Cerebellar Lobules IX and X of Otoconia-Deficient B6Ei.GL-*Nox3*^{het}/J Mice

Leonardo A. Duque, Sarath Vijayakumar, and Sherri M. Jones

Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC

The peripheral vestibular system is located within the temporal bone and its primary function is to encode head movement and position. The otolith organs, utricle and saccule respond to linear acceleration (ex. gravity) and static tilt positions of the head. This process depends on otoconia which provide the mass necessary for the macular organs to have gravity sensitivity; both organs have sensory hair cells that encode this sensory information and transmit it to vestibular primary afferents. Primary and secondary afferents from the vestibular system transmit signals related to movement and location of the head to the cerebellum. The head tilt (B6Ei.GL-*Nox3*^{het}/J) mutant homozygotes lack otoconia; therefore, hair cells cannot be stimulated, producing gravity sensory deprivation. *Nox3* mice were studied to investigate the adaptive changes of gravity deprivation in the morphology of Purkinje cells. Vestibular signaling pathway predominantly involves AMPA type glutamate receptors; of the different receptor subtypes, GluR2/3 is highly expressed in the Purkinje cells. In the present study, immunoreactivity of GluR2/3 was examined in lobules IX and X of the cerebellum of heterozygous and homozygous *Nox3* mice and C57BL/6JEiJ background strain aged 3, 7 and 10 months. The total number of Purkinje cells, cell line length, linear cell density and GluR2/3 receptor density were compared between the groups. No significant change in the Purkinje cell count, length of the cell line and linear cell density was observed in the heterozygous and the homozygous animals across age groups. GluR2/3 receptor density was significantly lower in the homozygous animals (87.87±3.89 vs 77.53±2.64 gray scale intensity), but only at 7 months suggesting that there may be a down-regulation of the AMPA receptors. Further studies are required to confirm this finding. Overall, based on the stereological methods used in this study, gravity deprivation does not appear to impact the morphology of Purkinje cells in lobules IX and X;however, it remains to be determined if developmental differences exist at younger ages. The research was supported by NIDCD 5R01DC006443-05 and 3R01DC006443-04S1.

Actin-Based Neuronal Morphogenesis is Regulated by Rho GTPase Signaling Pathways

Amy Friesland, Qun Lu

Department of Anatomy and Cell Biology, The Brody School of Medicine at East Carolina University, Greenville, North Carolina 27834

Development and maintenance of the neuronal morphology is a complex and dynamic process that is crucial for normal brain function and relies heavily upon cytoskeletal modification. Members of the Rho GTPase subfamily of proteins, including RhoA and Cdc42, are known to be important regulators of actin reorganization. RhoA promotes the retraction of cellular processes, while Cdc42 induces the extension of filopodia/spines. Through these cytoskeletal changes, Rho GTPases mediate migration, adhesion, cell shape, as well as a number of other cellular processes. Our laboratory has begun to investigate the regulatory roles of these small GTPases in the control of neuronal morphogenesis. Previous studies in our laboratory have shown that inhibition of RhoA activation increases axonal and dendritic morphogenesis, implicating a possible role of RhoA signaling in neurodegeneration. Much less is understood, however, about the effects of Rac-1 and Cdc42 on neuronal morphogenesis due to the lack of specific activators and inhibitors of Cdc42. Therefore, we have applied computer-assisted, virtual high through-put in silico screening of a large library of small molecules to examine their ability to bind the surface groove of Cdc42 that is critical for their activation. The highest ranked of these molecules were then characterized both morphologically and biochemically for their ability to either inhibit or activate Cdc42. Examination of the small molecules resulted in one possible Cdc42 activator and two possible Cdc42 inhibitors. Compounds were then applied to a dissociated primary neuronal cell culture prepared from the one day old mouse cerebrum (cortex and hippocampus) and examined by both immunofluoresence and time lapse video light microscopy. Initial characterization showed that Cdc42 plays important roles for growth cone morphology and dynamics. Supported by NIH AG026630 and CA111891.

