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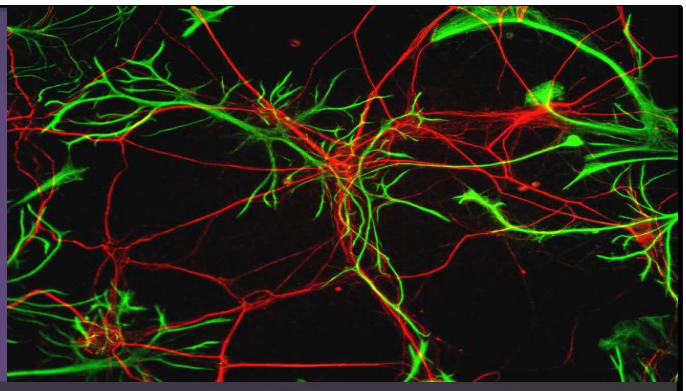

17th Annual Neuroscience Symposium
Catalyst for Collaboration

Featuring:

F. Scott Hall, Ph.D.

Department of Pharmacology
and Experimental Therapeutics
University of Toledo

"Dopamine Transporter Knockout Mice: What Does This Model Tell Us About the Causes and Treatment of Attention Deficit Hyperactivity Disorder?"

Tuesday, November 3rd, 2015
East Carolina Heart Institute
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The Society for Neuroscience

Neuroscience Symposium Schedule

November 3rd, 2015

Schedule:

9:00 – 10:00 Registration **(atrium)**
Breakfast with students and Dr. F. Scott Hall **(conference room)**

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

11:30 – 11:45 Opening remarks (Dr. Clemens, Dr. Tran) and Dr. Mary Farwell, Biology, ECU **(conference room)**

11:45 – 12:45 F. Scott Hall, PhD

University of Toledo, Dept of Pharmacology & Experimental Therapeutics

"Dopamine Transporter Knockout Mice: What Does This Model Tell Us About the Causes and Treatment of Attention Deficit Hyperactivity Disorder?"

12:45 – 1:00 Coffee break / Vendor exposition **(atrium)**

1:00 – 3:00 Faculty / student presentations **(conference room)**

1:00 – 1:20 Katie Clements, Issa Lab, Department of Biology, ECU
"Social Status Modulates Dopaminergic Pathway in Escape Circuit in Zebrafish (Danio rerio)"

1:20 – 1:40 Annalise vonderEmbse, DeWitt Lab, Department of Pharmacology, ECU
"Developmental Toxicant Exposure and Early Neuroimmune Susceptibility to Alzheimer's Disease"

1:40 – 2:10 Douglas Powell, PhD, College of Pharmacy and Health Sciences, Campbell University
"Neural vs. Non-Neural Contributions to Rigidity in Parkinson's Disease"

2:10 – 2:30 John Norbury, MD, Physical Medicine & Rehabilitation, ECU
"Taking the Pain out of Clinical Pain Management"

2:30 – 2:50 Daniel Goldberg, JD, PhD, Bioethics & Interdisciplinary Studies, ECU
"Our Brains Are Not Us" — On the Dangers of Neuro-Reductionism in Treating People in Pain"

3:00 – 4:30 Poster session 2 / Vendor exposition / Coffee and snacks **(atrium)**

4:30 – 5:00 Closing remarks and Awards **(auditorium)**

Podium Presentations

(in order of presenter)

Dopamine Transporter Knockout Mice: What Does This Model Tell Us About the Causes and Treatment of Attention Deficit Hyperactivity Disorder?

F. Scott Hall, PhD

Department of Pharmacology and Experimental Therapeutics, College of Pharmacy and Pharmaceutical Sciences, University of Toledo, Toledo, OH, USA

Attention deficit hyperactivity disorder (ADHD) is a psychiatric condition that is characterized by impaired attention, hyperactivity, impulsivity and impaired executive function. The aetiology of the disorder is uncertain, but appears to include both genetic and environmental components that lead to a disruption of corticostriatal function, with hypofunction in prefrontal brain areas and concomitant hyperfunction of ventrostriatal brain areas. Genetic deletion of the dopamine transporter in mice produces behavioral impairments that may model aspects of this disorder, including hyperactivity, deficits in attentional processing and impairments in executive function. Moreover, all of these deficits are ameliorated by drugs that are used to treat ADHD, while the same drugs produce impairments in normal mice – giving the model substantial predictive validity. Moreover, changes in subcortical dopamine function and corticostriatal connections indicate that similar structural and functional brain connectivity changes may occur in the DAT KO model as are seen in individuals with ADHD. Surprisingly, the basic mechanism of action of psychostimulants in the treatment of ADHD has been uncertain. Work with this model suggests that psychostimulants (as well as non-stimulant norepinephrine transporter blockers) act in the prefrontal cortex of DAT KO mice to reverse the corticostriatal imbalances in dopaminergic function resulting from the neurodevelopmental reductions in DAT function in the striatum. If the model is truly representative of the mechanisms underlying ADHD, this not only provides an explanation for the actions of these drugs, but may suggest ways to identify non-stimulant ADHD medications acting through alternative mechanisms within the same neurocircuitry.

Social Status Modulates Dopaminergic Pathway in Escape Circuit in Zebrafish (*Danio rerio*)

Katie Clements[1], Thomas Miller[1], Eoon Hye Ji[2], Fadi Issa[1]

[1]Department of Biology, East Carolina University
[2]Department of Physiology, David Geffen School of Medicine, UCLA

Zebrafish form social hierarchies that consist of either socially dominant or submissive fish. Once social status is established the behavior patterns and social displays between competing males reflect the their social standing. The objective of this project is to determine the neural bases of social behavior by identifying brain circuits that are influenced by social experience. When zebrafish are startled, they produce a stereotypical escape response called the C-start escape behavior. The underlying neural circuit that mediates C-start escape is well characterized and is centered around the Mauthner command neuron that is activated via auditory input. We hypothesized that social experience will affect the sensitivity of zebrafish escape behavior and the activation threshold of underlying Mauthner neural circuit. To test our hypothesis, two male zebrafish of similar age and size were paired for one week during which their social interactions were observed daily to monitor their social relationships. We then tested the zebrafish C-start escape response by delivering a brief auditory pulse at increasing decibels (70-105dB). Escape behavior produced in response to the auditory pulses was recorded via bath electrodes placed in the testing chamber, which measured the field potentials generated during escape behavior. Response sensitivity and habituation to auditory pulses of dominant and submissive animals were compared to control communal fish. Initial results show that dominants are less sensitive compared to submissive zebrafish. In conjunction, communal zebrafish, when chosen at random, are more likely to behave like dominant zebrafish. This suggests the neural change that occurs in the zebrafish is that of the subordinates. Furthermore, submissive fish are less likely to habituate to repeated stimulation compared to their dominant counterparts. We are currently teasing out the dopaminergic pathway involved with the Mauthner escape response in hopes of a better understanding of the reconfigurations in the vertebrate central nervous system based on social status.

