



The Eastern Carolina Chapter of the Society for Neuroscience

# PROGRAM AND ABSTRACTS

IX ANNUAL ECU NEUROSCIENCE SYMPOSIUM

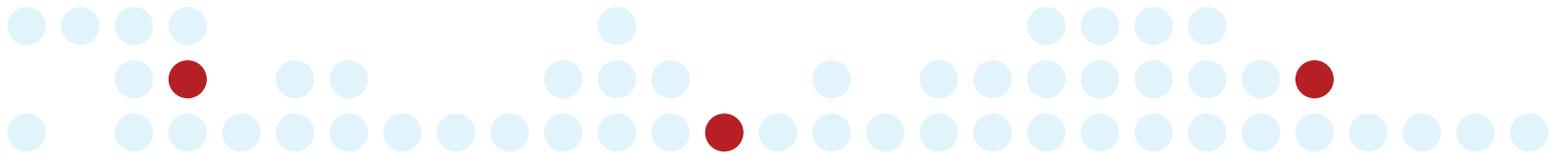


*CATALYST FOR COLLABORATION  
AT EAST CAROLINA:  
TODAY AND TOMORROW*

Thursday, November 29, 2007,

The Willis Building, East Carolina University

Greenville, North Carolina



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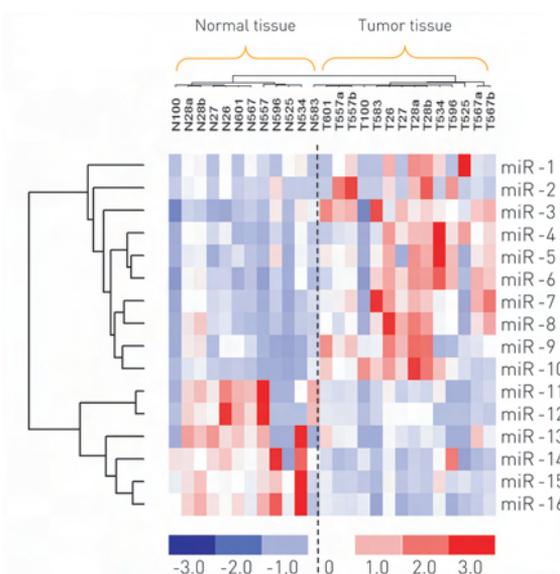
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Unsupervised hierarchical clustering and heatmap show that microRNA expression profiles clearly distinguish normal tissue from primary tumors. The paired samples of normal and tumor tissue were collected from 11 patients. The microRNA numbers are arbitrary.

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# **THE EASTERN CAROLINA CHAPTER OF THE SOCIETY FOR NEUROSCIENCE (ECCSFN)**

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IX ANNUAL NEUROSCIENCE SYMPOSIUM:**

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# IX ANNUAL ECU NEUROSCIENCE SYMPOSIUM

## CATALYST FOR COLLABORATION AT EAST CAROLINA: TODAY AND TOMORROW

### MEETING SCHEDULE

- 8:00-8:20 AM           **REGISTRATION**
- 8:20 –8:30 AM        WELCOME AND INTRODUCTORY REMARKS,  
Dr. Phyllis N. Horns, Interim Vice Chancellor for  
Health Sciences and Interim Dean of the Brody  
School of Medicine at East Carolina University
- MORNING ORAL SESSION**  
(Chaired by Qun Lu, Ph.D., Department of Anatomy  
and Cell Biology, ECU)
- 8:30 AM**               **Genetic Dissection of RAF/MEK/ERK and GSK-3  
Signaling Pathways in the Nervous System**  
William Snider M.D.,  
*Director UNC Neuroscience Center, UNC School of  
Medicine Chapel Hill, NC*
- 9:20 AM                Auditory and Vestibular Function in a Mouse Model  
for DFNA9  
Sherri M. Jones, Bruce E. Mock, Nahid Robertson,  
Cynthia Morton  
*Dept. of Communication Sciences and Disorders,  
ECU*
- 9:40 AM**               **Regulators of Topographic Axon Targeting in the  
Retino-collicular Projection**  
Patricia F. Maness, Ph.D.,  
*Professor, Department of Biochemistry & Biophysics,  
University of North Carolina, Chapel Hill,NC*
- 10:30AM                Early Cannabinoid Exposure Persistently Alters  
FoxP2 Expression within Brain Regions Important for  
Zebra Finch Vocal Learning and Control  
Ken Soderstrom and Bin Luo  
*Dept. of Pharmacology and Toxicology, ECU*
- 10:50 – 11:00AM      **COFFEE BREAK**

11:00–12:00 PM

**KEYNOTE ADDRESS**

(Introduced by Alex Murashov, M.D., Ph.D.,  
Department of Physiology, ECU)



**Molecular Mechanisms Of Neuronal Growth  
Cone Guidance**

Alex Kolodkin, Ph.D.

*Professor of Neuroscience*

*The Solomon H. Snyder Department of  
Neuroscience*

*Johns Hopkins University School of Medicine  
Baltimore, MD 21205*

12:00–12:45 PM

**BUFFET LUNCH/NETWORKING**

12:45–1:30 PM

**POSTER SESSION / VENDOR SHOW**

**AFTERNOON ORAL SESSION**

(Chaired by Jian Ding, Ph.D., Department of  
Physiology, ECU)

1:30PM

**A Comprehensive Model of Alzheimer's Disease**

Michael P. Vitek, Ph.D.

*Division of Neurology, Duke University, Medical  
Center, Durham, NC, President, Cognosci, Inc.,  
Research Triangle Park, NC*

2:20PM

Structural Studies of the APP Transmembrane  
Domain

David P. Cistola

*Departments of Clinical Laboratory Science and  
Biochemistry & Molecular Biology, ECU*

2:40PM

**HIV-1: Mechanisms of Neurotoxicity and  
Interaction with drugs of abuse**

Rosemarie Booze, Ph.D.

*Professor and Bicentennial Endowed Chair of  
Behavioral Neuroscience, Dept of Psychology and  
Dept. of Pharmacol. Physiol., and Neuroscience,  
University of South Carolina, Columbia, SC*

3:30PM

Perinatal Choline Supplementation Mitigates Trace  
Eyeblink Conditioning Deficits Associated With 3rd  
Trimester Alcohol Exposure In Rats

T.D. Tran, C.C. Richardson, J.D. Thomas

*Dept. of Psychology, ECU*

3:50PM Cross Tolerance following Chronic Intracerebellar Nicotine and Acute  $\Delta$ 9-THC Cerebellar Ataxia: Role of mouse Cerebellar Nitric Oxide  
M. Saeed Dar and Aaron Smith  
*Dept. of Pharmacology and Toxicology, ECU*

**4:10PM** AWARDS, CLOSING REMARKS  
Abdel A. Abdel-Rahman, President-Elect of Eastern Carolina Neuroscience Chapter of the Society for Neuroscience.

PODIUM PRESENTATIONS should allocate 15 minutes for the talk and 5 minutes for questions.



THE GRASS FOUNDATION  
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Visit by Dr. Alex Kolodkin was directly funded by 2007 Grass Traveling Scientist Program

The symposium was jointly sponsored by:  
Society for Neuroscience Chapter Grant



North Carolina Biotechnology Center Event Grant



For further information about neuroscience research at East Carolina University email [neuroscience@ecu.edu](mailto:neuroscience@ecu.edu) or visit <http://www.ecu.edu/neurochapter/>

# Keynote Address

## MOLECULAR MECHANISMS OF NEURONAL GROWTH CONE GUIDANCE

Alex Kolodkin, Ph.D.

*Howard Hughes Investigator, Professor of Neuroscience, The Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205*

Complex neuronal connectivity patterns develop through the action of guidance cues and their neuronal receptors. Multiple cues, including semaphorin proteins, provide repulsive and attractive influences that sculpt developing neuronal trajectories. Studies in invertebrates and vertebrates reveal the receptors and intracellular signaling mechanisms semaphorins use to guide neurons and regulate their morphology. Importantly, both extrinsic and intrinsic signaling mechanisms are capable of modulating responses to these guidance cues, greatly expanding the potential for semaphorins to sculpt neuronal connectivity. This work shows how semaphorins function in neuronal and non-neuronal cell types and suggests strategies for promoting neuronal regeneration.

# MOLECULAR MECHANISMS OF NEURONAL GROWTH CONE GUIDANCE

Alex Kolodkin, Ph.D.



Researchers dream of helping patients with permanent nerve damage to walk—or run, or swim—again. Before they can rebuild damaged nerves, however, scientists must truly understand how healthy nerves develop in the first place.

That's where Alex Kolodkin comes in. His goal is to learn how proteins act as "guidance cues" for growing nerves, alternately repelling and attracting growth to keep a developing nerve on the right track inside the body. To define the basic principles of complex nervous system organization, his lab works to identify genes in a fruit fly model and studies those newfound molecules in mice for a clearer picture of how they spur mammalian development.

