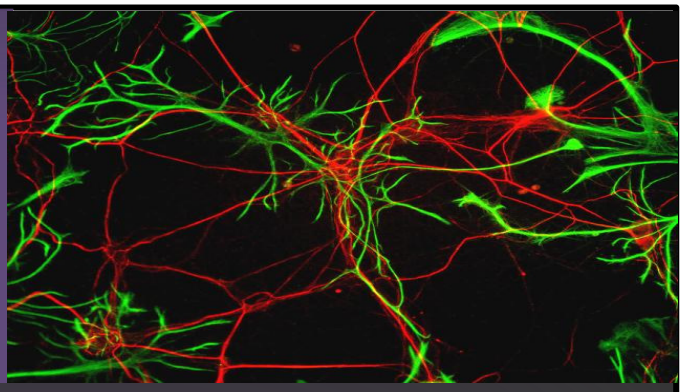
Eastern Carolina Chapter of the Society for Neuroscience Presents:



15th Annual Neuroscience Symposium
Catalyst for Collaboration

Featuring:

The Society for Neuroscience Distinguished Travelling Scientist

Richard Allen, Ph.D.

Associate Professor of Neurology

Johns Hopkins University

"Brain Iron Relation to Dopamine and Glutamate in Restless Legs Syndrome"

Tuesday, October 29th, 2013

East Carolina Heart Institute

9:00 am – Registration

11:45 am – Opening Remarks

12:00 pm – Keynote Address: Dr. Allen

1:30-2:45 pm- ECU Faculty Presentations

2:45-4:45 pm – Poster Session

4:45 pm – Closing Remarks and Awards

Registration and program information at: www.ecu.edu/neurochapter

The Officers and Council Members of the Eastern Carolina Chapter of the Society for Neuroscience would like to express their sincere gratitude to the following entities for their support of the 2013 Annual Neuroscience Symposium:

Eastern Carolina Chapter of the Society for Neuroscience

World Wide Medical Supplies

The Society for Neuroscience

The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Diseases Research

The North Carolina Biotechnology Center

Charles Rivers Laboratories

Multidisciplinary Studies Program in Neuroscience, ECU

Neuroscience Student Association, ECU

Harris Teeter Grocery Store, Greenville, NC

Officers:
President: Dr. Tuan Tran
President-Elect: Dr. Stefan Clemens
Past-President: Dr. Kori Brewer
Treasurer: Dr. Brian McMillen
Secretary: Dr. Sonja Bareiss

Council Members:
Dr. Kelly Harrell
Dr. Heather Wright
Dr. Xiaoping Pan

Student Council Members:
Erin Connolly

PROPOSED 2013 Neuroscience Symposium: Catalyst for Collaboration

Presented by:

The Eastern Carolina Chapter of the Society for Neuroscience

Schedule of Events:

9:00-11:45	**Registration:** ECHI Hallway
10:30-11:45	Brunch with Keynote and Students: ECHI Classroom
11:45-12:00	**Opening Remarks:** Michael Van Scott, PhD, Interim Associate Vice Chancellor, Division of Research and Graduate Studies
12:00-1:15	**Keynote Address:** Richard Allen, PhD, Dept. of Neurology, Johns Hopkins Medical Center "**Brain Iron Relation to Dopamine and Glutamate in Restless Legs Syndrome"**
1:15-1:30	**Break & Vendors w/snacks**
1:30-1:55	Hu Huang**,** PhD, ECU Human Performance Laboratory **"ROCK ROLE in CNS Control of Metabolism"**
1:55-2:20	Raymond Dionne**,** PhD, ECU Department of Pharmacology & Toxicology **"Phenotyping Chronic Pain for Discovery Research and Directed Therapeutics"**
2:20-2:45	Xiaoping Pan, PhD, ECU Department of Biology **"Identification of Circulating microRNAs as Biomarker for Blast-induced Neurotrauma"**
2:45-4:45	**Poster Session/Vendors** (w/ hors d'oeuvres)
4:45	**Closing Remarks and Awards**

Oral Presentations

Brain Iron Relation to Dopamine and Glutamate in Restless Legs Syndrome

Richard Allen

Department of Neurology, Johns Hopkins Medical Center, Baltimore, MD

The restless legs syndrome (RLS) (AKA Willis Ekbom Disease) is a common and to some a disabling neurological disorder significantly disrupting lives of 2 -3 % of adults in North America and Europe. It occurs with marked sleep loss, decreased work productivity, impaired quality of life and increased risk of cardiovascular disease. It has a well-documented brain iron deficiency affecting mostly the substantia nigra and striatum. Medications treating RLS indicate possible underlying biological dopaminergic and glutamatergic abnormalities.

The medical and neuroscience question: How brain iron deficiency produces the neurobiological abnormalities of RLS. Cellular and animal models of brain iron deficiency reveal somewhat unexpected processes altering the dopaminergic and glutamatergic systems. These processes reveal pathways to the disease that include hypoxic pathway activation and changes in gene expression. Clinical and autopsy studies confirm and indicate clinical utility of these findings for guiding treatment development.

This presentation after a brief video introduction to RLS will review evidence for hyper-dopaminergic state in RLS (pre-synaptic and extra-cellular dopamine increase) with post-synaptic adjustments. Iron deficiency and hypoxic pathway involvement relate to genetic findings and contribute to an expanded clinical presentation of RLS. Next will be consideration of possible alternate aspect of brain iron deficiency and RLS with glutamate involvement and hyperarousal. These combine to provide a more complete view of RLS and effects of brain iron deficiency. Finally iron treatment effects on brain iron and RLS will be noted in relation to issues of brain iron regulation and possible RLS treatment development.

ROCK ROLE in CNS-Control of Metabolism

Hu Huang[1,2], Christian Bjørbæk[3], Bradford B Lowell[3] and Young-Bum Kim[3]

[1]Department of Kinesiology and Physiology
[2]East Carolina Diabetes and Obesity Institution, East Carolina University, Greenville NC
[3]Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA

Leptin regulates energy balance. However, knowledge of the critical intracellular transducers of leptin signaling remains incomplete. Here we report that Rho-kinase 1 (ROCK1) regulates leptin action on body weight homeostasis by activating JAK2, an initial trigger of leptin receptor signaling. Leptin promotes the physical interaction of JAK2 and ROCK1, thereby increasing phosphorylation of JAK2 and downstream activation of Stat3 and FOXO1. Mice lacking ROCK1 in either POMC or AgRP neurons, mediators of leptin action, display obesity and impaired leptin sensitivity. In addition, deletion of ROCK1 in the arcuate nucleus markedly enhances food intake, resulting in severe obesity. Of note, ROCK1 is a specific mediator of leptin, but not insulin, regulation of POMC neuronal activity. Our data identify ROCK1 as a key regulator of leptin action on energy homeostasis.