Developmental Toxicant Exposure and Early Neuroimmune Susceptibility to Alzheimer's Disease

Annalise vonderEmbse, Z.W. Hinton, Q. Hu, and J.C. DeWitt

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

The role of developmental immune dysfunction in neurodegenerative diseases, such as Alzheimer's disease (AD), may be due to an early disruption of regulatory signals for activation of microglia, the resident macrophage of the central nervous system (CNS). Although microglia are thought to contribute to AD pathology by perpetuating chronic inflammation, there are data to suggest pathological contribution is due to a decrease in neuroprotective capabilities rather than neurotoxicity. We previously demonstrated that a "double-hit" model of postnatal lead (Pb) exposure in a triple transgenic mouse model for AD (3xTgAD) lead to microglial activation abnormalities and exacerbated late-stage AD pathologies. Here, we included a wildtype (WT) comparison and investigated the persistence and dysregulation of microglial activation states following toxicant exposure from postnatal day (PND) 5-10. Surface markers for classical, pro-inflammatory M1 activation (CD86) and alternative, anti-inflammatory M2 activation (CD209) were analyzed at PND 120, 180, and 240, with greater pro-inflammatory markers at the earliest timepoint (PND 120) and lower anti-inflammatory markers following postnatal Pb exposure. Furthermore, double-positive ($M1^+/M2^+$) stained microglia, indicative of dysregulated activation, increased with both time and Pb exposure regardless of genetic predisposition. Taken together, these data suggest regulation of microglia activation is perturbed during development due to toxicant exposure, which then persists into adulthood and impairs neuroprotection via an initial decrease in M2 surface markers and aberrant activation profiles at later stages of pathogenesis. Further investigation of the mechanisms by which regulation of microglial activation is altered following an early immune insult will provide critical insight into the precarious developing neuroimmune system and its potential role in AD etiopathology.

Neural vs. Non-Neural Contributions to Rigidity in Parkinson's Disease

Douglas Powell, PhD

Department of Physical Therapy, Campbell University

Rigidity (muscle stiffness) is one of the most disabling symptoms in Parkinson's disease (PD). It is clinically defined as an increased resistance to passive movement of a joint. There is a fundamental gap between mechanistic and applied approaches to understanding this symptom. The objective of the current study was to apply a system identification and modeling approach to differentiating the contributions of neural (enhanced muscle reflex) and non-neural (altered mechanical properties of muscle fibers) factors to rigidity. Six patients participated in the study. The wrist joint torque and muscle activities of the wrist muscles were measured during externally induced movements. Each subject was tested in the Off- and On- medication states. System identification and modeling approach was applied to separate the neural from the non-neural component with respect to the overall stiffness. Results show that both factors are responsible for rigidity in PD. Neural-related reflex component is the predominant factor in overall rigidity. Medication therapy decreased the level of reflex component to overall rigidity.

Keywords: *Parkinson's disease; rigidity; System identification; anti-Parkinson medication*

Taking the Pain out of Clinical Pain Management

John Norbury, MD

Physical Medicine and Rehabilitation, Brody School of Medicine, East Carolina University

Pain is defined as an "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." As clinicians, it is incumbent upon us to approach the chronic pain patient in a holistic fashion. This starts with making an appropriate diagnosis through a careful history and physical examination. A systematic approach to the history and physical is presented which can be very helpful in guiding the process. A plethora of diagnostic tests are available to aid us in caring for the chronic pain patient, but judicious use of these tests is important from both a cost and a patient care standpoint. Opioids remain a powerful tool for chronic pain, but require diligence to prevent harm to patients and protect providers. Methadone can be an especially powerful tool in pain management when utilized appropriately. Non opioid options for pain include other medications such as anti-epileptic medications and anti-depressant medications, relative rest, modalities, exercise, injections, surgery, alternative medicine, and most importantly lifestyle changes. The best chronic pain treatment programs are interdisciplinary and combine elements from these categories as appropriate to help the patient achieve their goals and improve function.

Our Brains Are Not Us - On the Dangers of Neuro-Reductionism in Treating People in Pain

Daniel S. Goldberg, JD, PhD

Department of Bioethics and Interdisciplinary Studies, Brody School of Medicine, East Carolina University

This presentation explores the potential and the danger of what has been termed "neuro-reductionism" in improving the inequitable undertreatment of pain in the U.S. The talk begins by explaining the idea of neuro-reductionism – roughly, that our identities or our minds are reducible to our physical brains. Although the evidence shows that the equivalence of mind with brain is widely accepted among neuroscientists, the issue is extremely controversial within other scholarly communities (anthropologists, sociologists, philosophers, etc.) The presentation moves on to explain several of the more compelling criticisms of neuro-reductionism, centering on the work of philosopher and neuroethicist Walter Glannon. The final portion of the presentation applies these criticisms to the devastating and inequitable undertreatment of pain in the U.S. Although many hold out the hope that the attempt to objectify pain in the brain may result in improved treatment and diminished stigma for pain sufferers, the problems with neuro-reductionism suggest that such efforts may be ineffective or even counterproductive.

Poster Session 1

(in alphabetical order by presenting author)

Coming Soon to Neurooncology: The 2016 World Health Organization Classification of Primary Tumors of the Central Nervous System with Incorporation of Molecular Diagnostic Data

Boyer, Philip J[1], Dvorak Jonathan D[2], Ma, Shuhua[1]

[1]Department of Pathology, East Carolina University and Vidant Medical Center, Greenville, NC, United States
[2]Brody School of Medicine, East Carolina University, Greenville, NC, United States

To date, the classification of primary neoplasms of the central nervous system (CNS) has incorporated a subjective, phenotypic, histologic approach with the gradual incorporation of immunohistochemical data (e.g. expression or lack of expression of glial or neural antigens). Molecular changes in specific neoplastic subsets have gradually been identified and validated (e.g. concomitant deletions of chromosomes 1p and 19q). However, the World Health Organization (WHO) 2007 CNS neoplasm classification scheme which establishes gold-standard diagnostic criteria used around the world, incorporating a consensus of international neurooncology experts and literature-supported data, did not incorporate molecular data (1). A subgroup of this WHO expert group published a consensus paper in late 2014 describing changes which will likely be incorporated into the updated WHO 2016 consensus classification scheme including the standard use of specific, validated molecular data sets (2). Using illustrative cases, this presentation summarizes the key anticipated changes and describes their application to the diagnostic process at an academic medical center. Incorporation of molecular data (1) recognizes early changes that take place in most adult astrocytomas and oligodendrogliomas, (2) acknowledges the limitations of phenotypic classification by histology and immunohistochemistry alone, and (3) reduces some of the subjective elements in the diagnosis of CNS neoplasms. The proposed changes help to optimally distinguish "primary" glioblastomas and small cell astrocytomas, which follow an aggressive course, from low grade astrocytoma and oligodendroglioma and "secondary" glioblastoma entities. A key anticipated change is the elimination of the controversial and subjective category oligoastrocytoma which has served as a "wastebasket" classification for neoplasms with overlapping phenotypic features. Highlighting the need for periodic updates to the diagnostic workup, a significant subset of neoplasms will remain unclassified by the proposed scheme including, (1) among mostly adult-presenting neoplasms, a proportion of low grade astrocytomas and, (2) among mostly childhood-presenting neoplasms, most astrocytomas and oligodendrogliomas. The publication by the WHO expert subgroup of likely changes to the 2016 classification criteria gave the neurooncology community a "heads up" regrading likely changes and has allowed for early adjustment to the diagnostic workup in anticipation of these changes.