Kolodkin launched his career with work that has already come to be regarded as classic. As a postdoctoral fellow, he led the discovery of the largest known family of repulsive guidance cues—a family of proteins called semaphorins, which can function to prevent neurons from extending or migrating into the wrong areas.

Kolodkin followed that work with a half-dozen more major finds, some surprising. In addition to finding key semaphorin receptors, his lab discovered a potential therapeutic target for regeneration of nerve axons: flavoprotein oxidoreductase, which helps to regulate semaphorin-mediated repulsion. He and his colleagues also demonstrated that the protein Sema7A binds to receptor molecules known as integrins to encourage axon growth rather than inhibit it—surprising many scientists, who had assumed a different receptor and action for Sema7A.

Semaphorins are just one of the protein families that guide nerve growth. Kolodkin hopes to define the fundamental principles that all such families of guidance cues use to build, maintain, and modulate neural connections.

## Featured Guest Speakers

### **HIV-1: MECHANISMS OF NEUROTOXICITY AND INTERACTION WITH DRUGS OF ABUSE**

**Rosemarie M. Booze, Ph.D.**



Dr. Booze's research focuses on the mechanisms by which the central nervous system adapts to injury and disease. In one set of studies, we are investigating the neurobiology of drug abuse. The molecular mechanisms which underlie chronic drug abuse are being studied with respect to the dopaminergic receptor systems in the brain. We have recently found that cocaine regulates a novel form of the dopamine receptor system, the D3 receptor, and may provide key evidence for how cocaine and other stimulants act in the brain. Of particular concern in these studies is the use of drugs, such a cocaine, by pregnant women and determining the long-term consequences for the offspring of these mothers. Research progress along these lines of investigation may identify the neurobiological basis for drug abuse and help develop new treatments for drug addicts. Additionally, intravenous drug use is a risk factor for HIV infection and we are studying the interactions between the HIV virus and cocaine in the brain.

*Dr. Rosemary Booze, is a Professor and Bicentennial Endowed Chair of Behavioral Neuroscience, Dept of Psychology and Dept. of Pharmacol. Physiol., and Neuroscience, University of South Carolina, Columbia, SC*

# **REGULATION OF CORTICAL INTERNEURON DEVELOPMENT BY NCAM IN A MOUSE MODEL WITH ABNORMAL BEHAVIORS RELEVANT TO SCHIZOPHRENIA**

**Patricia F. Maness, Ph.D.**



Patricia F. Maness, Ph.D., received her doctorate in Biochemistry in 1975 from the University of Texas. She was an Anna Fuller Fund Postdoctoral Fellow from 1978-1980 at the Rockefeller University in the laboratory of Dr. Gerald M. Edelman, and Assistant Professor until 1980. Since then Dr. Maness has served on the faculty of the University of North Carolina School of Medicine-Chapel Hill, where she is currently Professor in the Department of Biochemistry and Biophysics. She is a member of the UNC Lineberger Cancer Center, Neuroscience Center, Mental Retardation and Developmental Disabilities Research Center, and Curricula in Neurobiology and Toxicology. Dr. Maness's research is focused on the development of the mammalian nervous system; specifically, mechanisms of axon and dendritic growth and their disruption in neuropsychiatric disorders such as schizophrenia and mental retardation. Dr. Maness has received national recognition for her research, including the Jefferson-Pilot Award in Academic Research, an NIH Career Development award, and a Hilton Distinguished Investigator Award from the National Alliance for Research on Schizophrenia and Depression (NARSAD).

*Dr. Patricia Maness is a Professor at the Department of Biochemistry & Biophysics, University of North Carolina, Chapel Hill, NC*

## **GENETIC DISSECTION OF RAF/MEK/ERK AND GSK-3 SIGNALING PATHWAYS IN THE NERVOUS SYSTEM**

**William Snider, M.D.**



Work in my laboratory is directed at the role of neuronal growth factors in the development and regeneration of axons. We employ sensory neurons of the DRG as a model system. Sensory neurons are unique in elaborating a peripheral axon that regenerates readily after injury and a central axon projecting in the spinal cord that does not. This work is directly relevant to a major NINDS goal of achieving spinal cord repair. DRG neurons are powerfully regulated by members of the NGF and GDNF families of neuronal growth factors. We have recently defined critical functions for NGF and NT3 in the development of DRG peripheral and central projections (Neuron 25: 345–357; Neuron 38: 403-416). We have also recently shown critical but separable functions for Raf/Erk and PI3K signaling during sensory axon growth in vitro (Neuron 35: 65-76). A major difficulty in this field has been that signaling mediator mutants are embryonic or early postnatal lethal due to abnormalities in development of multiple organs. We have now generated conditional mutant mice where Raf/ERK signaling will be abolished only in DRG neurons. The development of axon projections in these mice is likely to be highly informative.

*Dr. William Snider is a Director of UNC Neuroscience Center, UNC School of Medicine, Chapel Hill, NC 27599*

## A COMPREHENSIVE MODEL OF ALZHEIMER'S DISEASE

**Mike Vitek, Ph.D.**



The overall interest of my laboratory is to identify the cellular mechanisms underlying neurodegenerative diseases and then to modify these processes in such a way as to return them to their healthy and functional state. One general approaches to study these mechanisms is by creating transgenic animals that mimic one or more essential features of human disease. The other approach has been to study the form and function of apolipoprotein E where the apoE4 protein isoform is associated with a number of neurodegenerative conditions including Alzheimer's disease, traumatic head injury, multiple sclerosis, etc.

Currently, our data are pointing to a relationship between apoE, testosterone, estrogen and oxidative stress which combine to modulate nitric oxide and tumor necrosis factor alpha production in a defined manner. To further test this idea, we have created transgenic mice expressing the entire human NOS2 gene which will now be tested in various models of neurodegeneration and inflammation.

*Mike Vitek, Ph.D. - Founder, Director, President, and Chief Scientific Officer of Cognosci Inc. Dr. Vitek is an Associate Professor at Duke University Medical Center, Department of Neurobiology and the Department of Medicine, Division of Neurology.*

# Abstracts for Oral Presentations

## **Auditory and Vestibular Function in a Mouse Model for DFNA9**

Sherri M. Jones<sup>1</sup>, Bruce E. Mock<sup>1</sup>, Nahid Robertson<sup>2</sup>, Cynthia Morton<sup>2</sup>

<sup>1</sup>Department of Communication Sciences and Disorders, East Carolina University, Greenville, NC, <sup>2</sup>Departments of Pathology, Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Genetic causes account for 50 to 60% of hearing loss. Genetic hearing impairment may be present at birth or may develop during childhood or adulthood. DFNA9 is a nonsyndromic hearing impairment with a dominant mode of inheritance that leads to progressive sensorineural hearing loss beginning in the second to fifth decade of life. Vestibular impairment (i.e., dizziness or imbalance) may also be prevalent. The gene for DFNA9 is COCH, which encodes the protein cochlin. Cochlin is found in various supporting structures and is the most prevalent protein in the inner ear. The purpose of the present research was to measure inner ear function in a COCH knockin (KI) mouse model. Homozygote (n = 17) and heterozygote (n = 14) *Coch*<sup>G88E/G88E</sup> (KI) mice as well as wild-type controls (n = 15) were studied at five ages: 11, 13, 17, 19 and 21 months. Animals were anesthetized to complete the following noninvasive functional measures: auditory brainstem response (ABR), vestibular evoked potentials (VsEPs) and distortion product otoacoustic emissions (DPOAEs).

At all ages tested, VsEP thresholds were elevated compared to wild-type littermates. At the oldest ages, two *Coch*<sup>G88E/G88E</sup> mice had no measurable VsEP. Age-related changes were also observed for wild-type and affected mice; however, the affected homozygotes clearly showed elevated thresholds at every timepoint tested. ABR thresholds for *Coch*<sup>G88E/G88E</sup> mice were substantially elevated at 21 months but not at the younger ages tested. At 21 months, four of eight *Coch*<sup>G88E/G88E</sup> mice and two of three *Coch*<sup>G88E/+</sup> mice had absent ABRs. DPOAE amplitudes of *Coch*<sup>G88E/G88E</sup> mice were substantially lower than wild-type and absent in the homozygotes with absent ABRs. These results suggest that vestibular function is affected beginning as early as 11 months when cochlear function appears to be normal, and dysfunction increases with age. Hearing loss declines substantially at 21 months of age and progresses to profound hearing loss at some to all frequencies tested. This is the only mouse model developed to date where hearing loss begins at such an advanced age, providing an opportunity to study progressive age-related hearing loss and possible interventional therapies. Supported by NIH R01 DC004477 and R01 DC006443.