Identification of Circulating microRNAs as Biomarker for Blast-induced Neurotrauma

Xiaoping Pan

Department of Biology, East Carolina University, Greenville, NC

In the context of developing novel non-invasive diagnostic and prognostic tools for mild traumatic brain injury (mTBI), this study aims to develop circulating microRNAs (miRNAs) as peripheral biomarkers for blast exposure and neurological health effects. It has long been recognized that TBI induces a complex genetic response that are implicated in lingering neurologic and cognitive problems. MiRNAs, a class of endogenous non-coding small RNAs, have emerged as a major gene regulator in most biological processes. Aberrant expression of miRNAs is implicated in neurodegenerative diseases and other CNS disorders. Therefore, a dynamic miRNA network regulating TBI-induced gene expressions may represent the molecular signature of TBI. A mild brain injury rodent model was generated using the advanced blast simulator located at East Carolina University. As an acute response to 70 kPa over ambient pressure, the expressions of vitamin D receptor, progesterone receptor, and all selected inflammatory parameters were misregulated in brain. Our preliminary work has also histologically verified the mild brain injury and evaluated the memory and cognitive deficits using the Morris Water Maze. Compared to sham-controls, intraventricular hemorrhages are common observations and blast-injured animals showed signs of depression behaviors. Twenty four miRNAs display aberrant expressions in blood of blast-injured animals collected seven days post-blast. miRNA markers target various neurological, inflammatory, and endocrinological pathways. For example, twenty miRNAs act on 96 genes in the neurotrophin signaling pathway initiated by the brain-derived neurotrophic factor (BDNF), implicated in neuroregeneration during TBI recovery and long-term potentiation associated with the posttraumatic stress disorder (PTSD).

Poster Presentations

(in alphabetical order by first author)

Examination of Neurophysiological Correlates of the Behavioral Activation (BAS) and Behavioral Inhibition (BIS) Systems

Kelly Bickel[1,2], Jonathan M. Highsmith[1], Eric Watson[1], James Loveless[1], Alexandra J. Stephenson[1,2], Katie A. Lehockey[1], and D. Erik Everhart[1]

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

The relationship between neurophysiology, human individual differences and behavior has received increase interest in the past few decades. Reinforcement Sensitivity Theory (RST) describes individual differences through human neurophysiological mechanisms. This theory assumes that three independent biological systems govern behavior, each with their own distinct neural pathways. RST consists of three interrelated systems: the Behavioral Activation System (BAS), the Fight-Flight-or-Freeze System (FFFS), and the Behavioral Inhibition System (BIS). The theory distinguishes amongst distinct kinds of approach and withdrawal systems, outlining a neurobiological nexus between personality traits, positive and negative affect and individual responsiveness to stimuli. The Behavioral Activation and Behavioral Inhibition Systems are classically associated with relative left and right frontal activity (as measured by resting alpha power), respectively, which is also associated with respective approach and withdrawal related behavior. Of note, less is known about how the specific subfactors of Behavioral Activation, which include Reward (RR) Responsiveness, Drive (D), and Fun Seeking (FS) may relate to anterior cortical activity patterns. The purpose of the current study was to replicate previous findings of anterior asymmetry and to examine the relationship between the subfactors of BAS (RR, D, and FS). It was hypothesized that RR, D, and FS would all be associated with relative left frontal activity, whereas BIS would be associated with relative right frontal activity. Sixteen young right-handed adults (9 women) completed the Behavioral Activation and Behavioral Inhibition scales. Baseline EEG data were collected, with specific emphasis on alpha (8-13Hz) power. As hypothesized, significant positive correlations (ranging between .44 and .58) were observed between RR, D, FS, and left anterior alpha power, although these relationships varied in magnitude as a function of specific electrode site (frontal pole, mid frontal, and posterior frontal) and subcomponent of alpha power (low, middle, high). No significant relationships were observed for BIS and frontal cortical activity. Thus, hypotheses are partially supported. The implications for these findings as they pertain to the neurophysiological underpinnings of individual differences in approach and withdrawal related behavior are discussed.

GSK-3β Activation for Modulation of Abnormal Neuronal Sprouting and Chronic Pain after Spinal Cord Injury

Lindsey Cannon SPT[1], Heather Lauth[1], Kori Brewer Ph.D.[2], Sonja K. Bareiss PT, Ph.D.[1]

[1]Department of Physical Therapy, East Carolina University, Greenville, NC
[2]Emergency Medicine, East Carolina University, Greenville, NC

Spinal cord injury (SCI) results in chronic pain and sensory dysesthesias in the majority of SCI patients. Recently, we showed that excitotoxic induced spinal cord lesions result in DRG neurite outgrowth that correlated with the presence of sensory dysesthesias. The purpose of this study was to administer a GSK-3β activator, LY-294002 (LY), to SCI animals in order to modulate abnormal sprouting of sensory neurons and prevent the development of pain. Long-Evans male rats received an intramedullary injection of quisqualic acid (SCI) or saline (sham operated control). Immediately after the SCI or sham surgery, a flexible catheter was inserted subdurally to deliver either LY or vehicle (veh) to the level of the lesion once per day for 3 days. Rats were divided into three groups: sham/veh (n=9), SCI/veh (n=12), or SCI/LY (n=11). Following operations, rats were observed daily for the presence of overgrooming, a spontaneous at level pain related behavior. Fourteen days post SCI, DRGs ipsilateral to the site of injection were harvested and cultured for 20 hours. The cells were stained with a neuronal specific marker (TubIII) and analyzed for overall neurite outgrowth and length. Overgrooming was observed in 5 of the 12 (42%) SCI/veh treated animals, whereas 0 of the 11 SCI/LY rats showed evidence of grooming. Consistent with previous reports, SCI/veh grooming animals (131.0 ±14.2 μm) showed a significant ($p<0.001$) increase neurite length compared to SCI/veh nongrooming animals (75.0 ± 8.7 μm) and sham/veh animals (58.1 ±9.8 μm). Treatment with LY significantly decreased ($p<0.0001$) the length of neurites (30.9 ± 2.2 μm). We showed that intrathecal drug delivery of a GSK-3b activator, LY-294002, prevented the development of at level pain behavior (overgrooming) and reduced neurite outgrowth to noninjured levels after SCI, suggesting that GSK-3b may be an effective therapeutic target. There are currently no effective physical therapy or pharmaceutical interventions for managing chronic pain and dysesthesias associated with SCI. This study provides information on a new therapeutic target for reducing excessive sensory neuronal sprouting to prevent the development of SCI pain.