*STUDENT PRESENTATION

Effects of Ketorolac (Toradol) on Mammalian Linear Vestibular Sensory Evoked Potentials (VsEPs)

G. Chris Gaines[1], Timothy A. Jones[1]

[1]Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC

The non-steroidal anti-inflammatory drug (NSAID) ketorolac (Toradol) is a candidate for use as a supplemental analgesic during major surgery in anesthetized rodents. The use of ketorolac during surgery is believed to reduce the anesthetic dose required to achieve and maintain an adequate surgical plane and improve the physiological conditions and survival of animals during long experimental procedures. Ketorolac has reported side effects including dizziness, "ear pain", hearing loss, tinnitus and vertigo in humans, but there has been no report examining ketorolac's effect on the auditory and vestibular system in animal models. Thus the use of ketorolac during studies of the inner ear in anesthetized animals is subject to question. Our aim was to evaluate the effect of ketorolac on vestibular compound action potentials in the mouse (C57BL/6J). This was accomplished by recording linear vestibular sensory evoked potentials (VsEPs) during ketorolac administration. The effective analgesic dose range for ketorolac is reportedly 0.1 – 5 mg/kg in rodents with a lethal dose 50 (LD50) of 200 mg/kg (half-life ~3 hours). We evaluated the effect of ketorolac on VsEPs during a series of doses (5 mg/kg, 10 mg/kg, 20 mg/kg, 40 mg/kg, 80 mg/kg) administered at 20 minute intervals in mice anesthetized (0.007 ml/g) with a mixture of ketamine (18 mg/ml)/ xylazine (2 mg/ml). VsEP results for ketorolac were compared to those from a sham group receiving the same volume of Ringer's solution at the same intervals and a control group maintained under anesthesia over a similar time period. Changes in VsEP latencies and amplitudes were examined as a function of time (linear regression over ~2 hour period). Responses were unaffected by ketorolac. Regression slopes for amplitudes and latencies were unchanged by the ketorolac dose regimen (MANOVA). These findings demonstrate that ketorolac can be used as an analgesic to supplement anesthesia in mice without concerns of modifying the linear VsEP. Supported by NIH NIDCD 3 R01 DC006443-04S1.

*STUDENT PRESENTATION

Down-regulation of Rostral Ventrolateral Medulla PI3K/Akt Signaling Underlies the Central CB_1R-Evoked Pressor Response in Conscious Rats

Badr M. Ibrahim and Abdel A. Abdel-Rahman

Brody School of Medicine, East Carolina University, Greenville, NC, USA

We have shown that intracisternal (i.c.) injection of the cannabinoid receptor (CB_1R) agonist WIN55,212-2 (3-30 μg) elicited dose-related increases in MAP. However, the exact cellular mechanisms that underlie CB_1R-evoked pressor/sympathoexcitatory effects are unclear. Studies have implicated PI3K/Akt signaling in some pharmacological responses mediated by CB_1R activation both in *vivo* and in *vitro*. Therefore, we hypothesized that CB_1R modulation of PI3K/Akt signaling in the brainstem underlies its centrally mediated sympathoexcitation in freely moving rats. WIN55,212-2 reduced Akt phosphorylation (PI3K downstream molecular target) in the rostral ventrolateral medulla (RVLM) and nucleus tractus solitarius (NTS) 5 min post injection, which coincides with or precedes WIN55,212-2-evoked peak pressor response ($P < 0.05$). The basal level of activity was restored 30 min post WIN55,212-2 injection. The hemodynamic and neurochemical responses were mediated via CB_1R because they were abrogated by the selective CB_1R antagonist AM251 (30 μg, i.c.). More intriguingly, Akt phosphorylation was significantly elevated in the NTS of animals that received AM251 prior to WIN55,212-2. Furthermore, i.c. Wortmannin (PI3K inhibitor, 0.42 μg) pretreatment dose dependently augmented WIN55,212-2 (7.5 and 15 μg, i.c) evoked pressor and neurochemical effects. Taken together, our findings suggest that that CB1R-evoked sympathoexcitation is mediated, at least partly, via inhibition of brainstem PI3K/Akt signaling pathway.