## **Regulation of Cortical Interneuron Development by NCAM in a Mouse Model with Abnormal Behaviors Relevant to Schizophrenia**

Patricia F. Maness

Department of Biochemistry & Biophysics, University of North Carolina, Chapel Hill, NC

Neural cell adhesion molecule (NCAM) exerts influences on axon growth, branching, and synaptic development by homophilic and heterophilic interactions involving its extracellular (EC) domain. The EC domain can be cleaved in normal brain by a disintegrin and metalloprotease ADAM10, releasing a soluble fragment that acts as a dominant negative to perturb NCAM function. Ectodomain shedding of NCAM in neurons is normally regulated by tyrosine kinase and ERK MAP kinase signaling pathways. Soluble NCAM has been found at elevated levels in the brain of individuals with schizophrenia, and NCAM polymorphisms are associated with neurocognitive deficits in schizophrenia populations. To study the effects of excess soluble NCAM in brain, we generated a transgenic mouse with elevated expression of the soluble EC fragment in developing and mature neurons. GABAergic interneurons display stunted outgrowth and branching of neuronal processes in the frontal cortex of NCAM-EC transgenic mice, resulting in reduced numbers of GABAergic synapses, including those of basket interneurons that regulate pyramidal cell output. The mice exhibit abnormal behaviors relevant to schizophrenia including increased locomotor activity, enhanced responses to amphetamine, and defects in sensory gating, working and emotional memory. Thus NCAM shedding may serve normally to regulate the development of inhibitory cortical circuitry, whereas its dysregulation may perturb the appropriate excitatory/inhibitory balance.

Maness, PF and Schachner, M. Neural recognition molecules of the immunoglobulin superfamily: signaling transducers of axon guidance and neuronal migration. Nature Neuroscience 10 (2007) 19-26.

Sullivan, PF, Keefe RSE, Lange LA, Lange EM, Stroup TS, Lieberman J, and Maness PF. NCAM1 and Neurocognition in Schizophrenia. Biological Psychiatry, 61 (2007) 902-910.

Pillai-Nair, N, Panicker, AP, Rodriguiz, RM, Miller, K, Demyanenko, GP, Huang, J, Wetsel, WP, and Maness, PF. NCAM-Secreting Transgenic Mice Display Abnormalities in Interneurons and Behaviors Related to Schizophrenia. Journal of Neuroscience 25, 4659-4671, 2005.

Hinkle, C.L., Diestel, S., Lieberman, J., and Maness, P.F. Metalloprotease-induced ectodomain shedding of neural cell adhesion molecule (NCAM). Journal of Neurobiology 66 (2006) 1378-1395.

## **Early Cannabinoid Exposure Persistently Alters FoxP2 Expression within Brain Regions Important for Zebra Finch Vocal Learning and Control**

Ken Soderstrom and Bin Luo

Department of Pharmacology and Toxicology, East Carolina University, Greenville, NC, USA

Early cannabinoid exposure is associated with changes in zebra finch vocal learning. This learning is dependent upon successful progress through distinct developmental stages. Physiological changes associated with this development include changes in expression of the transcription factor, FoxP2. In addition to evidence for a role in zebra finch vocal learning, FoxP2 mutations are associated with specific language disorders in man. Cannabinoid-altered song learning and FoxP2 involvement in normal processes of vocal development led us to investigate a potential mechanistic relationship.

Following song tutoring by an adult male, groups (n = 15) of young male zebra finches were injected with vehicle or the cannabinoid agonist WIN55212-2 (WIN, 1 mg/kg IP) from 50-75 days of age (approximately adolescence) and allowed to age to young adulthood (at least 100 days) in visual isolation. Songs were recorded and groups were further divided into three groups (n = 5) to receive: no further manipulation; a vehicle injection or; 3 mg/kg of WIN. Ninety minutes following these final treatments brains were processed for immunohistochemistry using an anti-FoxP2 antibody. Immuno-positive nuclei per unit area were determined and compared across treatment groups.

In adults, acute WIN treatment resulted in significantly increased FoxP2-expressing cells within zebra finch striatum and distinct regions of thalamus. Similar patterns of increased expression were seen following early, chronic WIN exposure, demonstrating changes that persisted from adolescence through early adulthood. Persistent changes in FoxP2 expression following early WIN exposure suggest that cannabinoid-altered vocal development involves gene expression under FoxP2 control.

## **Structural Studies of the APP Transmembrane Domain**

David P. Cistola

Departments of Clinical Laboratory Science and Biochemistry & Molecular Biology, School of Allied Health Sciences and Brody School of Medicine, East Carolina University, Greenville, NC 27858

The amyloid precursor protein (APP) and the Notch receptor are single-pass transmembrane proteins that are sequentially processed by a series of secretase enzymes. The gamma-secretase cleavage reaction occurs within the transmembrane domain of APP and releases the well-known pathogenic A $\beta$  peptides that accumulate in Alzheimer's disease. Single-site mutations in APP adjacent to this cleavage site cause the familial, early-onset form of the disease. Diet- and/or apoE4-induced changes in membrane lipids may further contribute to the altered processing of APP, especially in the more common, late-onset form of the human disease. Similar mutations in Notch cause aberrant cleavage and abolish cell signaling in cells and whole animals.

The structures of the APP and Notch transmembrane domains are not known, and the mechanisms by which they are cleaved by gamma-secretase remain enigmatic. Based on preliminary findings, we hypothesize that the transmembrane domains of Notch and APP are structurally adaptable and may sample several conformational states. Changes in substrate amino acid sequence and/or membrane lipid environment may shift that conformational equilibrium, altering the degree of water and enzyme accessibility of the substrate, the efficiency and specificity of gamma-secretase cleavage, and the production of pathogenic A-beta peptides.

High-resolution structure determination of transmembrane proteins has been long hampered by difficulties in obtaining diffraction-quality crystals for X-ray analysis or biologically relevant membrane systems suitable for NMR. Here we are testing a novel approach: to characterize the APP and Notch transmembrane domains embedded in nanodisks. These native-like bilayer membranes are sufficiently small, stable and monodisperse for high-resolution solution-state NMR methods. Their lipid composition can be varied to mimic various conditions found in native biological membranes.

In this presentation, our early results will be described. This work is supported by Investigator Initiated Research Grant Award IIRG-07-59562 from the Alzheimer's Association.

## **Perinatal Choline Supplementation Mitigates Trace Eyeblink Conditioning Deficits Associated with 3<sup>rd</sup> Trimester Alcohol Exposure in Rats**

T.D. Tran<sup>1</sup>, C.C. Richardson<sup>1</sup>, J.D. Thomas<sup>2</sup>

<sup>1</sup> Department of Psychology, East Carolina University, Greenville, NC 27858,

<sup>2</sup> Department of Psychology, San Diego State University, San Diego, CA 92120

Children exposed to alcohol prenatally may suffer from a range of fetal alcohol spectrum disorders, which include facial dysmorphology, neuropathology and behavioral alterations. The identification of interventions/treatments that can reduce the severity of these fetal alcohol spectrum disorders is critically important, as pregnant women continue to drink alcohol despite warnings. Using a rodent model, we previously reported that perinatal choline supplementation can reduce the severity of hyperactivity and learning deficits associated with developmental alcohol exposure, suggesting that choline may alter ethanol's effects on the developing hippocampus. In the present study, we examined choline's effects on "trace" eyeblink classical conditioning (TECC), which requires the functional integrity of the cerebellum, but more importantly, the hippocampus. The hippocampus allows for proper timing of conditioned responses (CRs) during the trace interval, a temporal gap between the conditioned stimulus (CS) offset and unconditioned stimulus (US) onset that requires the subject to bridge this difficult association. Thus, we examined whether choline supplementation could reduce the severity of alcohol-related deficits in a hippocampal-dependent learning task. Long-Evans rats were exposed to alcohol from postnatal day (PD) 4-9 via oral intubation (5.25 g/kg/day), a period of brain development equivalent to the 3rd trimester. Sham intubated controls were included. On PD 10-30, subjects were treated with 100 mg/kg choline chloride or saline vehicle. On PD 32, subjects were trained on TECC. Acquisition of CRs by ethanol-exposed subjects was significantly impaired, an effect that was mitigated with choline supplementation. In fact, learning performance of ethanol-exposed subjects treated with choline did not differ significantly from that of controls. These data indicate that perinatal choline supplementation may reduce the severity of behavioral alterations associated with hippocampal functioning, a finding with important implications for the treatment of fetal alcohol spectrum disorders.

This study was supported by East Carolina University to TDT and NIH #AA12446 to JDT.