Characterization of Blast-Induced Brain Injury and Identification of Circulating Biomarkers

Erin Connolly, Dorothy L. Dobbins, Xiaoping Pan

Department of Biology, East Carolina University, Greenville, NC

mTBI accounts for 75% of the injuries in returning veterans and are often missed in diagnosis due to insufficient diagnostic and prognostic techniques. This project aims to develop a novel diagnostic and prognostic tool for blast/high pressure air wave induced mTBI, which potentially lead to important findings for medical applications to enhance mTBI resiliency and rehabilitation, improving lives of many veterans and their families. Through the use of a rat model, we are able to look at the behavioral, molecular and histological impacts of mTBI's. Firstly a mild brain injury rodent model is generated using the Advanced Blast Simulator. The blood specimen are collected 1, 7 and 14 days post-blast to detect the temporal expressions of marker miRNAs. Meanwhile behavioral assays are performed to test the animals' spatial memory and cognitive status using the Morris Water Maze. Histological examinations are also conducted to test the integrity of neurons. Preliminary results show slight hematomas pooled around the choroid plexus within the lateral ventricles of blasted animals, which are consistent with current mTBI models. No significant difference was observed in general blood chemistry of mTBI versus sham-controls. However blood miRNAs of injured animals have shown aberrant expressions. Some miRNA are known to target important genes such as BDNF, which is important in neuroregeneration and recovery of mTBI.

Mechanisms of Antigen-Specific Tolerance Induction in a Rodent Model of Multiple Sclerosis

Alan D. Curtis, II[1,2], S.M. Touhidul Islam[1,2], Mark D. Mannie[1,2]

[1]Department of Microbiology and Immunology and The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Disease Research
[2]East Carolina University Brody School of Medicine, Greenville, NC

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) of putative autoimmune origin characterized by focal inflammatory lesions together with demyelinating plaques in the periventricular and perivascular regions of the CNS white matter. Antigen-specific vaccines represent promising approaches for therapeutic intervention and would preserve the integrity of the T cell repertoire and adaptive immunity by specifically targeting auto-reactive T cell clones. Previous studies have shown that single-chain fusion proteins comprised of granulocyte-macrophage colony stimulating factor (GM-CSF) as the N-terminal domain and a neuroantigen (NAg) epitope as the C-terminal domain are potent, NAg-specific inhibitors of experimental autoimmune encephalomyelitis (EAE). The purpose of this study was to better understand the scope and mechanism(s) of these therapeutic vaccines.

Myelin oligodendrocyte glycoprotein (MOG) is a transmembrane protein of oligodendrocytes, which have an important role in myelin formation and maintenance. Amino acids 35-55 (MOG35-55) are used to drive chronic-progressive EAE in C57BL/6 (B6) mice. MOG has also proven to be an important antigen in non-human primate EAE and is implicated in human MS. A GMCSF-MOG35-55 fusion protein effectively reversed established disease in passive or active models of EAE in wildtype B6 mice and in CD4-deficient or B cell-deficient mice. Thus, GMCSF-MOG reversed the action of pre-established effector T cells by mechanisms that did not require CD4 or B cell antigen presenting cells (APC). Further mechanistic studies revealed that GMCSF-MOG35-55 alters the ratio of MOG-specific and wild type T cells in the 2D2 transgenic mouse model of EAE. Treatment of these mice withGMCSF-MOG35-55 also down-regulated T cell surface expression of CD3ε, an important component of the T cell receptor (TCR) complex. These data begin to resolve the mechanisms behind the ability of GMCSF-MOG to induce antigen-specific tolerance. Together, these data provide evidence that GMCSF-MOG35-55 is a potent, antigen-specific tolerogen in EAE that does not involve CD4 or B cell APC and may sequester MOG-specific T cells from the blood. This study was supported by a Research Grant from the National Multiple Sclerosis Society (M.D.M.) and by the National Institute of Neurological Disorders and Stroke (R15-NS075830 and R01-NS072150, M.D.M.).

Role of Inflammation in High-Fat Induced Cognitive Deficits

Eric Guendner, Katherine Baumgartner, Carolyn Diaz, and Rachel A. Kohman

University of North Carolina Wilmington, Department of Psychology, Wilmington, NC, USA

Obesity is a risk factor for several life threatening diseases and neurological disorders including cardiovascular disease, diabetes, metabolic syndrome, dementia and Alzheimer's disease. One factor that may contribute to the increased risk for these conditions is the development of chronic inflammation. The current study evaluated whether consumption of high fat diet affects cognitive performance, measures of neural plasticity and alters the inflammatory response to an immune challenge. Adult C57BL/6J mice that were fed ad libitum either a high fat diet (HFD; 60% fat) or control diet (CD; 10% fat) for five months. Results indicate the consumption of HFD impaired acquisition of spatial learning task relative to mice consuming CD. Further in response to the endotoxin lipopolysaccharide (LPS) HFD mice showed a similar or attenuated inflammatory response compared to CD mice. The immune challenge increased the proinflammatory cytokines interleukin-1**β** (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in splenic and adipose tissues, but did not differ by diet. LPS challenge increased IL-6 and IL-1β in the hippocampus. IL-6 levels did not differ by diet, however, HFD mice showed decreased IL-1β in the hippocampus following LPS. Collectively, data indicate that HF diet consumption did not induce a chronic inflammatory state as previously reported. HF mice showed decreased expression of brain derived neurotropic factor (BDNF) and synaptophysn, a synaptic vesicle protein. We hypothesize that the cognitive deficit noted in subjects consuming the HFD may be related to the changes in neurotropic factor levels and synaptic plasticity. The data indicate that the consumption of a HFD impairs cognitive function and these deficits may occur independent of changes in the immune response.