*STUDENT PRESENTATION

Exercise after spinal cord injury increases BDNF expression in the spinal cord but does not reduce pain behaviors

Rich Lamm[1]; Brian Whitfield[2], Mackenzie Tuzzo1o[1], Sonja Bareiss, PT, Ph.D[3]and Kori Brewer, Ph.D.[2]

[1]Brody School of Medicine Summer Scholars Research Program, [2]Department of Emergency Medicine, Brody School of Medicine, [3]Department of Physical Therapy, College of Allied Health Sciences

Previous experiments have shown that experimental spinal cord injury (SCI) leads to significant loss of peripheral sensory neurites within the dermatome associated with injury. This neurite loss correlates with overgrooming behavior that has been purported to be a correlate of at-level SCI pain. Specific forms of exercise have been shown to preserve normal sensory function after SCI through upregulation of neuronal growth factors that protect against neuronal death and support neurite outgrowth.

The current experiment examined if a forced exercise protocol would alter the expression of the neuronal growth factor, BDNF, and what impact the change in BDNF expression would have on peripheral denervation and the corresponding development of overgrooming behavior after experimental SCI in rats. We hypothesized that daily treadmill exercise would increase BDNF in the spinal cord and dorsal root ganglia (DRG), preventing the loss of peripheral neurites and reducing the incidence of both spontaneous and evoked pain syndromes.

15 animals underwent SCI through intramedullary injection of quisqualate (QUIS) into the dorsal gray matter. Animals were then randomized into exercise or non-exercise (sedentary control) groups. Experimental animals received 14 days of exercise treatment consisting of 40 min/day medium intensity treadmill running at 15m/sec. Both groups were examined daily for overgrooming behavior and hyperalgesia indicated by altered thermal pain thresholds. Animals were sacrificed 14 days post-SCI. Skin biopsies, DRGs and spinal cords were collected from the level of injury. Skin samples were histologically analyzed for PGP9.5 expression. Spinal cord and select DRG samples were analyzed for BDNF expression by Western blot. The remaining DRGs from the level of injury were cultured for morphometric analysis.

Both exercised and sedentary animals showed loss of peripheral innervation at the level of injury. Fifty percent of the rats in both the exercise and sedentary groups developed overgrooming behavior. Exercised animals showed a significant reduction in both ipsilateral and contralateral pain thresholds ($p=0.002$ and $p=0.011$ respectively). Qualitative histological analysis of DRGs from exercised subjects showed marked increases in nerve sprouting. Exercised animals also showed a significant increase in BDNF expression in the cords ($p=0.013$). These data suggest that, in our model, forced exercise increased BDNF expression in the spinal cord after SCI and sprouting of DRGs, but did not preserve peripheral innervation or protect against the development of post-SCI pain behaviors.

Maternal Iron Status is a Critical Modulator of Outcomes in Fetal Alcohol Spectrum Disorders

Dorothy H. Lloyd[2], Ellen M. Sheffer[1,2], Iola D. Conchar[1,2], and Tuan D. Tran[1,2]

[1]Department of Psychology, East Carolina University, Greenville, NC 27834
[2]Multidisciplinary Studies Program in Neuroscience, East Carolina University

Alcohol consumption during pregnancy may result in fetal alcohol spectrum disorders (FASD) such as fetal alcohol syndrome (FAS) and alcohol-related neurodevelopmental disorder (ARND). Studies have shown that fetal alcohol exposure causes life-long cognitive, behavioral, and sensorimotor deficits. This type of insult affects many brain regions including the cerebellum and hippocampus, which are both particularly vulnerable to teratogens during the brain growth spurt - a period of accelerated brain development that corresponds to the third trimester in humans and early postnatal period in rats. Because alcoholics are often malnourished with respect to micronutrients, it is reasonable to consider whether these deficiencies could synergize with alcohol to worsen fetal brain damage. We hypothesized that maternal iron deficiency (ID) would worsen fetal alcohol-induced damage because it is the most common nutritional deficiency in pregnant women. Moreover, maternal ID causes behavioral deficits that strongly parallel those seen in FASD, suggesting that ID and alcohol may synergize to heighten alcohol's neurotoxicity. We used an established model of fetal alcohol exposure that disrupts cerebellar- and hippocampal-based forms of learning. Iron-sufficient (IS) or ID rats were generated from mothers that were administered with either IS (100 ppm Fe) or ID (20 ppm Fe stepped to 4 ppm Fe) during pregnancy and through postnatal days (PD) 7. The pups received alcohol (0, 3.5, 5.0 g/kg) in milk solution or sham intubations (SI) from PD 4-9. Overall, there were 4 treatment groups per litter. At ~PD 30, they were surgically prepared for either delay or trace eyeblink classical conditioning (ECC). Two days later, they received either forms of ECC testing for three consecutive days. At ~PD 38, their cerebella or hippocampi were prepared for histology, and then examined for cell loss in the Purkinje cell layer of hemispheric lobules I-VI or hippocampal region CA1, which are known to contribute to either delay or trace ECC, respectively. Results showed that ID exacerbated alcohol-induced learning deficits and these deficits correlated with Purkinje cell loss. Examination for cell loss in region CA1 is currently underway. The preliminary findings suggest that maternal iron status is an important modulator of fetal alcohol-induced neurobehavioral damage.