## **Cross Tolerance Following Chronic Intracerebellar Nicotine and Acute $\Delta^9$ -THC Cerebellar Ataxia: Role of Mouse Cerebellar Nitric Oxide**

M. Saeed Dar<sup>1</sup> and Aaron Smith

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

We have previously demonstrated that acute intracerebellar nicotine and RJR-2403, a selective  $\alpha_4\beta_2$  nAChR subtype agonist, dose-dependently attenuate  $\Delta^9$ -THC-induced ataxia (Smith & Dar, 2006). This investigation was intended to study the effect of chronic intracerebellar nicotine and RJR-2403 on acute  $\Delta^9$ -THC ataxia. Male CD-1 mice, their body weights ranging between 24-28 g, were microinfused nicotine, RJR-2403 and  $\Delta^9$ -THC directly into the cerebellum followed by their evaluation for motor coordination. Rotorod was used to evaluate motor incoordinating response of  $\Delta^9$ -THC. Drug microinfusions were made via stereotaxically implanted stainless steel guide cannulas. Presently, we have shown that chronic intracerebellar nicotine (1.25, 2.5, 5 ng; once daily for five days) and RJR-2403 (250, 500, 750 ng; once daily for five days) significantly attenuate cerebellar  $\Delta^9$ -THC-induced ataxia dose-dependently. These results suggested the development of cross-tolerance between nicotine and RJR-2403 with  $\Delta^9$ -THC. Intracerebellar RJR-2403 (750 ng) microinfused for 1, 2, 3, 5, and 7 days (once daily) significantly attenuated  $\Delta^9$ -THC ataxia in the 3, 5, and 7-day treatment groups; optimal cross-tolerance was evident at day 5 and persisted till 36 h after the last RJR-2403 microinfusion. Intracerebellar microinfusion of hexamethonium (nAChR antagonist; 1  $\mu$ g) or DH $\beta$ E ( $\alpha_4\beta_2$  nAChR antagonist; 500 ng) for 5 days 10 min prior to daily intracerebellar nicotine or RJR-2403 microinfusion virtually abolished cross-tolerance between nicotine or RJR-2403 and  $\Delta^9$ -THC, indicating nAChR participation. Additionally, microinfusion of antagonists 10 min after daily intracerebellar nicotine or RJR-2403 failed to alter the cross-tolerance, suggesting possible involvement of downstream cerebellar second messenger mechanisms. Finally, cerebellar concentration of nitric oxide products (NO<sub>x</sub>; nitrite + nitrate) was increased after 5-day intracerebellar nicotine or RJR-2403 treatment, which was decreased by acute intracerebellar  $\Delta^9$ -THC treatment. The “nicotine or RJR-2403 +  $\Delta^9$ -THC” treatments significantly increased cerebellar NO<sub>x</sub> levels as compared to  $\Delta^9$ -THC alone, supporting a functional correlation between cerebellar nitric oxide production and cerebellar  $\Delta^9$ -THC-induced ataxia and suggest participation of nitric oxide in the observed cross-tolerance between nicotine/ RJR-2403 and  $\Delta^9$ -THC.

# Abstracts for Poster Presentations

## Poster 1.

APOE PROMOTER G-219T POLYMORPHISM IS ASSOCIATED WITH CONCUSSION IN COLLEGE ATHLETES

T.Terrell<sup>1</sup>, R.Bostick<sup>2</sup>, R.Abramson<sup>3,4</sup>, D. Xie<sup>5</sup>, W. Barfield<sup>6</sup>, R.Cantu<sup>7</sup>, M.Stanek<sup>5</sup>

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The APOE gene on chromosome 19 encodes production of apolipoprotein (ApoE). APOE has 3 alleles: E2, E3, and E4. The APOE E4 polymorphism is associated with an increased risk of poor short-term and long-term functional outcome after severe traumatic brain injury (TBI). No prior study has assessed for an association between a history (hx) of concussion and genetic polymorphisms in college athletes. To assess the association of APOE, the APOE promoter G-219T, and tau protein exon 6 polymorphisms (<sup>His47</sup>Tyr and <sup>Ser53</sup>Pro) with a self reported hx of prior concussion. Methods: Cross sectional study of 160 college football and 36 soccer players. Written questionnaires and blood or mouthwash samples for DNA for genotyping by RFLP/PCR. Participants were 19.9 years (1.5), 54% white, 44% black, 3% Hispanic/other, 91% men and 9% women. Seventy-two (34.7%) had an hx of  $\geq 1$  concussion(s) and 124 had no concussion. For  $\chi^2$ , sport-years  $\geq 5$  had a significant association with hx of prior self-reported concussion. APOE E2,E3 and E4, APP, and tau genes are not significantly associated with an hx of  $\geq 1$  concussion. Multivariate logistic regression analysis adjusted for age, sport, school, and years in sport showed no significant association between APOE or tau genotypes/alleles and concussion. The tau <sup>Ser53</sup>Pro polymorphism has a trend toward significance (OR 2.1 [ 0.3 - 14.5] 95% CI). If APOE G-219T GG genotype is referent (OR=1.0), then TT is significantly associated with an hx of self-reported concussion, especially more severe concussions(OR=2.8 [1.1-6.9] 95% CI; p=0.03). GT increases risk slightly (OR=1.1 [0.5-2.5]). Risk of prior concussion increases with presence of the T allele in a dose-dependent fashion. If the athlete had  $\geq 5$  sport-years and TT genotype, this increased risk of concussion (OR=2.5 [1.0-6.5] p,0.05). G-219T TT genotype is statistically significantly associated with a self reported history of  $\geq 1$  concussions in college FB/soccer players. Weaknesses include possible recall bias of the concussion hx by participants and inadequate power. This study supports the need for a prospective study of genetic factors and the incidence of and sequelae from concussions in college athletes.

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**Poster 2.**

**METHODOLOGY TO OBTAIN A SENSORY COMPONENT OF THE SCIATIC NERVE IN A MURINE SPECIMEN**

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The increasing interest in the use of mouse models in electrodiagnostic research requires for techniques and reference values to be established. This study focuses on establishing sensory nerve conduction technique and reference values for the sensory fibers of the sciatic nerve. Mice were deeply anesthetized with intraperitoneal Ketamine Xylazine. Temperature was maintained at 37 +/- 1 Celsius. Based on pilot testing using various techniques, we chose an orthodromic method using a TECA Electromyographer. The recording and reference electrodes were two 1.2 cm stainless steel needle electrodes inserted subcutaneously, fixed 1 mm apart at the level of knee. The cathode was 1.5 cm caudal to the active recording electrode. The ground electrode (unshielded Ag-Ag Cl with contact posts) was placed underneath the abdominal area of the mouse. Stimulation was performed with stainless steel annealed wire loops around the ankle, with the cathode placed 5 mm proximal to the anode. All experiments were conducted in accordance with the Animal Care and Use Committee of our institution. Two males (2 months) and one female (4 months) mice were used. Values were taken from the average of 10 recordings in each hind limb. The mean distal latency and amplitude were 1.53 ms (+/- 0.32) and 7.06 microvolts (+/- 2.2) respectively. We propose a novel and reliable method to measure SNAP in mice. Additional research is necessary to determine whether this method can be used for diagnostic and treatment approaches in sensory nerve pathology.

**Poster 3.**

**EXCITOTOXIC SPINAL CORD INJURY INDUCES APOPTOSIS IN THE BRAIN OF RATS**

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Spinal cord injury is known to have effects that reach beyond the cord to all levels of the nervous system. These changes in the system are suggested to contribute to the development of post-injury sensory syndromes. The current study used Western blot analysis to determine if excitotoxic spinal cord injury induces apoptosis in brain regions associated with the transmission and perception of pain. Male, Long-Evans rats received intraspinal injection of 1.2ul quisqualic acid to produce an excitotoxic lesion within the intermediate dorsal gray matter. Control animals received an equal volume of normal saline. Animals survived for 14 days, or for 1 day after the onset of spontaneous pain behaviors (i.e. overgrooming). Expression levels of caspase-3, a marker of apoptosis, were measured in three areas of the brain: the rostral ventral medulla (RVM), anterior cingulate cortex (ACC), and the periaqueductal gray (PAG). Preliminary data from the 1 day post grooming animals indicated an increase in caspase-3 levels in the ACC and RVM compared to control animals. Western blots for the 14 day non-grooming animals are pending. The preliminary results suggest that excitotoxic spinal cord injury leads to apoptosis in regions of the brain associated with the processing of sensory information, especially pain. Loss of neurons in these regions may result in abnormal processing and the development of chronic sensory syndromes. It is important to determine if this is an effect of the injury, itself, or if apoptosis only occurs in those animals that display pain behaviors after injury.

#### Poster 4.

COMPARISON OF THE DEVELOPMENT OF TOLERANCE IN THE GUINEA PIG ILEUM LONGITUDINAL MUSCLE-MYENTERIC PREPARATION FOLLOWING CHRONIC *IN-VIVO* EXPOSURE TO OPIOID (MORPHINE) VERSUS CANNABINOID ((+)WIN-55, 212-2) AGENTS.