1. Lewis DN et al. IARC Press: Lyon, 2007.
2. Louis DN et al. Brain Pathol. 2014 Sep;24(5):429-435.

Cocaine Conditioned Place Preference Alters Neuronal Activity in Zebra Finch Song Regions

Nariman Emadzadeh and Ken Soderstrom

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

Zebra finches learn a form of vocal communication during adolescent development; this allows these animals to be used to assess effects of abused drugs on development of this learned behavior. In avian species it is difficult to measure drug self-administration, which is a disadvantage to the model. We have worked to create a conditioned place preference method to assess reinforcing properties of drugs in these birds. We have used expression of c-Fos as a marker for neural activity.

For conditioning, animals were placed in a two-sectioned chamber, separated by a divider, with each chamber distinguished by color (yellow vs. green) and by perch texture (rough vs. smooth). Pre-treatment preference was determined by placing finches between chambers without a divider and measuring time spent in each chamber over 15 min. This evaluation was repeated over five consecutive days. Following determination of preferred chambers, conditioning was initiated by placing animals in alternating preferred and non-preferred chambers and following treatment via IM injection with either vehicle or cocaine. Conditioning proceeded over eight consecutive days, alternating vehicle or cocaine administration. Following conditioning, animals were allowed free access to both chambers and time spent in each recorded over 15 min. Time spent in the cocaine-paired chamber was compared across treatment groups via ANOVA. The day following place preference tests, animals were placed within cocaine-paired chambers for 15 min. and then euthanized, transcardially perfused, and brains prepared for c-Fos immunohistochemistry. Densities of c-Fos-reactive nuclei were compared across treatment groups.

Results indicate that reinforcing properties of cocaine can be measured through conditioned place preference in zebra finches. Also, a dose-dependent increase in neuronal activity was observed within motor- and learning-related brain regions following post-conditioning placement in cocaine-paired chambers.

Findings expand the utility of the zebra finch model for studying drug reward. Future experiments will evaluate the ability of developmental drug exposure, which alters vocal learning, to also modify drug reward in adulthood.

Depletion of CD4$^+$ CD25$^+$ Regulatory T Cells Confers Susceptibility to Experimental Autoimmune Encephalomyelitis (EAE) in GM-CSF Deficient *Csf2*$^{-/-}$ Mice

Debjani Ghosh[1], Alan D. Curtis II[1], Daniel S. Wilkinson[1], and Mark D. Mannie[1,2]

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[2]The Harriet and John Wooten Laboratory for Alzheimer's and Neurodegenerative Disease Research

Previous studies have established that GM-CSF deficient (*Csf2*$^{-/-}$) mice exhibit profound resistance to EAE. This study addressed whether the resistance of *Csf2*$^{-/-}$ mice was due to a presumptive requirement for GM-CSF in the effector phase of EAE or whether EAE resistance instead reflected functional imbalance of effector and regulatory T cell subsets in the disease process. The main observation was that, in both active and passive models of EAE, treatment with the anti-CD25 mAb PC61 rendered *Csf2*$^{-/-}$ mice fully susceptible to severe paralytic EAE with disease incidences and severities equivalent to that of wildtype mice. When both donors and recipients were treated with PC61, adoptive transfer of myelin-specific *Csf2*$^{-/-}$ T cells into *Csf2*$^{-/-}$ recipients resulted in a non-resolving chronic course of severe paralytic EAE. The *Csf2*$^{-/-}$ T cell repertoire was marked by accumulations of naive CD44$^{\text{null-low}}$ CD4$^+$ and CD8$^+$ T cells but essentially normal frequencies of CD4$^+$ CD25$^+$ FOXP3$^+$ T cells. In conclusion, this study revealed that GM-CSF is not an obligatory cytokine in the effector phase of EAE. Rather, these data suggest that EAE resistance of *Csf2*$^{-/-}$ mice is due to a relative deficiency of effector T cell function that can be unleashed upon depletion of regulatory T cells.

Pelvic Nerve Injury Reduces Bladder Autonomic Innervation and Smooth Muscle in a Rat Model of Radical Hysterectomy

Shelby A. Powers, Elena Pak, Johanna L. Hannan

Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, United States

Radical hysterectomy (RH), a common non-obstetric surgery in women, frequently results in damage to the nerves controlling bladder function and lower urinary tract disorders that severely impact quality of life. The aim of this study is to characterize changes in bladder autonomic innervation and smooth muscle (SM) following bilateral pelvic nerve injury (BPNI), a model for RH. We hypothesized SM area and nerve terminals expressing vesicular acetylcholine transferase (VAChT), tyrosine hydroxylase (TH) and neuronal nitric oxide synthase (nNOS) would decrease post-BPNI. Female Sprague-Dawley rats (n=2-5/group, 12 weeks) underwent a modified "clock-crush" BPNI to simulate RH. Bladders were excised 3, 7, 14 and 30 days following injury. Sham animals underwent surgery to identify, but not injure the pelvic nerves. Bladders were formalin fixed, paraffin embedded and sections were stained with Masson's trichrome. Additional sections underwent immunofluorescence staining for alpha smooth muscle actin, and nerve terminals expressing VAChT, TH and nNOS. Two blinded individuals counted nerve terminals on 4 areas of each bladder section. The number of nerve terminals expressing VAChT significantly increased 3 days after BPNI and significantly decreased at 14 and 30 days (S: 36±5.5, 3d: 51±4.5, 7d: 29±3.4, 14d: 14±0.0, 30d: 21±2.6; $p<0.05$). Following injury, nerve terminals expressing TH were significantly decreased in all bladders compared to shams (S: 32±3.7, 3d: 13±5.8, 7d: 11±1.6, 14d: 11±1.0, 30d: 15±3.6; $p<0.05$). A trend towards a decrease in the number of nNOS nerve terminals was observed but was only significant at 30 days (S: 33±3.0, 3d: 19±5.7, 7d: 23±6.2, 14d: 23±0.5, 30d: 15±1.2; $p<0.05$). BPNI bladders had significantly decreased SM area at all time points compared to sham (S: 45%±4.1, 3d: 28%±1.0, 7d: 20%±2.7, 14d: 29%±2.1, 30d: 29%±1.1; $p<0.005$). BPNI leads to an early increase and then decline in parasympathetic (VAChT) innervation, a decline in sympathetic (TH) and nitrergic (nNOS) innervation, and marked SM decrease in rat urinary bladders. These data suggest that impaired voiding following RH could be due to diminished autonomic innervation and SM. Therapeutic strategies preventing nerve injury-mediated decline in neuronal input and SM may prevent the development of urinary incontinence and improve the quality of life after RH.

Electromyographical Analysis of Muscle Activation Using a Nitrogen-Filled Piston Resistance Training Device Compared to Isotonic Resistance

Nicole J. Hernandez[1], Madison Sanderford[1], Jennifer A. Bunn[1], Douglas W. Powell[2]

[1]Department of Exercise Science, Campbell University
[2]Department of Physical Therapy, Campbell University

Resistance training has been shown to improve muscle mass, muscle strength, and muscle power. Resistance training is especially important for populations at risk of sarcopenia and osteopenia such as older adults or astronauts working in microgravity. A limitation of common forms of resistance training is that they are gravity-based and lack efficacy in microgravity. Therefore an alternative solution must be sought. One alternative is the use of a gas-filled piston-based resistance training system. The goal of the current study is to assess the effectiveness of a Nitrogen-filled piston-based resistance training system (NitroForce) compared to isotonic resistance training.