Changes in Blood Biomarker Profiles Following Exercise in a Triple-Transgenic Mouse Model of Alzheimer's Disease

Morgan Haskins[1], Terry E. Jones[1], Morgan M. Pearce[1], Tuan D. Tran[2], Qun Lu[3], and Sonja K. Bareiss[1]

[1]Department of Physical Therapy, [2]Department of Psychology, [3]Anatomy and Cell Biology at East Carolina University

Exercise has been shown to protect against cognitive decline and Alzheimer's disease (AD) progression. Despite the wealth of epidemiological evidence of this therapeutic approach, the dose of exercise required to protect against AD has not been determined. Recent studies show that the pathological processes leading to AD cause characteristic alterations in blood signaling inflammatory proteins that are associated with the progression of AD. The purpose of this study was to determine the impact of increasing dosages of exercise on AD plasma signaling protein profiles. Triple transgenic AD mice (3xTg) bearing the PS1-M146V, APP-Swe, and tauP301L mutations and non-transgenic mice were assigned to one of the following groups: sedentary controls, one time per week, or three times per week forced wheel running. All exercise groups received 12 weeks of exercise at a moderate intensity of 8.0 m/min for 60 minutes. Blood was drawn at the start (3 months) and end of the exercise intervention. Blood was analyzed using the Meso Scale Discovery System testing for the following analytes: monocyte chemotactic protein-1 (MCP-1), tumor necrosis factor-alpha (TNF-α), and regulated and normal T cell expressed and secreted (RANTES). Our results show that alterations in serum inflammatory cytokines are evident as early as 3 months of age in 3xTg-AD mice, changes that precede the well characterized cognitive and pathological hallmarks in this AD model. Exercise dose-dependently improved cognitive function and restored serum inflammatory profiles in 3xTg-AD mice. This study provides evidence to support that increasing exercise frequency may be an important therapeutic tool in combating or delaying cognitive decline associated with AD.

Assessment of Persistent Neuronal Morphological Changes after Chronic CB_1 receptor Antagonist SR141716A or Antidepressant Treatment in Zebra Finches

Tessa L. Holland, Marcoita T. Gilbert, Ken Soderstrom

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

Zebra finches possess a developmental sensitive period of vocal learning in which a decrease in dendritic spine density occurs in brain song regions. Previously, we observed that chronic developmental treatment with CB_1 receptor agonist WIN55,212-2 prevented this normal reduction, but adult treatment had no effect, suggesting WIN55,212-2 possibly disrupted developmentally significant endocannabinoid signaling. To evaluate the effects of CB_1 receptor antagonism during vocal development, in a similar experiment we treated developing and adult zebra finches (n=4) with CB_1 receptor antagonist SR141716A (6 mg/kg) for 25 days followed by 25 days of no treatment (until maturation) and created 3D neuronal reconstructions using Golgi-Cox staining and Neurolucida software. Surprisingly, both developmental and adult treatment persistently increased spine densities. Adult treatment produced more dramatic increases, suggesting tonic inhibitory endocannabinoid control of song region spine densities is perhaps important to maintain mature neural phenotypes. Another interpretation is the increased spine densities are a consequence of the mood depressant effect of SR141716A, which was used clinically to treat obesity and observed to cause depression in patients. To discover if antidepressant treatment resulted in different effects on neuronal morphology, we treated zebra finches (n=3) with the monoamine oxidase inhibitor (MAOI) phenelzine (1 mg/kg) for 25 days followed by 25 days of no treatment. Chronic phenelzine treatment had no persistent effect on spine density, and antidepressants may help maintain stable spine populations. Preliminary results indicate co-administration of phenelzine prevents SR141716A-induced increased spine densities, suggesting an association between spine density and the mood effects of these drugs.

Dopamine D3 Receptor Dysfunction Alters the Functional State of Mu-Opioid Receptors and Induces a Morphine-Tolerant Phenotype in the Spinal Cord

Grace Loeffler[1], Kori Brewer[1], Christine Baran[1] Marley Jensen[2], Stefan Clemens[2]

Departments of Emergency Medicine[1] and Physiology[2] Brody School of Medicine @ East Carolina University

Mice lacking a functional D3 receptor (D3KO) have decreased withdrawal latencies from painful thermal stimuli compared to wild type (WT) mice. We tested the hypothesis that this increased pain sensitivity in D3KO may result from alterations in the functional opioid system.

Baseline thermal thresholds were assessed in D3KO and WT mice via the Hargreaves method. Thresholds were reassessed after a single dose of morphine (2mg/kg, i.p.). Mu-opioid receptor (MOR) and phosphorylated MOR (p-MOR) protein levels in the lumbar spinal cords of each group were measured using Western Blotting. Electrophysiological methods were used to determine spinal reflex amplitudes (SRAs) in isolated spinal cords from D3KO and WT mice before and after bath-application of morphine.

We confirmed that D3KO exhibited lower thermal thresholds at baseline compared to WT. Administration of morphine *in vivo* significantly increased thermal thresholds in WT mice, but had no effect in D3KOs. WT and D3KO had similar protein expression levels of MOR, however, D3KO mice had a significantly higher proportion of p-MOR than WT. *In vitro*, SRAs were increased in D3KO vs. WT mice, and morphine had no effect on SRAs in D3KOs.

Our data suggest that a dysfunctional D3 receptor system promotes morphine tolerance in the spinal cord of morphine-naïve animals. This phenomenon is associated with changes in spinal MOR functional states in D3KO. These characteristics make the D3KO mouse a suitable animal model for the study of chronic pain states and morphine tolerance.

Small Molecules Targeting Rho GTPase Signaling Altered in Alzheimer's Disease

Qun Lu, Amy M. Friesland, Jonathan Lee, Kyle Fulk and Yan-Hua Chen

Department of Anatomy and Cell Biology; The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Diseases Research, The Brody School of Medicine, East Carolina University, Greenville, North Carolina USA 27834

Background: Altered intracellular protein transport and synaptic remodeling are two of the most prominent cellular processes that are affected in Alzheimer's disease (AD) as well as other neurodegenerative diseases. Rho subfamily of the small GTPases within the Ras superfamily is essential for modulating these functions. Research has increasingly established the RhoA, Rac1, and Cdc42 subclasses of small G-proteins as controlling elements of secretase trafficking, Aβ production, neurotransmitter receptor signaling, and the synaptic cytoskeletal dynamics. Understanding how they operate in the context of memory and learning is pivotal for the development of Rho-GTPase based therapeutic treatments of AD.