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Tolerance is a phenomenon that develops following chronic treatment with several drugs including cannabinoids and opioids. Heterologous tolerance develops in the guinea pig ileum following *in-vitro* exposure to either opioids or cannabinoids (Basilico *et. al.*, 1999) and after *in-vivo* treatment with opioids. However, few studies have compared the nature of tolerance that develops in the guinea pig ileum following chronic *in-vivo* exposure to morphine, an opioid, with that produced by *in-vivo* exposure to (+)WIN-55,212-2, a cannabinoid. To induce tolerance, morphine was injected in escalating doses twice daily for 7 days and in a separate experiment, (+) WIN-55,212-2 was injected i.p. at a dose of 6mg/100g body weight once daily for 5 days. The guinea pig ileum longitudinal muscle-myenteric plexus preparation (LM/MP) was used to assess the degree and character of tolerance by comparing the ability of 2-Chloroadenosine [2-CADO], D-Ala<sup>2</sup>, N-Me-Phe<sup>4</sup>, Gly<sup>5</sup>-ol [DAMGO] and (+)WIN-55,212-2 to inhibit neurogenic contractions. The sensitivity of the LM/MP to a stimulating agent, nicotine, was also assessed by comparing the concentration required to produce 50% of the maximum contractile response. Ileae obtained from animals exposed to morphine *in-vivo* showed significant tolerance to all inhibitory agonists with the ratio of the IC<sub>50</sub> values being 4.8 for DAMGO, 3.6 for 2-CADO, and 4.9 for (+)WIN 55,212-2. In contrast, *in-vivo* (+)WIN-55,212-2 exposure resulted in the development of tolerance to (+)WIN-55,212-2 only (the magnitude of shift was 10.5) that was also accompanied by a reduction in the maximum response. No significant shift in either the IC<sub>50</sub> value or maximum response was observed for either DAMGO or 2-CADO. Furthermore, *in-vivo* treatment with (+)WIN-55,212-2 had no effect on the sensitivity of the LM/MP to nicotine. Therefore, we conclude that the development of heterologous tolerance in the guinea pig ileum only occurs after *in-vivo* treatment with opioids and may involve non-receptor coupled changes. Neither heterologous tolerance nor increased sensitivity to nicotine developed following *in-vivo* (+)WIN-55,212-2 exposure. The reduced IC<sub>50</sub> and maximum response only to cannabinoid agonists suggests that the development of the tolerance following chronic *in-vivo* (+)WIN-55,212-2 exposure may involve a receptor-dependent modification.

**Poster 5.**

**ARE ADENOSINE OR ADRENERGIC RECEPTORS ALTERED IN RAT BRAIN FOLLOWING CHRONIC ASPARTAME CONSUMPTION?**

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Anecdotal evidence has implicated the artificial sweetener aspartame (NutraSweet™) in a variety of adverse conditions from brain tumors, seizures and headaches to memory loss. Scientific evidence for these accusations however is lacking. Many studies have been conducted looking at aspartame's effects on short-term memory and have found no adverse effects, but very few have addressed possible long-term detrimental effects. Considerable evidence has implicated alpha, beta and adenosine receptors in learning and memory. The specific aim of this study was to determine if long-term aspartame administration in rats would cause a change in brain adrenergic or adenosine receptor densities, as a possible biochemical explanation for previous results that suggested aspartame could cause memory loss. 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 and preparations made and frozen. Receptor numbers were determined using radioligands to label the receptors. Whole brain preparations, from aspartame-treated rats showed a 12% increase in total apparent adenosine A1 receptor numbers when compared to controls. No significant differences were seen between control and treated animals for any of the adrenergic receptors measured. A trend was seen, however, for the alpha1 receptors to be slightly decreased in the treated animals. More animals and various brain areas need to be studied to further define any differences. Studies such as these are needed to identify possible adverse effects of aspartame and correlate these with changes with memory or learning.

## Poster 6.

### EFFECT OF PERIADOLESCENT EXPOSURE TO NICOTINE ON ADULT RAT RESPONSE TO DIAZEPAM IN AN ELEVATED PLUS-MAZE.

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Research indicates a strong association between nicotine exposure during adolescence in mammals and self-administration of nicotine and/or other drugs of abuse in the post adolescent/adult period. In rats particularly, adolescence represents a critical ontogenetic period during which nicotine exposure could very well predict responses to drugs of abuse later in life. In an attempt to further understand this link, this experiment was designed and conducted to explore the effects of early exposure to nicotine in rats, on the response to another potential drug of abuse diazepam, later in life. One group of rats was exposed to 0.4 mg/kg i.p. nicotine or vehicle daily from PD (postnatal day) 35-44 and a second group from PD 60-69. All rats were tested for five minutes in an elevated plus-maze at PD 80 thirty min after a 1.0 mg/kg s.c. diazepam or vehicle injection. This dose of diazepam is sub- or at threshold in this test. The prediction that early nicotine exposure would sensitize the PD 35-44 rats to diazepam was confirmed by an increased "open-arm activity." In the rat groups that were treated at PD 35-44, there was a significant difference ( $p < 0.05$ ) between the nicotine/diazepam rats and the other two groups of rats that were dosed with nicotine/vehicle or vehicle/vehicle ( $1.1 \pm 0.5$  vs.  $5.1 \pm 0.7$  open arm entries and  $10.5 \pm 6.6$  vs.  $53.9 \pm 13.5$  sec in the open arm for veh/veh vs. nic/diaz treatments, respectively). However, statistical analyses of the data from the rat group that was exposed to nicotine at PD 60-69 revealed that between-group comparisons of the nicotine/diazepam, nicotine/vehicle and vehicle/vehicle sub-groups did not yield significant differences at the  $p < 0.05$  level in their open-arm activity. These data suggest that exposure to nicotine during the onset of adolescence affects the response of the young adult to a different drug of abuse. It also suggests that nicotine exposure at PD 35-44 is a better predictor of drug responses in adulthood than nicotine exposure at PD 60-69. These data further emphasize that the periadolescent period is a vulnerable developmental period.

## Poster 7.

### NICOTINE-ETHANOL INTERACTION: REGULATION OF TWO DISTINCT NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) SUBTYPES IN MOUSE CEREBELLUM

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We previously demonstrated cross tolerance between nicotine and ethanol and attenuation of ethanol ataxia by intracerebellar (ICB) nicotine. Using rotarod test and direct ICB microinfusion technique in stereotaxically cannulated CD-1 mice, this study investigated the role of predominant nAChR subtypes(s) such as  $\alpha_4\beta_2$  and  $\alpha_7$  in nicotine-ethanol interaction. RJR- 2403 [32.25, 62.5, 125ng] a  $\alpha_4\beta_2$ -selective agonist, markedly attenuated ethanol (2g/kg; i.p) ataxia dose dependently that was blocked by ICB dihydro- $\beta$ -erythroidine (DH $\beta$ E; 500, 250, 125 ng), a potent  $\alpha_4\beta_2$  –selective antagonist, suggesting the involvement of  $\alpha_4\beta_2$  subtype. DH $\beta$ E (500ng; ICB) also significantly attenuated nicotine-induced attenuation of ethanol ataxia reinforcing the role of  $\alpha_4\beta_2$  subtype in nicotine-ethanol functional interaction.

Also, ICB microinfusion of PNU 282987 (250ng-2.5ug), a novel  $\alpha_7$  subtype selective agonist reduced ethanol ataxia thus indicating the probable role of  $\alpha_7$  nAChR subtype in this context. Moreover, pretreatment with methyllylcaconitine (MLA; 3-12ng; ICB), a  $\alpha_7$ -subtype selective antagonist, effectively prevented PNU's and nicotine's attenuation of ethanol ataxia confirming the contribution of  $\alpha_7$  subtype in ethanol's motor incoordination. Both DH $\beta$ E and MLA per se had no effect on ethanol's motor incoordination precluding any tonic role of nAChR subtypes in ethanol ataxia. Taken together, our results support a comparable role of nAChR  $\alpha_4\beta_2$  and  $\alpha_7$  subtype in nicotine-induced attenuation of cerebellar ethanol ataxia. Future treatment strategies for ethanol induced ataxia might require manipulation of nAChR subtypes i.e. to lessen alcohol related motor abnormalities and its associated accidental mortality.

**Poster 8.**

**EXOCRINE PANCREATIC SECRETION AND EFFECTS ON SNARE COMPONENTS BY SCORPION VENOM.**

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Pancreatic exocrine secretory discharge *in vitro* studies in our laboratory reveal significant alterations to release of newly synthesized zymogen proteins and to subcellular ultrastructure when scorpion venom or its bioactive components are used as secretagogues. Our previously published accounts of similarly treated tissues demonstrate linear dose response curves but reveal diminished discharge at elevated levels of venom and its bioactive components. These changes appear to be reflected as well when aliquots of homogenates of treated tissues are separated by polyacrylamide gel electrophoresis using Laemmli buffer systems. The more striking changes included both the diminished staining and disappearance of lower molecular weight components. Effects are apparent as early as 5 minutes, even at 4° C using excised pancreatic lobules. Western blots revealed positive phosphotyrosine content for these bands and presumptive identity as the SNARE component VAMP2. Electron microscopy immunocytochemistry has confirmed both SNARE identity as VAMP2 and the marked decrease in colloidal gold particles on conjugated secondary antibodies that are predominantly associated with trans-Golgi zymogen granules. Subsequently, we repeated these results with both isolated zymogen granules and zymogen granule membranes. In order to identify the site of cleavage we employed chromatographically purified scorpion venom proteins and expressed cloned truncated soluble SNARE proteins that are reassembled into detergent-resistant complex. Venom protease cleavage of the SNARE protein VAMP2 demonstrates the shortest known cytoplasmic products. Continuing studies are expected to describe effects on all SNARE components as well as reassembled SNARE complexes.