Methods: Thirty recreational athletes performed five repetitions of squat and toe-raise exercises, independently, in each of two experimental conditions: NitroForce and Isotonic. The NitroForce condition was characterized by resistance of approximately 70% of max (1RM) resisted by the NitroForce system. Isotonic condition included participants performing each exercise with 70% of 1RM with resistance provided by free weights. Surface electromyography (sEMG) was recorded from the vastus medialis (VM), vastus lateralis (VL), and the lateral (LG) and medial heads of the gastrocnemius (MG). Root mean square with a 20 ms smoothing window was used to rectify and smooth the sEMG signal. Mean sEMG amplitude normalized to MVC was used to quantify muscle activation intensity in each muscle in each condition. Paired samples t-tests were used to compare mean sEMG values of each muscle in each condition. Significance was set at $p < 0.05$.

Results: In the squat exercise, VM activation was significantly greater in the NitroForce compared to isotonic condition ($p = 0.039$), but no differences were observed in the VL ($p = 0.367$). No significant differences were observed between the NitroForce and isotonic conditions for the LG ($p = 0.400$) or MG ($p = 0.442$) in the toe-raise exercise.

Discussion: These data demonstrate that the NitroForce provides a similar level of resistance and overload to standard isotonic resistance training loads. However, due to the non-gravity-based nature of the NitroForce, it may provide a better resistance training solution in microgravity environments.

Acute Restraint Stress Alters Adult Zebra Finch Song Performance and Neuronal Morphology: Potential Model for Evaluation of Neurobiological Effects of Developmental Stress

Tessa L. Holland and Ken Soderstrom

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

During a sensitive period of development, male zebra finches learn a complex song that is important to courtship. During early stages of vocal development they listen to and memorize an adult's song, forming a template. Later in development they gradually improve the song by practicing and using auditory feedback. In adulthood, the song has low variability and is stable. This process parallels language acquisition in humans. We are interested in the effect of psychological stress on vocal development in zebra finches.

Currently, we evaluated the effect of acute restraint stress on song performance and neuronal morphology in adult zebra finches. We hypothesized that stress would alter spectral and temporal features of the adult's song. Zebra finches ($n = 4$-5) were administered 30 minutes of restraint stress or 30 minutes of no stress, immediately followed by one hour of audio recording. A female bird was presented as a social stimulus to promote singing. Songs were compared to previously obtained baseline recordings in a paired design. Sound Analysis Pro software was used to analyze songs. Song syllables of the stress group had significantly higher pitch and longer duration when compared to baseline recordings (Wilcoxon signed-rank test, $p < 0.05$). The no stress group had no significant differences from baseline recordings.

In a complementary experiment, adults were administered acute restraint stress ($n = 5$), and following cessation of the stressor, brains were collected for histological experiments. One hemisphere was Golgi-Cox stained in order to evaluate changes in dendritic spine density as a morphological measure related to neuronal activity. Additionally, one hemisphere was used for the immunohistochemical analysis of c-fos, a protein that acts as a marker of neuronal activity. In auditory cortex regions L2 and NCM, changes in dendritic spine density following stress corresponded to changes in c-fos density (Student's t-test, $p<0.05$). Stress may alter neuronal activity in auditory regions as an adaptive, fight-or-flight response.

We are working to evaluate the effects of developmental stress on vocal learning in the songbird model and if co-treatment with THC, the partial agonist, principal constituent of *Cannabis*, will mitigate the persistent effects of developmental stress.

Evaluation of Thermal Pain Thresholds in MEIS1 Mice, a Possible New Animal Model for Restless Legs Syndrome (RLS)

Samantha Meneely, Mai-Lynne Dinkins, and Stefan Clemens, PhD

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

Restless Legs Syndrome (RLS) is a chronic sensorimotor disorder characterized by discomfort and the uncomfortable urge to move the legs. Symptoms occur most often in the evening or at night and can severely disrupt sleep. The cause of RLS is unknown, but one theory is pointing to a role of genetic factors surrounding the MEIS1 (MEIS homeobox 1) gene. The first line of drug therapy uses dopaminergic agents that primarily target inhibitory D3 receptors, but no behavioral data are available that have assessed the effects of these compounds on thermal pain withdrawal latencies as a model for enhanced sensitization or pain. We recently observed that D3 receptor stimulation altered D1 receptor expression; therefore we here tested the effects of both D3 and D1 receptor modulators.

We compared pain withdrawal latencies in MEIS1 knockout mice and their appropriate wild type controls (WT) using a Hargreaves' apparatus (IITC Inc.). Animals received intraperitoneal injection of 0.9% NaCl (sham control), levodopa (a precursor to dopamine, L-dopa, 10 mg/kg), pramipexole (a selective D3 receptor agonist, 0.5 mg/kg), SCH 39166 (a selective D1 receptor antagonist, 0.1 and 0.5 mg/kg), and SKF 38393 (a selective D1 receptor agonist, 1 mg/kg).

We found that MEIS 1 knockout animals L-dopa, pramipexole, and SCH 39166 significantly increased thermal withdrawal latencies, while the D1 receptor agonist, SKF 38393 did not have any effect. In contrast, pain withdrawal latencies in wild type controls only increased under L-dopa and pramipexole, but after injection with the D1 receptor agonist or antagonist.

These preliminary suggest that MEIS 1 may play a role in the modulation of D1 receptor but not D3 receptor pathways, and that the proven effectiveness of D3 receptor modulators in RLS may be independent of the MEIS 1 gene.

Effects of Minocycline on Nicotine-Induced ΔFosB Expression in Brain Regions Relevant to Drug Reward and Sensitization

PS Nagchowdhuri, KT Lane, HL Williams, BA McMillen

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

Nicotine use during adolescence is considered to be a 'gateway' that leads to the brain's sensitization to other illicit substances in the future. Psychoactive drugs such as nicotine are known to induce the expression of the transcription factor ΔFosB which facilitates this sensitization process. Previous studies have shown that a once daily, 10-day administration of nicotine during periadolescence in rats (PND 35-44) at an intraperitoneal (i.p.) dose of 0.4 mg/kg induced the expression of ΔFosB in the memory and reward subset of the rat brain, and this expression persisted into adulthood (PND 80), especially in the nucleus accumbens (NAc) and dentate gyrus of hippocampus (DG) (Soderstrom et al. 2007). Our long term goal is to elucidate the role of microglia in periadolescent sensitization to nicotine *in vivo*. Further research is needed to determine if there are cross-talks between microglia priming and ΔFosB induction. In this study, minocycline, a tetracycline antibiotic commonly used to suppress the activation of microglia, is injected into periadolescent Sprague Dawley (SD) male rats at a dose of 30 mg/kg, 30 minutes prior to nicotine (0.4 mg/kg) injections for the 10 days that bracket the onset of puberty. The minocycline pretreatment resulted in a reduction in the densities of primed microglia and ΔFosB-labeled nuclei in the DG and PFC 24 hours following the last set of injections when compared to nicotine treatment alone. The results thus far imply that microglia priming may play a role in the induction of ΔFosB, and this may have relevance to the memory aspect of sensitization to drugs of abuse.