*STUDENT PRESENTATION

Epigenetic Effect of Paternal High Fat Diet on Offspring Susceptibility to Glucose Intolerance in Mice

Matthew P. Parker[1], Maneesh Jeyakumar[1], Elena Pak[1], Dianne Walters[1], Darrell Neufer[1-2] and Alexander K. Murashov[1]

[1]Department of Physiology, [2] East Carolina Institute for Diabetes and Obesity, East Carolina University, Greenville, NC

National statistics demonstrate continued increases in overweight and obesity among children over the past three decades (Loomba et al., 2008). Several lines of evidence indicate that obese (Loomba et al., 2008) and diabetic fathers (Harjutsalo et al., 2006) are likely to have obese offspring predisposed to diabetes. The molecular basis for these nongenetic transgenerational effects is unknown, but likely involves epigenetic modifications in the methylation state of DNA, histones and microRNAs (Handel et al., 2010). Our current research tests the hypothesis that exposure of male mice to high fat diet will increase susceptibility of their offspring to obesity and glucose intolerance. We further presume that this phenotypic change will be linked to alteration in DNA methylation, gene and miRNA expression.

Four-week old C57 Black male mice were divided into three groups: a group with 60%-fat chow, a control group with 10% fat chow and an exercise group on 10% fat chow. The mice were exposed to their diet regiment for 12 weeks. Glucose tolerance tests and metabolic cages assessed changes in their metabolic profiles. An MRI was used to measure changes in body composition. After 12 weeks of treatment, the body weight increase in mice differed between groups: 27.6% control, 114% fat diet, 39.8% wheel group. The data from the glucose tolerance tests showed that one hour after injection with 50% dextrose, the glucose levels of the mice changed over the 12 weeks: the control group had a 32.7% increase, the fat diet group had a 70.3% increase, the exercise group had a 6.2% decrease. This data shows that male mice on high-fat diet have lower tolerance to glucose and a higher body fat composition than control and exercise groups.

All males were mated with normal C57Black 7-week old female mice. The offspring will be monitored for changes in developmental pattern and body weight curve. Upon reaching 5 weeks, half of the offspring will be subjected to 60% fat diet for 12 weeks and half will be used as controls on regular mouse chow. Tissue collected from fathers and offspring will be screened for genome-wide changes in DNA methylation pattern. We anticipate differences in susceptibility to diabetes between offspring of high-fat fathers and offspring of control and exercise groups. This research was supported by ECDOI Pilot Grant to AKM