**Poster 9.**

**INVESTIGATING RHO GTPase FUNCTION IN CISPLATIN INDUCED NEUROTOXICITY**

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Cisplatin is one of the oldest and most commonly used chemotherapeutics available. Although the drug has proven to be consistently effective against a wide range of cancers, it has a known side-effect of peripheral neuropathy and has also been implicated in chemotherapy related cognitive impairment. Our laboratory has utilized primary neuronal cell cultures to investigate the effects of cisplatin treatment on nerve cells. We have found that cisplatin causes a dramatic decrease in the number and length of dendritic processes in primary rat hippocampal and cortical neurons. A similar effect was also found when dorsal horn/dorsal root ganglion co-cultures were used. The effect of the therapeutic dose did not result in neuron cell death but was still effective in killing CWR22rv1 prostate cancer cells, which provides evidence of a separate mechanism of action for neurotoxicity. The overall simplification of dendritic arbors under cisplatin treatment correlated with abnormal actin cytoskeleton arrangement at the growth cones, indicating the potential involvement of Rho GTPases. To investigate this hypothesis, a specific inhibitor of Rho GTPase downstream effector Rho kinase, Y-27632, was used in conjunction with cisplatin treatment. The results demonstrate a recovery of normal dendritic morphology with Y-27632 treatment, indicating that inhibiting Rho GTPase signaling is beneficial for maintaining normal neuronal morphology during cisplatin treatment. To determine if the results of the primary neuronal based model could be extended to *in vivo* studies, C57BL6 mice were treated with cisplatin and neuropathy was determined using Semmes-Weinstein monofilament testing and Sural nerve sensory nerve conductance. Cisplatin treatment caused a significant deficit in hind-paw plantar touch sensation and a decrease in sensory nerve conductance. Preliminary results indicate Y-27632 effectively reversed the neuropathy, further validating the potential for Rho pathway inhibition in neuroprotection from drug induced neuropathy.

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**Poster 10.**

**ALTERATION OF CIRCADIAN RHYTHM IN HIV-INFECTED PATIENTS AND TRANSGENIC MOUSE EXPRESSING THE HIV PROTEIN TAT IN THE BRAIN.**

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We monitored the circadian rhythms of HIV-infected patients and healthy subjects as controls with actigraphy over a period of 5 years. Alteration of circadian rhythm occurs in patients in different disease stages and with a wide range of CD4 counts. Although HIV-1 neuropathogenesis remains poorly understood, viral proteins (gp41, gp120, and Tat) are thought to play a major role. Among these viral proteins, Tat is important because it can be secreted from infected cells to the extracellular space, subsequently affecting nearby neurons. Since our previous study demonstrated that Tat could reset the circadian clock in the brain, the suprachiasmatic nucleus (SCN), we hypothesize that the disruption of circadian rhythm and sleep during HIV infection could be a consequence of Tat on the SCN. To test our hypothesis, we used a transgenic mouse model that can selectively induce the expression of Tat in the brain when doxycycline is administered to the animals. RT-PCR confirmed Tat expression in the mouse SCN two weeks after feeding the animals with the doxycycline diet. Interestingly, the alteration of circadian rhythm in the Tat-expressing mouse is comparable to that found in the HIV-infected patients, characterized with reduced motor activity and impaired synchronization to the environmental light dark cycle. This work is supported by NIH grants (NS47014-01) to JM Ding.

**Poster 11.****δ-CATENIN: A NOVEL MEMBER OF THE GSK3 SIGNALING COMPLEX THAT PROMOTES β-CATENIN TURNOVER IN NEURONS**

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Through a multiprotein complex, glycogen synthase kinase3-β (GSK3-β) phosphorylates and thereby destabilizes β-catenin which is an important element for neuronal growth and proper synaptic function. As a dendritic specific member of the β-catenin superfamily, δ-catenin is also involved in regulating neurite outgrowth and modulating synaptic activity. Aberrant expression of δ-catenin is known to result in abnormal cognitive function and altered synaptic plasticity. However, mechanisms underlying how δ-catenin expression and stability are regulated are poorly understood. We investigated the possibility that δ-catenin expression may also be regulated by GSK3-β signaling and thereby participate in the molecular complex regulating β-catenin turnover. Here we show that GSK3-β co-immunoprecipitates with and phosphorylates δ-catenin in cultured primary rat cortical neurons and mouse brain homogenates. Immunofluorescent microscopy studies revealed a co-enrichment of GSK3-β, δ- and β-catenin in both the soma and dendrites of cultured neurons. Treatment of cultured neurons with LiCl, a GSK3-β inhibitor, resulted in increased δ- and β-catenin immunoreactivity and stability. We show that δ- and β-catenin immunoreactivity increased and protein turnover decreased when cultured primary neurons were treated with proteasome inhibitor Alln, suggesting that δ- and β-catenin stability are regulated by ubiquitin-dependent proteolysis. Co-immunoprecipitation experiments using PC12 cells stably overexpressing δ-catenin (PC12-δ-cat) revealed enhanced GSK3-β and β-catenin interactions compared to PC12 controls. In addition, PC12-δ-cat cells treated with Alln showed additional ubiquitinated β-catenin forms compared to controls. Consistent with the idea that δ-catenin facilitates the interaction of destruction complex molecules, cycloheximide experiments using PC12-δ-cat cells showed enhanced β-catenin turnover. These results identify δ-catenin as a new member of the GSK3-β signaling pathway involved in facilitating the interaction, ubiquitination and subsequent turnover of β-catenin in neuronal cells.

This study was supported by National Institute on Aging.

**Poster 12.****EXCITOTOXIC SPINAL CORD INJURY INDUCES MORPHINE TOLERANCE IN RATS**

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The development of neuropathic pain syndromes, which are intractable or become tolerant to treatment with opiates are common following spinal cord injury (SCI). Recent studies in peripheral pain models have demonstrated changes in the expression of the mu opioid receptor and  $\beta$ -endorphin in the spinal cord which are similar to changes seen during the development of morphine tolerance. We hypothesized that animals with excitotoxic spinal cord injury (eSCI) would exhibit some level of tolerance to exogenous morphine even though they are morphine naïve. Following an excitotoxic SCI (eSCI), thermal thresholds of male Long-Evans rats were tested using a 52°C hot plate. Baseline thresholds were determined by three consecutive days of hot plate testing prior to injury. Thresholds were reassessed at 7 days post-injury and again at either at 13 days post-injury or on the day of onset of “overgrooming” behavior (spontaneous pain behavior). Morphine responsiveness was then tested the following day (14D post-injury or 1D post-“overgrooming” onset (1D P-Gr)) by subcutaneous administration of 10mg/kg of morphine followed by behavioral testing 20 minutes later. Afterwards, rats were sacrificed and the anterior cingulate cortex, periaqueductal grey, locus coeruleus, and rostral ventral medulla were collected for molecular analysis. Following eSCI, the effect of morphine on thermal thresholds was significantly blunted in most rats in both the 14D and 1D P-Gr groups compared to controls (14.81±3.875 s and 12.41±4.422 s vs. 28.13±1.224 s,  $p < 0.001$ ). While the majority of animals showed evidence of morphine tolerance after eSCI, some animals maintained normal morphine responsiveness. Current research in the lab is focused on determining the molecular differences of these two populations, in particular examining differences in the opioid receptors and their ligands as well as the opioid antagonist cholecystokinin.

**Poster 13.**

**NOVEL KV3 GLYCOFORMS DIFFERENTIALLY EXPRESSED IN ADULT MAMMALIAN BRAIN CONTAIN SIALYLATED N-GLYCANS**

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The N-glycan pool of mammalian brain contains remarkably high levels of sialylated N-glycans. This study provides the first evidence that voltage-gated K<sup>+</sup> channels, Kv3.1, 3.3, and 3.4, possess distinct sialylated N-glycan structures throughout the central nervous system of the adult rat. Electrophoretic migration patterns of Kv3.1, 3.3, and 3.4 glycoproteins from: spinal cord, hypothalamus, thalamus, cerebral cortex, hippocampus, and cerebellum membranes digested with glycosidases were utilized to identify the various glycoforms. Differences in the migration of Kv3 proteins were due to the desialylated N-glycans. Protein expression patterns of Kv3.1 and 3.3 revealed similarities with the highest protein levels observed in cerebellum, and the lowest levels in thalamus and hypothalamus. However, Kv3.3 protein levels were higher in spinal cord than both cerebral cortex and hippocampus while those for Kv3.1 were quite similar to each other. The difference between the Kv3.4 protein and both Kv3.1 and 3.3 proteins was that both cerebellum and hippocampus expressed the highest levels while the other four regions expressed lower levels. We suggest that novel Kv3 glycoforms differentially expressed throughout the central nervous system may be an important component of these channels in: cell recognition events, targeting to specific subdomains of neurons, and in modulating channel activity.