The Role of miRNAs in Nicotine-Induced Early and Late-Onset Diseases

Krishna Patel, Sana Bharde, Robyn Alston, Faten Taki, Baohong Zhang, Xiaoping Pan

Department of Biology, East Carolina University

MicroRNAs fine tune the expression of target genes needed for homeostasis. Our pilot study showed that nicotine altered 17% miRNAs in C. elegans larvae. Here, we plan to investigate the role of microRNAs in mediating nicotine-induced adolescent and adult-onset phenotypes: larval pharyngeal pumping, adult germ line apoptosis, and survival. Worms were treated with nicotine during the postembryonic stages with either 20μM or 20mM. For the pharyngeal pumping assay, videos were recorded for at least 15 worms/group to compute average pumps/sec. For adult apoptosis and lifespan assays, worms were washed off treatment before gametogenesis and allowed to grow till adulthood on nicotine-free media. Day 1 adults were stained with SYTO12 or acridine orange and apoptotic cells were counted for at least 50 gonads per treatment group. The remaining 200 worms were counted and transferred every 1-2 days until all worms died. Kaplan-Meier curves were used to compare the survival curves across treatment groups. Our data suggests that post-embryonic nicotine exposure inhibited pharyngeal pumping, and increased germ cell apoptosis in WT worms which were rescued in mutant strains. Due to the conservation of those signaling pathways, our results provide insights for miRNA-based treatment strategies for nicotine disorders in early and late stages.

The Effects of High-Fat and High-Sugar Diets and Exercise on the Development of Drosophila First Generation Offspring

Michelle R. Pike[1], Oksana Williams[2], Henry Nicolae Alarco Jeri[3], Ajay Ajmera[3], Alexander K. Murashov[2]

[1]Department of Biology, East Carolina University
[2]Department of Physiology, Brody School of Medicine
[3]Department of Biology and Chemistry, East Carolina University

With the childhood obesity rates increasing, it is imperative to understand the effects of different diets and activity on offspring development. While studies have shown maternal high-calorie diet is a significant risk factor for the offspring's sexual dimorphism and development, emerging evidence suggests that obese and diabetic fathers may also contribute to offspring metabolic phenotype. In this study we questioned how different diets and exercise of fathers may produce an effect on offspring development; including time of development, probability of producing a higher ratio of males or females, and offspring activity levels. Drosophila Melanogaster represent a unique model for transgenerational studies because of its well-known genetics, short life cycle, well-defined developmental stages, and fast reproduction rates. Development of the F0 and F1 generations were analyzed by observing the first day of appearance of larvae, pupa, and adult, and by collecting daily larvae and pupa on each tube wall until the first adults hatched. The total number of adults was obtained by counting the number of males and females produced in each tube for nine days after the first adult appeared. Male Drosophila fathers in this research were put on high-fat, high-sugar, and control diets for 14 days. After 14 days of being on a diet, all male flies were mated with control virgin females on control food. The results showed there was a significant difference ($P<0.001$) in the time required for both larvae and pupa to first emerge between the high-sugar diet and all other groups in F0 and F1. We observed a significant acceleration in the time needed for adult flies to first emerge between F0 and F1 from high-sugar diets and F0 and F1 from control ($P<0.001$) and high-fat diets ($P<0.01$). The observed differences indicated a significant change in the rates of development in the offspring compared to one another and to the control father group due to paternal diet. No significant differences between the ratio of males and females and overall number of males and females produced from the F0 and F1 groups were observed. Overall, the data suggests different diets of fathers have impacts on the rate of development of their offspring.

Therapeutic Approaches to Attenuate Cognitive Decline in an Alzheimer's Disease Model

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Alzheimer's disease (AD) is the most common form of dementia in the elderly. It is characterized by progressive cognitive decline and accumulation of beta-amyloid (Aβ) plaques and neurofibrillary tangles within the brain. These pathological changes stimulate inflammation within the brain by activating microglia, the immune cells of the brain. Microglia can play dual roles in AD as they can aid in clearing Aβ plaques or can contribute to the progression of the disease. Previous work has shown that exercise can reduce inflammation and may protect against the cognitive impairments associated with dementia. Minocycline, an anti-inflammatory compound, inhibits microglial cell activation and has been shown to reduce Aβ levels. The present study investigated the potential additive effects of minocycline and exercise using a mouse model of AD. Adult (9 month) 3xTg-AD and wild type (WT) mice were housed with or without a running wheel and were administered minocycline or water in their water bottles for 4 weeks. During treatment, motor behavior and anxiety were assessed with the open field and novel object exploration tasks. Radial arm water maze (RAWM) was used to evaluate spatial learning and memory retention. Preliminary results indicate that there were minimal differences between the 3xTG-AD and WT mice on the behavioral tests. However, the 3xTg-AD, especially females, displayed increased anxiety-like behavior in the novel object test. In the RAWM all groups acquired the task, as shown by reduced distance and latency, but the 3xTg-AD showed an increase in swim speed across testing days. The lack of clear differences between the 3xTg-AD and WT mice makes it challenging to interpret the effects of the combined treatments. However, additional work is currently in progress to determine whether the individual or combined treatments altered inflammation and levels of Aβ plaques within the brain.

Protein Quality Control in Neurodegenerative Diseases and Identification of New Therapeutic Targets

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The only factor explicit about neurodegenerative diseases is the accumulation of misfolded proteins in the form of "amyloid". For example, deposition of Aβ peptide and tau protein as amyloid in the extra- and intra-cellular space defines Braak stages that are used to indicate the severity of Alzheimer's disease (AD) or pathological AD staging. Our studies have focused on elucidating the pathway normal proteins opt enroute their transformation to disease causing entities. Together with the vast amount of research reported in this area it turns out that for largely unknown stochastic reasons proteins misfold into a conformation that exposes their sticky patches (usually 6-10 residues) triggering intermolecular association into final ordered fibril structures called "amyloid". The journey from native protein to amyloid involves assembly of protein into transient oligomeric and proto-fibrilar intermediates. Being short lived and hard to study these intermediate species have created quite an intrigue and excitement within the area.

Running parallel along this is the protein quality control system that every physiological cell is equipped with and consists of various families of chaperones. We will consider Hsp70 family here. The Hsp70 protein of the family identifies unfolded/misfolded proteins (through a 6-8 residue patches) sometimes with the help of its Hsp40 counterpart and together with NEF protein helps it fold at the expense of ATP. If the unfolded protein fails to fold in one attempt the cycle keeps repeating until successful. If for some reason unfolded protein is unable to fold, Hsp70 tags it for degradation through CHIP mediated ubiquitination. The protein quality control is thus robustly maintained.

The interesting factor is both Hsp70 and amyloid prone proteins use a 6-8 residue patch. Is there any similarity, any overlap, any preference, any competition that is the question we deal with. And to investigate this we chose proteins implicated in amyloid/neurodegenerative diseases like Tau, Aβ, insulin and used also model proteins like albumin.

Our initial data indicates strong link between residue exposure and amyloid formation as characterized by biochemical and biophysical methods. Further, our data indicates a relation of these patches with chaperone binding. Together these proteins provide novel targets that we plan to screen for small drug like molecules. The larger aim of these projects is a rational drug design for intervention in neurodegenerative diseases.