*STUDENT PRESENTATION

Morphological Correlates of Gravity Receptor Functional Aging in CBA/CaJ Mice

Jessica E. Pierce, Sarath Vijayakumar, Sherri M. Jones

Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC

Cdh23^{ahl} (*Ahl*) is a genetic mutation located on mouse chromosome 10 that affects Cadherin23, a protein critical for sensory transduction. *Ahl* predicts age-related hearing loss; however, it may not predict vestibular functional aging. We are currently assessing vestibular functional and structural aging, and hypothesized that morphological aging of vestibular structures correlate with gravity receptor function as measured by vestibular evoked potentials (VsEPs). To test this hypothesis, we quantified hair cells, synaptic ribbons, and post synaptic receptor sites in the utricle of CBA/CaJ mice (6, 12, and 22 months of age, n = 4 to 6 specimens per age), and correlated the structural data with functional VsEP data from previous studies. CBA/CaJ mice have no known genetic mutations affecting the inner ear and serve as an aging control model. Utricles were dissected and stained with CtBP2 (a marker for synaptic ribbons) and Shank1a (a protein located within the post-synaptic density). Specimens were then imaged with a Zeiss LSM 510 confocal microscope. The number of hair cells, synaptic ribbons, and Shank1a were quantified and averaged for four distinct areas (~2300 μm^2) across each epithelium. The number of hair cells was similar at 6 (73.44 ± 6.41) and 12 months (77.32 ± 7.17), but declined significantly by 22 months (56.28 ± 8.57). The number of synaptic ribbons per hair cell declined significantly from 6 (5.23 ± 0.88) to 12 months (3.73 ± 0.76) and from 12 to 22 months (2.17 ± 0.67). Shank1a also declined significantly between 6 (7.40 ± 1.76) and 12 months (4.52 ± 1.01), but not between 12 and 22 months (3.40 ± 0.82). CtBP2 and Shank1a counts per hair cell were significantly correlated with VsEP thresholds in that the number of synaptic ribbons and Shank1a per hair cell declined as VsEP thresholds became significantly elevated with age. Results suggest that gravity receptor functional decline with age is associated with an age related decline in synaptic and neural elements. Research was supported by NIH R01 DC006443 and DC006443-04S1.

*STUDENT PRESENTATION

Nurses Provide the 411 to Prevent the 911 Building a Bridge to Promote Best Practice for Acute Stroke Care: Guiding Lipid Management

Cristine W. Small MPH, RHIA, CPHQ, Dawn Becker, BSN, RN, Sharon Watford, RN, BSN, Marie C. Welch RN, MSN, MS, CRRN, Vonda Kenlaw, RN, Karen Tucci-Herron, RN, MSN, Susan D. Freeman MSN, FNP-BC

Department of Surgical Services Neuroscience, Primary Stroke Center, Pitt County Memorial Hospital, Greenville, NC

Elevated blood cholesterol levels increase a person's risk for stroke and high cholesterol is often discovered during an acute stroke event. Best practice guidelines from the American Stroke Association /American Heart Association recommend stroke/ Transient Ischemic Attack (TIA) patients with an LDL ≥100 be discharged on cholesterol reducers. A collaborative approach to treatment of abnormal lipids will ultimately improve cerebrovascular morbidity and mortality. Nurses collaborate with health care providers to assure appropriate lipid management for EVERY stroke or TIA patient.

A retrospective analysis of clinical databases was undertaken. High reliability principles were implemented to promote quality improvement for stroke patients. Nursing empowerment, concurrent abstraction and immediate proactive intervention and interactive educational sessions are at the forefront of stroke improvements efforts, along with redundancies and decision aids. The electronic medical record (EMR) is an important tool for integration of evidence-based clinical guidelines, decision support, increased preventive care services, improved processes and outcomes, and the continuity of care.

The aim is toAchieve 100% Compliance on the LDL Stroke Measure: Ischemic stroke patients with LDL >= 100 mg/dL, or LDL not measured, or, who were on a lipid-lowering medication prior to hospital arrival are prescribed statin medication at hospital discharge.

Data illustrated an incremental increase in LDL measure compliance by 12% from Tier 1 Goal 86%, to Tier 2 Goal 98%, and 29% improvement in LDL measure compliance pre EMR and post EMR. Analysis of the LDL Achievement Measure, which includes TIA, showed a 5% increase in measure compliance from period July 2008 to June 2009 (91%) to July 2009 to June 2010 (96%).

With the current economic climate, high reliability principles such as use of the Plan-Do-Check-Act (PDCA) performance improvement methodology, lean methods, the EMR, and streamlined processes are pivotal to successful quality initiatives and alignment of programs with healthcare reform. In addition, aggressive TIA management is indicated as an important process for stroke prevention.

Age-Related Vestibular Dysfunction in Inbred Mouse Strains: Influence of the *Ahl* locus

Sarath Vijayakumar, Ph.D. and Sherri M. Jones, Ph.D.

Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC

The *Ahl* locus mapped to chromosome 10 has been identified as a major contributor to age-related hearing loss and has also been identified as a variant of the cadherin 23 gene ($Cdh23^{753A/G}$, Noben-Trauth et al., 2003). While this mutation may lead to profound hearing impairment in aged mouse strains, Mock et al. (2009) showed that it does not predict functional aging of the gravity receptor organs. For example, C57BL/6J mice maintain normal vestibular function despite losing hearing beyond 10 months of age. The purpose of the present study was to examine a potential role for *Ahl* in vestibular aging by characterizing gravity receptor function in the B6.CAST-$Cdh23^{Ahl+}$/Kjn (B6.CAST) strainthat carries the normal functioning Cdh23 allele. We assessed gravity receptor function by measuring linear vestibular evoked potentials (VsEPs) in 45 mice aged 2 to 20 months. Auditory function was also assessed with auditory brainstem response (ABRs) testing. VsEP parameters and ABR thresholds were compared to age-matched data from C57BL/6J mice previously studied in our lab. Synaptic architecture of the utricular hair cells was examined by immunolabeling the synaptic bodies (anti-CtBP2) and post-synaptic AMPA receptors (GluR2/3). Consistent with published studies, ABR thresholds revealed minimal age-related hearing loss in the congenic animals. VsEP thresholds ranged from -4.5 to -13.5 dB re: 1.0g/ms, and at +6 dB re: 1.0 g/ms, P1 peak latencies ranged from 1.25 to 1.63 ms while P1-N1 amplitudes ranged from 0.40 to 1.42 µV. Linear regression slopes for VsEP thresholds were -0.07 dB per month for the congenic mice compared to 0.12 dB per month for the C57BL/6J strain. Indeed, gravity receptor functional aging in both of these strains is significantly less than CBA/CaJ (model with no known genetic mutations affecting the inner ear). Preliminary histological findings do not indicate structural differences in the synaptic morphology. Thesedata out to 20 months suggest no significant effect of age on VsEP threshold or P1-N1 amplitudes for the B6.CAST. Furthermore, they suggest that the *Ahl* locus does not play a significant role in gravity receptor aging and the maintenance of function for both C57BL/6J and B6.CAST is likely due to other genetic modifiers that remain to be determined.
Research supported by NIDCD R01DC006443 and DC006443-04S1.

*POST-DOC PRESENTATION

The Importance of MicroRNAs during Regenerative Axon Growth

Di Wu, Elena Pak, and Alexander K. Murashov

Department of Physiology, East Carolina University, Greenville, North Carolina

Recently, functional and potent RNA interference (RNAi) has been reported in peripheral nerve axons transfected with short-interfering RNA (siRNA). In addition, components of RNA-induced silencing complex (RISC) have been identified in axotomized sciatic nerve fibers as well as in regenerating dorsal root ganglia (DRG) neurons in vitro. Based on these observations, and on the fact that siRNA and microRNAs (miRNA) share the same effector enzymes, we hypothesized that the endogenous miRNA biosynthetic pathway would respond to peripheral nerve injury. To answer this question, we investigated the function of miRNAs in response to peripheral nerve crush injury in mice. The experiments were performed on dissociated dorsal root ganglia (DRG) neuronal cultures after conditional sciatic nerve lesion in mice. Sciatic nerve crush was performed 5 days before DRG collection as our animal model to induce regenerative axon growth. Inducible Dicer (*CAG-CreERt:Dicer*$^{fl/fl}$) knockout mice and wild-type mice were used in this study. After overnight culture, neuronal cells and axons were stained with antibody against neuronal ß-tubulin. Comparison of axon length and the number of axon branches between Dicer knockout and wild type groups revealed that the deletion of Dicer, a key enzyme responsible for generation of microRNA dramatically reduced regenerative axon growth. In addition, microarrays performed on DRGs from injured versus naïve side identified a group of differentially expressed miRNAs. The injury-regulated pattern of miRNAs expression was confirmed using real-time RT-qPCR. The loss of function and gain of function analyses indicated that miR124, miR140, miR431 and miR744 are promising candidates for the key regulatory switches of peripheral axonal growth.
Taken together, our data provide further evidence in support of our hypothesis of miRNA involvement in peripheral nerve regeneration processes.

Acknowledgments: This study was supported by Harriet and John Wooten Laboratory grant to AKM.

*STUDENT PRESENTATION