Methods: Our recent studies demonstrated the remarkable alteration of actin cytoskeleton and its regulatory proteins in AD. These proteins include filamin (Lu et al., JAD, 2010), G-actin, and components of Rho-GTPase signaling. In order to establish a repertoire of small molecule modulators of Rho-GTPase signaling, we performed a computer-assisted *in silico* screening of the SPECS database using GLIDE program (Schrodinger) for chemical compounds that can disrupt the interaction of Cdc42 with intersectin (ITSN1), a Cdc42 specific guanine nucleotide exchange factor (GEF) (Friesland et al., PNAS, 2013). This study led to the discovery of small molecules targeting Rho GTPases including Cdc42, RhoA, and Rac1.

Results: The biochemical and imaging studies not only established the first small molecule ZCL278 targeting Cdc42-ITSN1 interaction, it also provided the proof-of-concept results showing the effectiveness of ZCL278 to interfere with Golgi organization and neuronal cytoskeleton. Additional studies on ZCL series compounds showed favorable drug tolerance, potential CNS effects, and distinct neuronal responses to staurosporine induced neuronal toxicity. Our other studies employ isolated primary cortical neurons from the triple transgenic (3xTg-AD) mice expressing AD-like mutations in presenlin 1 (M146V), APP (695swe), and TauP301L. In the 3xTg neurons, filamin, G-actin, and Cdc42 showed redistribution in the soma and the axonal or dendritic processes, further validating the potential significant involvement of actin cytoskeleton in AD pathogenesis.

Conclusion: The discovery of ZCL series chemicals could form a novel compound base to potentially develop into AD modifying therapeutics. Our studies are supported by grants from NIH and the Harriet and John Wooten Foundation for Alzheimer's Disease Research.

Atypical Cannabinoid Receptor, GPR18 Signaling and Its Interaction with Lipid Rafts in nPC12 Cells

Anusha Penumarti and Abdel A. Abdel-Rahman

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

Background and objectives: The abnormal cannabidiol (Abn CBD) receptor GPR18 mediates peripheral vasodilatation. Our previous studies were the first to demonstrate the anatomical expression of GPR18 in tyrosine hydroxylase-immunoreactive neurons in the rostral ventrolateral medulla (RVLM), and to suggest important function for RVLM GPR18 in blood pressure regulation. The latter is supported by the dose-related reductions and elevations in blood pressure (BP) caused by the GPR18 activation by abnormal cannabidiol (Abn CBD) and blockade by O-1918, respectively, in conscious rats. We also showed that activation of RVLM PI3K/AKT-ERK1/2-nNOS network and inhibition of cAMP play a causal role in GPR18 mediated hypotension. There is growing evidence that lipid rafts (LR) participate in the regulation of G-protein coupled receptor (GPCR) as well as cannabinoid 1 receptor binding and signaling. In the present study, we utilized nPC12 cells to test the hypothesis that GPR18 signaling is influenced by its interaction with lipid rafts in nPC12 cells.

Key results: The downstream effectors of GPR18 signaling in nPC12 cells include cAMP and specific kinases such as ERK1/2 and PI3K/Akt. Also, activation of GPR18 in nPC12 cells (Abn CBD/NAGly) caused an increase in nitric oxide (NO) and catalase levels and a decrease in NADPH oxidase levels leading to decreased oxidative stress. Blocking the receptor (O-1918) on the other hand, lead to a decrease in NO and catalase levels and an increase in NOX levels thereby increasing oxidative stress. Additionally, our confocal imaging findings show that GPR18 is associated with cholesterol- and sphingolipid-enriched membrane domains (rafts). Activation of GPR18 (Abn CBD or NAGly) caused dissociation of GPR18 from the LR and this response was abrogated by prior GPR18 blockade (0-1918).

Conclusions: Our findings suggest that lipid rafts dampen GPR18 signaling in nPC12 cells. Dissociation of GPR18 from LR is required for the GPR18-mediated activation of the PI3K/AKT-ERK1/2-NOS and inhibition of cAMP and oxidative stress.

Characterization of Dose-Specific Nicotine-Dependent Behaviors: Regulation of nAChRs in Caenorhabditis Elegans

J. Ryan Polli and Xiaoping Pan

Department of Biology, East Carolina University, Greenville, NC

The major compound in tobacco products responsible for addiction, nicotine, targets the nicotinic acetylcholine receptors (nAChRs) and leads to drug dependence. The nematode Caenorhabditis elegans' (C. elegans) genome encodes 28 conserved nAChR subunits, presenting an excellent model organism to investigate nicotine-induced cholinergic signaling in the context of drug addiction. To define the role of nAChR genes involved in nicotine-dependent behaviors in the whole animal, we first characterized the addiction-related behaviors: stimulation, dependence/withdrawal, and adaption/tolerance. Chronic (24 hr dosing) low-level (6.17-61.7 μM) nicotine exposure has been shown to change the activity of nicotinic receptors in a dose-specific manner. Worms exposed to 6.17 and 61.7 μM nicotine in liquid medium for 24 hr displayed nicotine-dependent behaviors; however, the 19.5 μM dosed worms did not exhibit the withdrawal behavior, which was accompanied by a concomitant general activation and increases in nAChRs abundance/activity. This study provides useful information regarding the comprehensive in vivo expression pattern of the 28 "core" nAChRs following different dosages of chronic nicotine treatments, which provide insights into the link between nAChRs and addiction neurophysiology.

Increasing Treadmill Velocity Does Not Evoke Unique Responses in Ankle Joint Mechanics in Males Compared to Females during Level Walking

Douglas W. Powell[1], Cora E. Scruggs[1], John D. Willson[2]

[1]Department of Physical Therapy, Campbell University, Buies Creek, North Carolina
[2]Department of Physical Therapy, East Carolina University, Greenville, North Carolina

Introduction: Emerging research has suggested that males and females may adopt unique motor strategies during the completion of dynamic tasks such as walking, running and landing. Specifically, it has been hypothesized that males perform dynamic activities with greater reliance upon hip musculature while females rely upon more distal ankle musculature. The purpose of this study was to compare changes in ankle joint moments and powers evoked by increasing treadmill velocity between males and females.