The Effect of Paternal High Sugar Diet and Exercise on Metabolic Phenotype of Offspring in Drosophila Melanogaster

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The recent increase in type II diabetes and obesity is primarily due to a sedentary lifestyle and unbalanced diet. The effects of maternal exposure to a high fat diet or malnutrition on a phenotype of offspring are supported by multiple studies. Recent observations suggest that epigenetic changes in fathers can also affect offspring metabolic phenotype. In our laboratory we investigate how paternal diet and exercise affect metabolic profiling of offspring. We used Drosophila Melanogaster as a model organism because of its powerful genetics, short life cycle, well defined developmental stages, and fast reproduction rates. In the current study we investigated effects of high sugar diet and exercise as environmental factors on offspring metabolic phenotype. The treatment groups included control, high-sugar, and exercise group. One-day old male flies, F0 generation, were placed on control diet for 3 days, and then challenged with 5 days of exercise (on control food), or subjected to high-sugar, or control diet. After exposure, 5 males from each group were mated with 5 virgin control females for F1 generation. The F1 generation 1 day old male flies were placed on control diet for 3 days and then divided into control and challenged groups. The control F1 groups were kept on control diet for 5 days and then mated with virgin females for F2 generation. The F2 generation male flies were also divided on control and challenged groups and treated similarly to F0 and F1 generations. The challenged F1 and F2 groups were placed on high sugar diet for 5 days and then collected and frozen for metabolic phenotype assessment. The metabolic phenotype of all generations was evaluated by measuring weight, triglyceride content, glucose and trehalose levels, motility of flies as well as endurance utilizing vertical test. We observed significant increase in weight in F0 fathers on high-sugar diet compared to F0 control fathers; surprisingly, the high-sugar father's F1 offspring had significantly lower weight, when maintained on control food. Triglyceride levels were significantly higher in F0 on high sugar diet compared to F0 on control diet. The F1 from high-sugar diet fathers had higher triglyceride levels than F1 from control and exercise fathers when on control food, no significant difference was observed between F1 challenged with high sugar diet. The F2 from exercise fathers had lower triglyceride content than F2 from high sugar fathers when on control diet. The preliminary results support our hypothesis that paternal epigenetic changes due to exposure to a high-sugar diet and exercise can affect F1 and F2 generation metabolic phenotype.

Estrogen Dampens Neuronal Oxidative Stress and Pressor Response Caused by Cannabinoid Receptor 1 Activation in Rostral Ventrolateral Medulla in Conscious Rats

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Background and objective: Our recent studies demonstrated that activation of the cannabinoid receptor 1 (CB_1R) in the rostral ventrolateral medulla (RVLM) elevates blood pressure in conscious male rats. It is known that estrogen (E_2) inhibits central sympathetic tone. However, whether dampening of the CB_1R-mediated sympathoexcitation accounts for this neuro-endocrinological effect of E_2 has not been investigated. The aim of the current investigation was to test this intriguing hypothesis in female rats exhibiting the highest endogenous E_2 level and following E_2 deprivation and replacement.

Methods: Female rats, surgically implanted with intracranial guide cannula and femoral artery catheter to permit intra-RVLM microinjections, and blood pressure measurement, respectively, were used under the following hormonal states: (i) Proestrus sham operation (SO) rats (highest endogenous E_2 level); (ii) E_2-deprived, ovariectomized (OVX) rats; (iii) OVX rats plus E_2 replacement ($OVXE_2$) rats. All rats were employed in the conscious state, and received intra-RVLM microinjection of the CB_1R agonist WIN55,212-2 (WIN) and/or the CB_1R antagonist AM25, or vehicle.

Results: Intra-RVLM WIN (100, 200 and 400 pmol) caused dose dependent pressor response in all rat groups that was inversely related to E_2 level. Compared with SO rats, the pressor response was significantly enhanced in E_2-deprived OVX rats, and restored to SO levels E_2 replacement. Intra-RVLM CB_1R activation had no effect on heart rate in SO rats, but increased heart rate in OVX rats. Following intra-RVLM WIN, ex vivo neurochemical findings revealed enhanced phosphorylation of Akt and nNOS, higher nitric oxide and reactive oxygen species levels in RVLM neurons of OVX rats. All these biochemical responses were attenuated in E_2 replaced ($OVXE_2$) rats. The blood pressure and neurochemical responses elicited by WIN were attenuated by prior RVLM microinjection of the CB_1R antagonist AM251.

Conclusions: These findings suggest that estrogen dampens the molecular events triggered by CB_1R activation, which lead to oxidative stress in RVLM neurons, and subsequent elevations of sympathetic activity and blood pressure. These novel findings might yield insight into a central mechanism for estrogen-dependent dampening of sympathetic activity and hypertension in females.

Poster Session 2

(in alphabetical order by presenting author)

Cortical Impact on the Dynamics of Subthalamo-Pallidal Networks

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Parkinson's disease pathophysiology is marked by increased oscillatory and synchronous activity in the beta frequency band in cortical and basal ganglia circuits. Less is known, however, about how these abnormal dynamics are correlated across these brain regions. This study explores the oscillatory interactions between cortical and basal ganglia networks in Parkinson's disease in the model of the basal ganglia. The patterns of responses of beta-band bursting in the model suggest that the experimentally observed beta-band synchronization in Parkinson's disease may be promoted by the simultaneous action of both cortical and subthalamo-pallidal network mechanisms.

The Role of Prenatal Hormone Exposure on Neurobehavioral Impairments in a Rat Model of Autism Spectrum Disorders

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Purpose: In an ongoing multidisciplinary collaboration with Dr. Krista McCoy's lab, the role of prenatal hormone exposure (PHE) in producing neurobehavioral impairments in rats, as an animal model for autism spectrum disorders (ASDs), is being studied. Both PHE and behavioral testing - in the form of trace eyeblink classical conditioning (ECC), a hippocampal-dependent task - have been completed by both of the labs. The possible link between loss of neuron number within the hippocampus and alterations in associative learning that were previously found using ECC will be examined. Neuron number is thus an important brain indicator of behavioral function and dysfunction.

Significance: Approximately 1 in 68 children are diagnosed with an ASD, and annual costs in the U.S. for each child are estimated at $2.3 million. While the precise causes for abnormal brain development in ASDs are not known, environmental contributions – particularly androgens and estrogens - may play an adverse role. Further, the effect of PHE on hippocampal function is not well-understood, allowing for studies that may help elucidate their involvement in ASDs.

Role & Methodology: Because the hippocampus is vulnerable to environmental teratogens, we hypothesize that PHE will impair associative learning in trace ECC. Pregnant Sprague-Dawley rats received daily injections of either dihydrotestosterone propionate (8 mg/kg), estradiol benzoate (50 μg/kg), or corn oil alone (vehicle) from embryonic days 15.5-17.5. Their adult offspring (two of each sex) were tested as adults (3 months of age) using trace ECC, which was carried out for 6 consecutive days. Afterwards, their brains were extracted and stored. Changes in neuron number using unbiased stereology within hippocampal cell layer CA1 will be examined.

Preliminary Results: The trace ECC testing has already been conducted, and preliminary results indicate impaired learning in PHE rats. Cell counting technique will be used to correlate brain and behavior measurements. Findings from this study may help to determine the link between cell loss and ASDs, so that treatments targeted at enhancing cellular function can be implemented.