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## **Poster 14.**

### **TEST-RETEST DIFFERENCES OF LOW-LEVEL EVOKED DPOAES**

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It is generally recognized that evoked otoacoustic emissions (OAEs) are more sensitive in revealing early or sub-clinical cochlear damage versus standard behavioral testing. OAEs reflect nonlinear distortion and linear reflection mechanisms in the cochlea generated by outer hair cell activity in response to acoustic stimulation. Distortion product otoacoustic emissions (DPOAEs) are intermodulation products produced by the cochlea when presented with two closely spaced simultaneous pure tones. DPOAE amplitude increases with increasing input levels until saturation and varies as function of stimulus frequency. Following cochlear insult, DPOAEs are reduced in amplitude or are absent. OAEs evoked to “low-level” primary tones may be more sensitive to cochlear insult; however, monitoring for cochlear dysfunction with DPOAEs is dependent upon test-retest reliability. The purpose of this study was to examine test-retest reliability of low-level evoked DPOAEs. Participants were 16 normal-hearing young females. Twelve primary tones with  $f_2$  frequencies from 1514-7568 Hz with four  $L_1/L_2$  levels between 57/45 and 51/30 dB SPL were utilized. Initial test; retest without probe removal; retest with probe removal; and retest by a second tester were obtained.

A three-factor repeated ANOVA was conducted to analyze differences in DPOAE S/N as a function of  $f_2$  frequency,  $L_1:L_2$  level, and test condition. Only significant main effects of frequency and level were found ( $p < .05$ ). In other words, DPOAE S/Ns varied as a function of  $f_2$  frequency and  $L_1/L_2$  stimulus levels as expected. DPOAE S/Ns, however, were not significantly affected by repeated measures with and without probe replacement nor with different testers. A three-factor repeated ANOVA was conducted to examine differences in test-retest differences of DPOAE S/N as a function of  $f_2$  frequency,  $L_1:L_2$  level, and tester. Intra- and inter test-retest differences were not significantly different. Critical differences, for determining whether two sets of DPOAE S/Ns are different as a function of  $f_2$  frequency,  $L_1:L_2$  level, and tester, were computed from the standard deviations of the test-retest differences. Values for the 95% confidence level representing critical differences ranged from approximately 4 to 20 dB. Critical differences for test-retest differences at the 95% confidence level were large questioning the clinical utility of low-level evoked DPOAEs.

**Poster 15.**

**OXYTOCIN MODULATION OF ZEBRA FINCH VOCAL DEVELOPMENT**

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Oxytocin is a nine amino acid neuropeptide involved in a variety of physiological processes including modulation of social behaviors. Accumulating evidence suggests a role for oxytocin in the etiology of autism, a disorder characterized by both altered social and vocal behaviors. The extent to which oxytocin signaling may be involved in vocal development has not been established, probably due to a lack of appropriate animal models. Since song birds like the zebra finch learn a form of vocal communication, they represent a promising model for evaluating effects of oxytocin-related peptides on vocal development.

To begin to establish the suitability of zebra finches as an animal model to study effects of oxytocin-related peptides on vocal development, we have been working to clone and functionally characterize the receptor likely involved. cDNA encoding 492 bases of the 3' end of the zebra finch mesotocin receptor (the avian ortholog of mammalian oxytocin receptors) have been isolated through degenerate primer PCR-based methods. The zebra finch mesotocin receptor cDNA sequence isolated to date is 85% homologous to that of the human oxytocin receptor, and the deduced protein sequence is 84% identical to that of the human. A zebra finch brain cDNA library has been constructed in order to determine the remaining coding sequence. Our cloning approach and rationale will be presented in detail.

Supported by the Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University.

**Poster 16.**

**THE ROLE OF THE ALPHA3 SUBUNIT ISOFORM OF THE SODIUM PUMP IN HETEROLOGOUS TOLERANCE IN THE GUINEA PIG ILEUM: CORRELATION OF PROTEIN ABUNDANCE WITH FUNCTIONAL TOLERANCE.**

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Previous studies from this laboratory have shown that chronic exposure to morphine via pellet implantation leads to the development of heterologous tolerance in the guinea-pig ileum which develops slowly (fully expressed by 4 days) and declines even slower (return to normal sensitivity over a period of more than 14 days). Other work from this laboratory has implicated a change in the resting membrane potential myenteric S neurons coincident with a reduction in the alpha3 subunit of the sodium pump. If the change in this specific protein was the basis for the functional change in responsiveness then that protein should change over a similar time course. The goal of the present study was to compare the abundance of three proteins in tissue homogenates of the guinea pig ileum at various times following a single pellet implantation procedure. In addition to the alpha3 subunit of the sodium pump, the abundance of the alpha1 subunit and beta actin was compared in the same tissue homogenates. Using a dot blot apparatus, tissue homogenates obtained from animals exposed to morphine for 1, 4, 7, 10, 14 or 21 days could be quantitatively examined simultaneously. The results of these experiments indicate that there is a time dependent reduction in the alpha3 subunit isoform of the sodium pump that is evident by 4 days after exposure and returns to normal levels by 14 days after exposure. In contrast, no differences in the abundance of either the alpha1 subunit isoform of the sodium pump or beta actin were observed at any time period. Therefore, the results of the present study identify a strong correlation between reductions in the abundance of the alpha3 subunit isoform and the appearance and decline of heterologous tolerance and suggest that this protein may play a critical role in the development of tolerance.

**Poster 17.**

**EVIDENCE OF DYSPHAGIA IN A TRANSGENIC MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS**

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Amyotrophic lateral sclerosis (ALS) is a fatal, incurable disease predominantly characterized by selective degeneration of upper and lower motor neurons, resulting in progressive paralysis of limb muscles as well as muscles involved in respiration, speaking, and swallowing. Sensory pathology of the central and peripheral pathways involved in limb sensation also has been well-documented for this disease. Insight into the pathogenesis of ALS has been acquired primarily through investigations using SOD1-G93A transgenic mice. However, it is currently unknown whether this animal model develops swallowing impairment (dysphagia) similar to humans with ALS. To date, dysphagia in ALS has been attributed solely to motor neuron degeneration. Furthermore, the possibility of sensory pathology contributing to dysphagia has not yet been explored. We hypothesized that SOD1-G93A mice will demonstrate behavioral and electrophysiological evidence of dysphagia that corresponds with neurodegeneration of the key motor and sensory regions involved in swallowing. To investigate this possibility, we evaluated the lick and mastication rates of SOD1-G93A transgenic mice (n=13; 8 females, 5 males) at three discrete time points of the disease: 60 days (pre-symptomatic stage), clinical onset (onset of hind limb muscle weakness; ~100 days), and end-stage (onset of hind limb paralysis; ~140 days). Age- and gender-matched nontransgenic littermates (n=14; 7 females, 7 males) were used as controls. At the final time point, electrophysiological and histological methods were performed on all mice (n=27).

Lick and mastication rates of transgenic SOD1-G93A mice were significantly ( $p < .05$ ) lower than those of WT mice at all three time points, providing evidence of oral stage dysphagia that begins quite early in this animal model. The pharyngeal stage of swallowing was impaired only for transgenic females, who required a significantly higher ( $p < .05$ ) stimulus frequency applied to the superior laryngeal nerve to elicit pharyngeal swallows. Male and female transgenic mice developed marked neurodegeneration of the brain stem motor nuclei involved in pharyngeal swallowing, which suggests that pharyngeal dysphagia should exist for both genders. Investigation of the sensory components of swallowing may provide insight into this unexpected gender difference.

**Poster 18.**

**SPONTANEOUS DISCHARGE PATTERNS OF VESTIBULAR NEURONS IN THE OTOCONIA-DEFICIENT MOUSE**

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Vestibular primary afferents in the normal mammal are spontaneously active and discharge properties have been well characterized. Such discharge patterns are believed to be independent of stimulation and thought to depend on excitation by vestibular hair cells due to a stimulus-independent background release of synaptic neurotransmitter. In the case of otoconial sensory receptors, it is difficult to study spontaneous activity in the absence of natural tonic stimulation by gravity. We investigated discharge patterns of single primary afferent neurons of the superior vestibular nerve in the absence of gravity stimulation using two mutant strains of mice that lack otoconia (head tilt. *het-<sup>-/-</sup>* Nox3, and tilted, *tl<sup>t</sup>Otop1*). Hair cells, primary afferents and synaptic apparatus were present in the maculae of these mutants. Spontaneous discharge activity was characterized in 201 neurons from anesthetized adult animals [neurons: *het(-/-)*n = 69; *tl<sup>t</sup>(-/-)*n = 47 and 85 neurons from control mice with normal otoconia *het(+/-)*]. The mean interval coefficient of variation (CVi = sd/mean) was similar for all groups and CVi values formed a bimodal distribution characteristic of the mammal. Mean discharge rates were significantly higher in otoconia-deficient strains for neurons with CVi's < 0.4 [ $p < 0.02$ ; rate in sp/s: *het(-/-)*= 82.2 +/- 21.6(53); *tl<sup>t</sup>(-/-)*= 84.5 +/- 22.9(36); *het(+/-)*= 73.4 +/- 22.9(51)]. These findings indicate that afferents in the superior vestibular nerve are spontaneously active and exhibit discharge rates higher than siblings with intact otoconia yet the distribution of CVi's appears to be normal. The vast majority of cells studied were unresponsive to rotational stimuli suggesting that a high percentage of cells innervated gravity receptors. The elevated rates are interesting in that they may reflect the presence of a functionally 'up-regulated' tonic excitatory process in the absence of natural sensory stimulation. Supported by NIH-NIDCD 005776