Methods: Ten healthy young adults (5 Male; 5 Female) between the ages of 18 and 30 years performed two 30-second treadmill walking trials at their preferred walking velocity and 120% of their preferred walking velocity. An 8-camera motion capture system (240 Hz, Qualisys, Sweden) and instrumented treadmill were used to collect three-dimensional kinematics and kinetics. Ankle joint moments and powers were calculated using Visual 3D (C-Motion, Inc.). A 2x2 (gender by velocity) repeated measures ANOVA was used to determine significant differences in peak ankle plantarflexor moments and powers between males and females with increasing treadmill velocity. A univariate ANOVA was used to compare absolute and relative changes in peak ankle plantarflexor moments and powers in response to increasing treadmill velocity. Alpha level was set at $p<0.05$.

Results: Males exhibited significantly greater peak ankle joint moments ($p < 0.01$) and powers ($p < 0.01$) compared to females in both velocities. Females did not exhibit disproportionately greater changes in ankle joint moments (Abs: $p = 0.264$; Rel: $p = 0.433$) or powers (Abs: $p = 0.313$; Rel: $p = 0.695$) compared to males in response to increasing treadmill velocity.

Discussion: These data do not suggest that males and females exhibit unique neuromuscular strategies in response to increasing treadmill velocity. However, further investigation should continue due to the small sample size used in the present study (N=5 per group).

Table 1. Peak ankle plantarflexor moments and powers in male and female participants.

	PF Moment (Nm/kg)		PF Power (W/kg)	
Group	Preferred	Fast	Preferred	Fast
Female	-0.72 (0.74)[a]	-0.61 (1.10)[a]	-0.66 (0.57 [a]	-0.64 (0.87)[a]
Male	-2.49 (0.41)	-2.89 (0.21)	-3.55 (1.59)	-2.81 (1.22)

Note: [a] – denotes significant effect of gender.

Activation of Central Nicotinic Acid Receptor GPR109A Increases Blood Pressure In Conscious Rats

Samar Rezq and Abdel A. Abdel-Rahman

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

The commonly used anti-hyperlipidemic drug nicotinic acid elicits unpleasant flushing reaction in patients due to the release of prostaglandins (PGs), and recent studies identified GPR109A as the nicotinic acid receptor. There are no reports on whether GPR109A is expressed and serves a functional role in cardiovascular regulating nuclei in the brainstem such as the rostral ventrolateral medulla (RVLM). These studies are clinically relevant because nicotinic acid crosses the blood brain barrier. An important finding of this novel study was the demonstration, for the first time, of GPR109A expression in the RVLM (Western blot). A functional role for RVLM GPR109A was sought following microinjection of its agonist nicotinic acid into the RVLM in conscious rats. Nicotinic acid was microinjected in an estimated dose range that is expected to reach this neuronal pool following systemic administration of therapeutic doses of nicotinic acid. Intra-RVLM nicotinic acid caused a sharp brief elevation in blood pressure and a reduction in heart rate, responses that are very similar to L-glutamate-evoked responses. Ongoing integrative and molecular studies will elucidate the role of neuronal L-glutamate and/or PGs release in the RVLM as potential mediators of the nicotinic acid (GPR109A) mediated pressor response. The findings are clinically relevant because they will elucidate the mechanisms of a centrally mediated deleterious effect of nicotinic acid on blood pressure, and yield new insight into the potential use of concurrent therapeutics to circumvent such deleterious effect.

Positive Lysosomal Modulator Targets a Single Pathway to Reduce Multiple Types of Pathogenic Proteins

Romine H[1], Ikonne US[1], Farizatto KL[2], Bullock MT[3], Ruiz S[1], Jackson R[1], Freeman P[1], Hwang J[1] and Bahr BA[1]

[1]Biotechnology Research Center, William C. Friday Laboratory, University of North Carolina – Pembroke, U.S.A.
[2]Depto. Genética e Biologia Evolutiva, Universitária São Paulo, Brazil
[3]Robeson Early College High School - Lumberton, U.S.A

Lysosomes are the cellular components involved in removing misfolded/aggregating proteins, but with aging lysosomes become less effective. Age-related protein accumulation disorders including Alzheimer's disease (AD), Parkinson's disease (PD), and other dementias, are suspected to involve imbalances between protein production and protein clearance. Strategies targeting the lysosomal system to enhance protein clearance are prime candidates for drug discovery efforts to reduce protein accumulation pathology and prevent the onset of dementia. The positive lysosomal modulator Z-Phe-Ala-diazomethylketone (PADK) enhances the trafficking and maturation of the lysosomal enzyme cathepsin B, thus to elicit protective clearance of toxic proteins in the brain. A key pathogenic factor in AD is the amyloid β peptide (Aβ), and cathepsin B was discovered to degrade Aβ42 via C-terminal truncation and was effective at reducing higher orders of Aβ assemblies (Mueller-Steiner et al. 2006, Neuron 51:703; Butler et al. 2011, PLoS One 6:e20501). Treating APPSwInd and APPswe/PS1ΔE9 mouse models of AD with PADK (ip, 18 mg/kg/d for 9-14 days) led to significant reductions in Aβ42 peptide in hippocampus, cortex, and other brain regions. Similarly, in the case of the A53Tα-synuclein mutant mouse model of PD, the administration of PADK led to reduced levels ofα-synuclein in TX-100 extracts of spinal cord, brainstem, midbrain, as well as hippocampus. Rat hippocampal slice cultures treated with 600 nM Aβ42 for 4 days exhibited enhanced levels of phosphorylated tau (pTau) which were also reduced by PADK-mediated cathepsin modulation. In the slice model and in both the AD and PD mouse models, PADK-mediated protein clearance was found to be associated with improved synaptic integrity. Synaptic pathology has long been considered the key event in age-related disorders that 1) leads to cognitive deficits and 2) contributes to early, gradual changes that constitute risk factors for dementia. In the disease models, PADK treatment improved the levels of synaptic proteins well known for being reduced with aging and in association with protein accumulation pathology. The evidence of synaptic recovery through lysosomal enhancement indicates a link between lysosomal capacity and the maintenance of brain function, providing a unique pathway to attenuate cognitive impairment and delay the onset of dementia. The results support a single strategy to promote cathepsin B-mediated clearance to offset multi-proteinopathy, providing a potential disease-modifying treatment for early and progressive dementias.