Contralateral Suppression of Transient Evoked Otoacoustic Emissions in Young Adults with Varying Degrees of Musical Ability

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Of all descending auditory pathways, the olivocochlear efferent system has been studied extensively. Two olivocochlear efferent pathways exist: lateral olivocochlear and medial olivocochlear (MOC). MOC and lateral olivocochlear neurons receive auditory innervation and both constitute pathways of olivocochlear acoustic reflexes. The MOC ipsilateral and contralateral reflexes are best understood. The role of the MOC efferent reflex has been suggested to be fourfold: shift the dynamic range of hearing, facilitate selective attention, protect the ear from acoustic trauma, and reduce the effects of masking noise. The functioning of the MOC reflex can be tested noninvasively in humans through contralateral suppression of transient evoked otoacoustic emissions (TEOAEs). TEOAEs are sounds emitted following acoustic stimulation to short/brief duration stimuli. TEOAEs provide a simple, efficient, and non-invasive objective indicator of healthy inner ear function. Contralateral suppression of TEOAEs refers to a reduction in amplitude of the emission with auditory stimulation of the contralateral ear. This effect is attributed to alteration of cochlear micromechanics by MOC efferents. The MOC reflex is more robust in musicians versus non-musicians as evidenced in greater contralateral suppression of TEOAEs. All previous research comparing musical ability and MOC efferent strength has defined musicianship dichotomously (i.e., high-level music students or professional classical musicians versus non-musicians). There is no data, however, to show this relationship on a continuum of ability in music. The objective of the study was to further explore contralateral suppression of TEOAEs among adults with a full spectrum of musicianship ranging from no history of musicianship to professional musicians. Participants were 32 normal-hearing young adults. TEOAEs were evaluated with 60 dB peSPL click stimuli with and without a contralateral 65 dB SPL white noise suppressor. Musicianship was assessed with the Brief Profile of Music Perception Skills (PROMS; Law & Zentner, 2012). There were no significant correlations or predictive linear relations ($p > .05$) between the amount of TEOAE suppression and the total Brief PROMS score or subscale Brief PROMS scores (i.e., melody, tuning, tempo, and accent). The results do not support the notion of a graded enhancement of MOC efferent suppression among adults with varied degrees of musicianship.

Metabolic Profile and Hypothalamic Neuron Interactions in the Triple Transgenic Mouse Model of Alzheimer Disease

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Background: Obesity and metabolic dysfunction are highly associated with the progression of dementia and Alzheimer Disease. The objective of the study is to observe the metabolic profiles of Alzheimer disease by using a highly established triple transgenic mouse model (3xTgAD) of Alzheimer Disease, and its association with hypothalamic neurons, specifically Pro-OpioMelanoCortin (POMC) and Tyrosine Hydroxylase (TH) expressing neurons in hypothalamus, that regulate metabolism.

Methods: Phenotypic analysis included measurements of food intake and body weight. Energy expenditure and body composition were examined by using the Comprehensive Lab Animal Monitoring System (CLAMS) and Magnetic Resonance Imaging (MRI) at ages of 10 weeks, 12 weeks, and 24 weeks. Furthermore, immunohistochemistry was utilized to identify amount changes for POMC and TH expressing neurons in hypothalamus.

Results: At 10 weeks of age, the 3xTgAD mice displayed increased food intake, and decreased locomotion activity, but displayed normal energy expenditure compared with control group, resulting in increased adiposity and body weight. Starting from 12 to 24 weeks old, the 3xTgAD mice showed significantly increased energy expenditure when compared with the control, despite having increased food intake, and decreased locomotion activity, leading to a significant body weight reduction in the 3xTgAD mice. There were significant reductions in the amount of TH expressing neurons at 3 months of age, and POMC expressing neurons at 6 months of age in the 3xTgAD mice compared with the control group suggesting that there may be a relationship between certain hypothalamic neuronal populations and metabolic activity associated with Alzheimer disease.

Conclusion: The results indicate a shift in metabolic profiles starting at 10 weeks of age, and become increasingly pronounced at 12 weeks of age, which is before Alzheimer disease normally develops within the 3xTgAD model, suggesting that there are metabolic abnormalities during the early stages of Alzheimer Disease and that it may be associated with alterations of dopaminergic neurons and POMC expressing neurons in the hypothalamus. Identifying pathways that influence brain pathology before the onset of overt neurodegeneration provides an avenue for possible preventative intervention of Alzheimer Disease.

Can Early Life Exposure to Lead Impact Synaptic Loss in a Transgenic Rodent Model with a Predisposition to Alzheimer's Disease?

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According to the NIH, Alzheimer's disease is ranked as the sixth leading cause of death in the United States, but further research estimates reveal it might be closer to the third leading cause of death for the oldest portion of our population (Alzheimer's Disease Education and Referral Center, 2015). Recent estimates from Rush University in Chicago propose that the number of deaths due to Alzheimer's may rise to 1.6 million by 2050, which is 43% of deaths in those aged 65 and over (Weuve, J., et al., 2014). Synaptic loss is being studied as an early marker in Alzheimer's disease. Therefore, the purpose of this project is to uncover the effects of how early-life exposure to lead (as lead acetate) impacts synaptic loss. Additional variables, including age, sex, and strain, will be analyzed to expose possible influences of these variables on synaptic loss. Brains were collected from male and female wild-type or transgenic animals exposed to a vehicle control or lead acetate in early postnatal life at 120, 180, or 240 days of age. Synaptosomes were prepared from whole brains minus hippocampuses, and synaptic loss was evaluated through total protein concentration of synaptosomes and relative abundance of synaptic proteins, including synaptophysin and SNAP25. The hypothesis is that with early life exposure to lead, the predisposed transgenic strain of rodent will have an early increase in synaptic loss relative to the wild-type strain as well as a more pronounced synaptic loss with age. This project is ongoing and will be in the data collection stages upon time of presentation. Uncovering the effects of exposure to early-life toxicants in both wild type and pre-disposed animal models could reveal, in large part, serious precursors and therefore earlier detection of a disease that takes on a large emotional and financial toll in the U.S.

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Restoring Loss of Communication for Individuals with Locked-In Syndrome (LIS)

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It is extremely difficult for individuals with locked-in syndrome (LIS) to communicate with others although they are cognitively intact. In order to help them communicate with their care-givers or family members, our collaborative research team plans to use an EEG-based noninvasive communication system, with which a user can spell alphabet letters simply by fixating letters on a display one at a time. By flashing the letters on the display in a random order, a special brainwave pattern, so called P300 event-related potential (ERP), can be elicited. The amplitude of P300 ERP tends to be larger when target letters flash where target letters are those that a user intends to spell by fixating. Then, a trained machine-learning algorithm can differentiate the P300 ERPs with large amplitude (i.e. targets) from those with small amplitude (i.e. non-targets). By checking the times when the P300 ERPs with large amplitude occurred, computer software can predict which letters a user was trying to spell.

The feasibility of this automatic spelling system for individuals with LIS has been widely suggested in the literature. However, there are very few case studies where those with LIS were able to spell letters of their intention using this system. In order to make it easier for them to use this system, two major problems need to be address. The first problem is the slow spelling speed. It is not uncommon that a user has to keep fixating one letter for several minutes until computer software can successfully predict the letter of intention. We believe that this problem can be addressed by adopting new flashing paradigms. On health subjects, some of new flashing paradigms haven been reported to increase the spelling speed up to ten times. The second problem is the unreliable performance of current machine-learning algorithms. Several new machine-learning algorithms are under development, which are specifically designed for P300 ERP pattern recognition. With new flashing paradigms and specialized machine-learning algorithms, we should be able to improve the overall performance of the automatic spelling system so that it becomes a more attractive communication device for individuals with LIS.