**Poster 19.**

**CRITICAL WINDOWS OF VULNERABILITY TO ALCOHOL EXPOSURE DURING DEVELOPMENT ON EYEBLINK CONDITIONING RESPONDING IN RATS**

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Fetal alcohol spectrum disorders (FASDs) are severe, lifelong conditions in children whose mothers consumed alcohol during pregnancy. Symptoms include brain and behavioral deficits, particularly cognitive dysfunction. One brain structure that is a target of early alcohol exposure in humans is the cerebellum, which mediates not only simple motor behaviors, but also some forms of learning and memory. For example, it is responsible for the acquisition of eyeblink classical conditioning (ECC), a form of associative learning. In “delay” ECC, a conditioned stimulus (CS) precedes, overlaps, and coterminates with an unconditioned stimulus (US) that naturally elicits an eyeblink response. After repeated exposure to these stimuli, the organism “learns” to produce an anticipatory eyeblink response that precedes the US, called a conditioned response (CR). Previously, we found that early postnatal alcohol exposure in rats (equivalent to the third trimester in humans) dramatically impairs ECC during either periadolescence or adulthood (e.g., Tran et al., 2007). No study has examined whether alcohol exposure during different periods of cerebellar development produces differential ECC impairments. This question is important as humans who do abuse alcohol during pregnancy, tend to binge drink more so during early stages of pregnancy and not as much during the third trimester. Findings from the use of an animal model of prenatal FASD may help elucidate the neurobehavioral findings in humans who suffer from heavy prenatal alcohol exposure. Pregnant rats were either treated with ethanol (5.25 g/kg/day) from gestational days 1-20, with maltose-dextrin, or left untreated. An additional group of non-treated pregnant rats produced offspring that were treated with 5.25 g/kg/day ethanol during postnatal days (PD) 4-9 – the third trimester-equivalent. On PD 30, offspring were surgically prepared for testing, and subsequently conditioned using long-delay ECC. Initial results showed that prenatal or postnatal alcohol exposure induces a pattern of ECC learning deficits, as indicated by lower CR percentages and CR amplitudes. The results suggest that ECC is a sensitive indicator of developmental alcohol-induced injury to the cerebellum, and that alcohol insult during either developmental period is deleterious to its function. More data will need to be collected to substantiate these findings.

This study was supported by ABMRF grant to TDT.

**Poster 20.**

**MODERATE MATERNAL IRON INADEQUACY WORSENS  
NEUROBEHAVIORAL OUTCOMES IN A RAT MODEL OF DEVELOPMENTAL  
ETHANOL EXPOSURE**

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Prenatal alcohol exposure causes behavioral and cognitive disorders collectively known as fetal alcohol spectrum disorders (FASDs). Prevention of alcohol use is difficult, thus therapies that could ameliorate alcohol's damage are desirable. FASD severity increases with parity, implying the depletion of a protective maternal factor, perhaps a nutrient. Iron deficiency (ID) is the most common nutritional deficiency in women and causes neurodevelopmental deficits which parallel those of FASD. We hypothesized that moderate maternal ID may exacerbate alcohol's effects upon the developing brain. We tested this hypothesis in ID or iron sufficient (IS) rat pups exposed to alcohol during the brain growth spurt period (postnatal days 4-9; PD 4-9). ID dams were mildly ID on PD 5 and were anemic by weaning, with increased red-cell distribution width and decreased liver iron and mean corpuscular hemoglobin, an iron profile that mimics that of many pregnant women. ID pups at PD 10 had more severe anemia, with significantly decreased hemoglobin, hematocrit, serum iron, transferrin saturation and liver iron. ID worsened the impact of alcohol upon cerebellar function at PD 32-34, as measured by frequency and strength of conditioned responding in eyeblink classical conditioning (ECC) – a well-studied form of associative learning in the neurosciences. Preliminary morphological data is discussed that may indicate a disruption in the cerebellum which is most severe in ID, alcohol-fed pups. Further studies are necessary to confirm these data, but the structure-function relationship demonstrated thus far may indicate that ID worsens the neuroteratogenicity of developmental alcohol exposure in the rat.

This study was supported by East Carolina University, NIH award T32 ES07015 to SS, and Palmer Fund of the Waisman Center at UWM to SS/TT.

**Poster 21.****ROLE OF CDK5 IN LIGHT ENTRAINMENT OF CIRCADIAN RHYTHM**

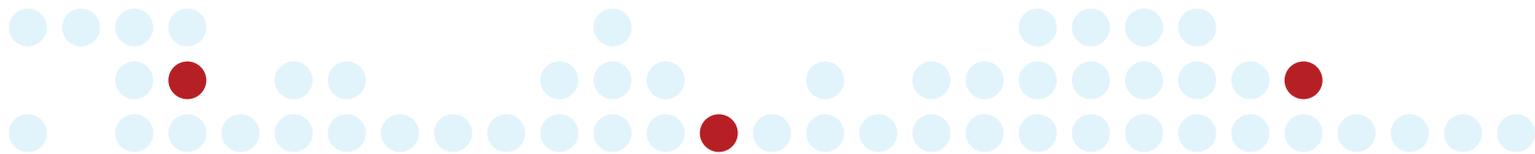
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Mammalian circadian rhythms are regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is synchronized to the environmental light:dark cycle through light-induced phase resetting. The SCN receives direct retinal input through a subpopulation of light sensitive but non-image forming retinal ganglion cells (RGC) that contain melanopsin. These RGCs project to the SCN via the retinal hypothalamic tract (RHT) and release glutamate (Glu) in response to light. Light exposure during the early or late night results in phase delays or advances respectively of circadian rhythms, but induces no change in phase during the day. The cellular mechanism underlying this bidirectional phase shift remains unclear. In the present study, we explored cyclin-dependent kinase 5 (CDK5), a cell signaling molecule that regulates diverse neural functions, such as modulating Glu neurotransmission, through the phosphorylation of a plethora of pre- and postsynaptic proteins. The kinase activity of CDK5 is regulated by two non-cyclin CDK5 activators, p35 and p39. In addition, accumulation of p25, the cleavage product of p35 can result in hyperactivation of Cdk5. Since Glu is the neurotransmitter of light entrainment, we investigated the role of CDK5 and its activators in the SCN. A 20 min light pulse (~100 lux) at ZT16 decreased both the protein levels of p25, p35, and the kinase activity of CDK5 in the mouse SCN. In contrast, a light pulse at ZT 22 increased CDK5 kinase activity and that of its two activators. A light pulse at ZT6 did not change CDK5 levels. When p25 was overexpressed in tetracycline-dependent transgenic mice, the magnitude of light-induced phase delay at CT 16 was increased, whereas the light-induced phase advance at CT 22 was decreased. Interestingly, the magnitude of phase resetting returned to basal level when the p25 transgene was turned off by switching to the doxycycline diet. Anatomical studies showed that the p25 transgene is present in vasopressin, pERK and unidentified cells and fibers of the SCN shell, and are in contact with GRP fibers. These results support a role of CDK5 in the regulation of light entrainment in the SCN. Supported by NIH grants (NS47014-01) to JM Ding and (NS37919) to R Silver.

**NOTES:**



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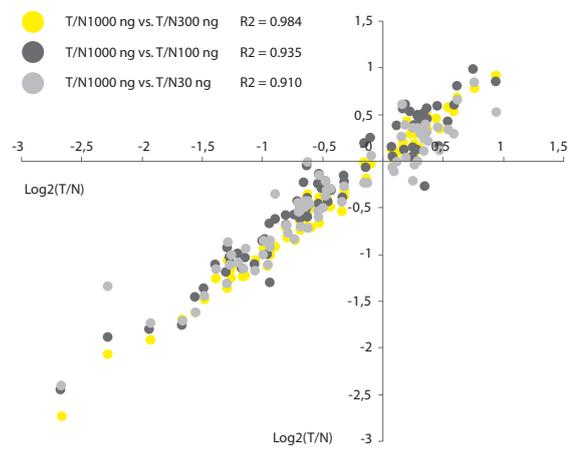
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Figure 1



An excellent correlation of 91% between 30 ng and 1000 ng total RNA samples. Log2 ratios of Tumour vs. Normal (T/N) adjacent tissue of a oesophagus cancer using 1000 ng input material is plotted against Log2 ratios in identical experiments using 300 ng, 100 ng, and 30 ng total RNA. The correlation is 91%, 93% and 98% with 30 ng, 100 ng and 300 ng, respectively.

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