Results of Functional Capacity Evaluations (FCEs) in Patients with Chronic Back Pain Following Temporary Placement of a Spinal Cord Stimulator

Clayton Self, SPT[1], Michael Odom, SPT[1], Jacob N. Thorp, PT, DHS, OCS, MTC[1] , C. Ervin Davis, PhD[2, 3], Brandon N. Kyle, PhD[4]

[1]ECU Physical Therapy [2]ECU School of Dental Medicine, [3]Vidant Pain Management Center, [4]ECU Psychiatric Medicine

Introduction: From 1992 to 2006, the prevalence of chronic low back pain (LBP) rose significantly from 3.9% to 10.2%. Also, the proportion of patients seeking care for chronic LBP increased from 73.1% in 2008 to 84% in 2009.1 Spinal Cord Stimulators (SCSs) have been shown to be an effective treatment option for pain at 3 year follow-up compared to back surgery.2 In a 2013 systematic review, over 50% of patients with failed back surgery and chronic back and leg pain reported 50% or more pain reduction at mean follow-up of 24 months post-SCS placement.3 A 2004 review concluded that use of SCSs is safe, because there had been over 3000 patients with this device and no instance of any major adverse effect.4 Functional Capacity Evaluations (FCEs) are commonly used to evaluate the physical performance of individuals with low back and leg pain. Therefore, FCEs could be a useful outcomes measure for effectiveness of SCS. Purpose: The purpose of this study was to examine the effects of SCSs on physical performance by comparing FCE scores pre- and post-SCS trial. Methods: FCE data were collected at a multidisciplinary outpatient pain management center from 34 patients with chronic neuropathic pain in leg and back before and after trial placement of a spinal cord stimulator. Patients underwent a modified FCE before SCS placement and approximately seven days later. The FCE consisted of sitting, standing, bending, navigating stairs, and walking. Patients were also asked to walk carrying a weight (up to 20 lbs.) for as far as possible. They were asked to do each task for as long or as far as possible, up to a maximum predetermined limit for each section. Additionally, patients gave a verbal pain rating (0-10) after each task. Mean pain ratings and mean task completion times, pre- and post-placement, were calculated and compared using paired t-tests. Results: Change in percentage of patients achieving maximum task time for sitting from pre- to post-SCS placement was non-significant ($p=0.09$), as well as change in stand time ($p=0.11$). Significant increases were found in time that patients were able to bend ($p= 0.003$), walk ($p=0.03$), and navigate stairs ($p=0.04$). Decreases in pain ratings with sitting, 5.93 to 3.35 ($p=5x10-5$), standing, 6.74 to 4.16 ($p=8x10-6$), bending, 3.93 to 6.93, walking, 7.21 to 4.54 ($p=1x10-6$), and stairs, 7.25 to 4.53 ($p=5x10-6$), were significant. The distance by weight product completed decreased significantly ($p=0.0024$), along with pain ratings during weight carry (7.09 to 4.34) ($p=8x10-6$). Conclusions: The results indicate that SCS trials for chronic back pain can significantly increase functioning while decreasing report of pain. Further research is needed to evaluate long-term effects of permanent SCS placement.

Effect of Increasing Gait Velocity on the Regularity of Center of Mass Movement During Treadmill Walking

Cora E. Scruggs[1], John D. Willson[2] and Douglas W. Powell[1]

[1]Department of Physical Therapy, Campbell University, Buies Creek, North Carolina
[2]Department of Physical Therapy, East Carolina University, Greenville, North Carolina

Introduction: Variability is an inherent characteristic of biological signals. Literature suggests that reductions in moment-to-moment variability are associated with aging and pathology. However, little is known regarding the effect of increasing mechanical demand on movement variability. Therefore, the purpose of this study was to quantify changes in moment-to-moment variability of center of mass motion in response to increasing walking velocity.

Methods: Ten healthy young adults between the ages of 18 and 30 years performed two 30-second treadmill walking trials at each of five walking velocities. Walking velocities were equal to the individual's preferred walking velocity, ±10% of preferred walking velocity and ±20% of preferred walking velocity. A single retro-reflective marker was used to estimate the individual's center of mass and was placed on the posterior, superior portion of center of the pelvis (approximately L5-S1 joint) and tracked using a motion capture system (240 Hz, Qualisys, Sweden). Approximate entropy (ApEn), a nonlinear measure of variability, quantifies moment-to-moment variability as a value between 0 and 2. An ApEn value of zero indicates a highly predictable signal such as a sine wave. Conversely, an ApEn value of 2 indicates a highly variable signal in which no point can be predicted from preceding points such as white noise. ApEn values were used to quantify variability within the marker trajectory in the mediolateral and vertical directions. The effect of treadmill velocity on ApEn values was determined using a univariate analysis of variance. Post-hoc t-tests were used to determine the source of a significant main effect of treadmill velocity. Alpha level was set at $p < 0.05$.

Results: Increasing treadmill velocity was associated with significant increases in center of mass variability in the mediolateral direction ($p = 0.003$). Additionally, increasing treadmill velocity was associated with significant increases in variability of center of mass motion in the vertical direction ($p = 0.020$).

Discussion: These data demonstrate that movement variability is significantly altered by mechanical demand. Further research may investigate the effects of load carriage on movement variability.

Mitigation of Organophosphate-Induced Neurobehavioral Impairments Using Naltrexone in Rats

Marie Shuter[1, 2], Lidia Ortega[1], Jessica Justus[1,2], JoColl Burgess[2], Kori Brewer[3], William Meggs[3], and Tuan Tran[1,2]

[1]Department of Psychology, East Carolina University, Greenville NC
[2]Multidisciplinary Studies Program in Neuroscience, East Carolina University, Greenville NC
[3]Deparment of Emergency Medicine, BSOM, East Carolina University, Greenville NC

Accidental poisoning with organopesticides used for agricultural purposes is commonly seen in rural communities, including many areas of eastern NC. It is documented that chronic nerve damage, including cerebral dysfunction and neuropsychological disabilities occur in humans after such poisonings. Unfortunately, organophosphate compounds are also used as nerve agents in chemical warfare and terrorist attacks. Some of the symptoms that persist after exposure include headaches, memory loss, confusion, and fatigue. Studies have shown acute poisonings can induce impairments on performance in neuropsychological tests. While acute physiological manifestations are well managed with atropine and pralidoxime, a large percentage of subjects eventually develop neurocognitive problems including, memory loss, confusion, anxiety disorders and increased aggression. An explanation is that an inflammatory cycle within the CNS may be a common mechanism of many neurological conditions. This suggests that novel anti-inflammatory drugs may be beneficial in minimizing the impact of inflammatory processes, thus reducing the onset of neuropsychological impairments. Naltrexone is a potent anti-inflammatory agent that is safe and readily available. Indeed, clinical trials have shown that naltrexone is effective in several inflammation-related diseases, such as neurogenic pain and movement disorders. This study involved a rodent model of acute organophosphate poisoning using diisopropylfluorophosphate (DFP), an irreversible acetylcholinesterase inhibitor, to determine if naltrexone can mitigate the development of neurocognitive problems in the weeks after exposure. Adult rats (n = 12/group) were given acute DFP (50 mg/kg), DFP + naltrexone (5 mg/kg), or naltrexone; rats were treated chronically with naltrexone for 12 weeks. Afterwards they underwent assessments for reference memory using the Morris maze (5 days of learning and 1 day of memory retention) and assess associative learning using trace eyeblink classical conditioning (6 days acquisition, 3 days extinction). Both tasks are known to be mediated by an intact hippocampus, which may be vulnerable to DFP. Preliminary results (n = 10/group) indicate that rats poisoned with DFP but treated with naltrexone show improvements in MM performance. Eyeblink assessments are still ongoing. Naltrexone has been shown to be neuroprotective against inflammation-mediated neurodegeneration and is therefore a good candidate in examining the prevention of neurological sequela from organophosphate poisoning.

Three Generations, One Future: Post-embryonic Nicotine Exposure Caused Trans-Generational Addictive Behavior in *C. elegans*

Faten A Taki, Xiaoping Pan, and Baohong Zhang

Department of Biology, East Carolina University, Greenville, NC 27858

Passive and active exposure to tobacco smoking among youth is directly associated with immediate as well as long term health deterioration. Despite all pubic heath policies and efforts, the percentage of teenage smokers is still relatively high, especially in developing countries. Very few, if any, studies have been done on the trans-generational effect of nicotine exposed during the more sensitive, early developmental stages. We employed *C. elegans* as a biological model to study the multigenerational impact of chronic nicotine exposure. Nicotine treatment was limited to the N2 hermaphrodites of the F0 generation. It was strictly treated to L1-L4 (~31 hours) period after which worms were transferred to a fresh NGM plate. L4 developmental stage was used for behavioral analysis across three generations: F0, F1, and F2. Our results show that nicotine was associated with changes in sinusoidal locomotion, speed, and body bends in L4 larvae in all three tested generations. Despite having different patterns, those behavioral alterations were not restricted to F0, but were observed in F1 and F2 generations which were never exposed to nicotine. Our study is the first to reveal that nicotine addiction is heritable using *C. elegans* as a model organism. These results underscore the sensitivity of early development stages, with hope to spread more awareness to encourage the avoidance of nicotine exposure, especially at a young age.

Developmental Lead Exposure and the Exacerbation of Alzheimer's Pathology: An Immunological Analysis

A.N. vonderEmbse, D. Johnson, J.N. Franklin, Q. Hu, and J.C. DeWitt

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

Although Alzheimer's disease (AD) is typically a late-onset neurodegenerative disorder, there may be a period of susceptibility early in brain development which, if disrupted, may exacerbate the disease pathologies. We hypothesize that early exposure to a known neurotoxic and immunotoxic chemical, such as lead acetate, may alter microglia function and/or phenotype during this critical window of development, which then promotes amyloidosis or decreases microglia phagocytosis of amyloid-β, the protein associated with AD neuropathology. The exact mechanism by which microglia are involved with amyloid-β deposition or removal is currently contested in the scientific literature, although there is significant evidence for the colocalization of microglia and amyloid-β plaques. We investigated this "double-hit" model of the concurrent effects of environmental toxicant exposure and critical windows of development susceptibility through the use of a genetically predisposed triple transgenic mouse model (3XTgAD). Pups were orally gavaged with a 100 parts per million of lead acetate and vehicle from postnatal day (PND) 5-15, a known postnatal period of vulnerability for microglia. At PND50, 90, and 180, hippocampus was isolated from brains of treated and control animals and stored for future analysis of microglia colocalization with amyloid-β. The remaining brain tissue was processed to measure microglial activity by flow cytometry. Activity of microglia from treated animals was increased relative to control animals. These initial data suggest that early-life exposure to an environmental agent, when given during a critical window of microglia maturation, changes microglial activity. We believe that these changes in activity alter the course of AD neuropathologies.

Elucidating the Mechanism of Cytokine-Neuroantigen TTVs Through the use of GMCSF(E21R)-MOG

Daniel S. Wilkinson, Ashton E. Thomason, and Mark D. Mannie

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

Multiple Sclerosis (MS) is an inflammatory demyelinating autoimmune disease of the central nervous system (CNS). The disease is characterized by focal inflammatory lesions and plaques of demyelination in the white matter of the brain and spinal cord. MS is estimated to affect around 400,000 people in the United States and around 2.5 million people in the world. The cause of MS is uncertain, but evidence suggests that environmental and genetic factors both play a role in its etiology. Current MS therapies rely on anti-inflammatory mechanisms or broad-spectrum immunosuppression, which can have serious adverse complications. The development of antigen-specific vaccines that induce tolerance to a particular self-antigen will avoid many of these complications. Our lab has taken a novel approach of inducing tolerance through the administration of cytokine-neuroantigen (NAg) fusion proteins. These fusion proteins consist of a native cytokine covalently linked to the major encephalitogenic epitopes of myelin (i.e., the NAg) that cause experimental autoimmune encephalomyelitis (EAE), the animal model of MS. Several of these fusion proteins inhibited disease incidence when given as prophylactics and halted disease progression when administered after onset of EAE. The cytokine fusion partner that showed the best inhibitory activity was granulocyte-macrophage colony stimulating factor (GM-CSF). In this study, murine GMCSF-NAg was mutated at a specific residue in GM-CSF that was previously shown to antagonize the biological activity of GM-CSF in humans (1). Our preliminary studies showed that the murine mutant fusion protein, known as GMCSF(E21R)-MOG, induced maximal proliferation of MOG specific T-cells indicating that the MOG antigenic domain was fully active. However, the GM-CSF(E21R) domain had substantially reduced cytokine potency compared to wildtype GM-CSF or GMCSF-MOG in a GM-CSF bioassay. Overall, these preliminary data indicated that GMCSF(E21R)-MOG targeted the MOG antigen to dendritic cells like the wildtype fusion protein but lacked the in vitro immunosuppressive activity normally associated with GMCSF-MOG. This mutant fusion protein will help elucidate the role of the CSF2RB beta-chain receptor in tolerance induction by cytokine-NAg fusion proteins in EAE.