Effects of Long-Term Dopamine D3 Receptor Agonist Treatment on Autonomic and Cardiac Function in Aged Animals

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Normal aging is associated with a gradually increasing risk of cardiovascular diseases, including hypertension, ventricular hypertrophy and fibrosis, and a decrease in overall dopamine (DA) levels. A role for DA in the pathophysiology of hypertension has been well established, and several mechanisms have been reported by which failure of the D3 receptor (D3R) system might account for an increase in blood pressure. Moreover, we have shown previously that a dysfunction of the dopamine D3R system is associated with aging-dependent changes in autonomic function and cardiac fibrosis. As patients suffering from the sensorimotor disorder Restless Legs Syndrome (RLS) often also manifest an increased risk of cardiovascular diseases, and as treatment with D3 receptor agonists can improve both RLS symptoms and the associated hypertension, we tested here the hypothesis if prolonged activation of the D3R system in aged mice can restore or improve autonomic and cardiac function.

Mice received daily intraperitoneal injection of the D3 agonist Rotigotine (0.05, 0.5, or 5 mg/kg/day) for 4-5 weeks and blood pressure was monitored 5x / week using a non-invasive tail cuff measurement system. Ultrasound echocardiograms were performed before and after treatment, to determine cardiac function parameters.

We observed, at the end of the 5-week treatment period, a dose-dependent decrease in heart rate that was associated with an increase in systolic and diastolic blood pressure and a decrease of ejection fraction and fractional shortening. However, during the first 2 weeks of treatment, high Rotigotine significantly decreased both systolic blood pressure and heart rate. This points to a bi-modal regulation of D3R pathways in controlling autonomic function.

As D3Rs are often functionally linked to the D1R system, we have begun to explore if interactions between D1Rs and D3Rs may be involved in the regulation of autonomic changes with age, rather than D3Rs alone.

Supported by UCB Pharma (IIS-2014-101512)

Functional Brain Imaging of Multisensory Integration During Computerized Dynamic Posturography in Older Adults Using Functional Near-Infrared Spectroscopy (fNIRS)

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Previous functional near-infrared spectroscopy (fNIRs) studies have suggested that young adults activate the temporo-parietal vestibular cortex during a balance task requiring primarily vestibular feedback control.(Karim et al., 2013) The purpose of this study was to examine what brain regions are activated in older adults during sensory integration balance tasks. Fifteen community-ambulating healthy older adults (mean age: 73 ± 5y; 7 male) participated in this study. A Smart Equitest™ platform was used to provide the sensory integration test conditions. A 32-channel continuous wave fNIRS instrument was used to record hemodynamic changes bilaterally over the dorsolateral frontal cortex, temporo-parietal cortex, and occipital cortex. An A–B–A block design (baseline-test-baseline) was used to elicit changes in balance control while subjects maintained balance during four pairs of sensory integration balance test conditions: 1) Eyes Open (EO) while standing on Fixed platform (Fixed) - Sway-referenced platform (SR) - Fixed platform; 2) Eyes Open in the Dark (EOD) during Fixed – SR – Fixed; 3) Fixed platform during EO–EOD–EO; and 4) SR platform during EO–EOD– EO. Each block lasted 40 seconds, and the four test conditions were randomly presented. The root-mean-square (RMS) of the center of pressure (COP) from the two baseline blocks was compared with the test condition. A repeated measures ANOVA was used to test differences in COP magnitude among the sensory integration conditions. fNIRS data were analyzed based on a spatial-temporal version of a general linear model. The greatest changes in COP occurred when the platform changed from Fixed to SR, both during EO and EOD. However, these conditions elicited relatively small changes in fNIRS activation. The most significant change in activation occurred during the transition from EO to EOD while standing on the SR platform. The locus of activation was centered in the supramarginal gyrus. In contrast with young adults, the older adults did not demonstrate a large change in activation when vestibular only control was produced with constant removal of visual input (Karim et al., 2013). These differences may reflect age-related changes in sensory weighting.

Shared Neural Substrates of Species Recognition Between Parental and Parasitic Songbirds

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In many social animals, early exposure to conspecific stimuli is critical for the development of accurate species recognition. For example, songbirds rely on conspecific 'tutors' for appropriate species-specific song development. Obligate brood parasitic birds, however, forego parental care and young are raised by heterospecific hosts. Having evolved from a non-parasitic ancestor, how do brood parasites recognize their own species? Studies of non-parasitic parental songbirds (e.g. zebra finch) have revealed that the primary and secondary auditory forebrain areas are critical in the differential processing of conspecific vs. heterospecific songs. Here we evaluate whether the same auditory brain regions underlie species recognition in adult pin-tailed whydahs (*Vidua macroura*), a brood parasitic songbird that is sister-taxa to non-parasitic estrilid finches, including the model species zebra finch (*Taeniopygia guttata*). First, we found captive whydahs to exhibit a greater behavioral response (vocal and movement) to conspecific vs heterospecific (zebra finch) song playbacks. Using functional magnetic resonance imaging (fMRI), we detected an increase in the mean volume of the blood oxygenation level dependent (BOLD) response to con- vs. heterospecific songs within the auditory forebrain. Finally, we found greater expression of the immediate early gene ZENK (zif268, egr-1, ngfi-a, krox24) within the auditory forebrain following exposure to con- vs heterospecific songs. Our study demonstrates that neural activation is located within similar forebrain regions as those of parental songbirds. The evolutionary transition to brood parasitism, therefore, likely involved changes to existing proximate mechanisms– "evolutionary tinkering"–rather than wholesale reworking of neural substrates for species recognition in songbirds. Thus, developmental shifts of experience-dependent neuroplasticity likely enable brood parasites to recognize their own species and avoid (mis)imprinting on the host's phenotype.

Temporal Changes in LPS-Induced Microglial Cell Activation in Adult and Aged Mice

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The brain's resident immune cells, microglia, can express distinct forms of activation including the classic pro-inflammatory (M1) and alternative anti-inflammatory and neuroprotective (M2) phenotypes. Prior work has shown that at distinct time-points after a stroke or traumatic brain injury microglia express different phenotypes, indicating a time-dependent shift in activation (Hu et al., 2012; Wang et al., 2013). Further, normal aging has been shown to prime microglia towards the M1 phenotype. As a result, aged subjects show an abnormal neuroinflammatory response to an immune challenge when compared with adults. Presently unknown is how these age-related changes in microglia activation differ across time. The current study evaluated whether young and aged mice show differential temporal profiles of microglia activation following an immune challenge. Aged (18-20 months) and young (4-5 months) female C57BL/6J mice were administered a single intraperitoneal injection of the bacterial endotoxin lipopolysaccharide (LPS) or saline. Alterations in locomotor and anxiety-like behavior were assessed 4, 24, 48, 72, or 168 hours following LPS or saline exposure. Immediately following behavioral testing, hippocampal samples were collected. Analysis of hippocampal gene expression data indicates that normal aging impacts the onset and duration of the M1 and M2 responses following LPS exposure.

Social Status-Dependent Regulation of an Identified Brain Circuit in Zebrafish

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In zebrafish (*Danio rerio*), social interactions between adult males consist of a series of aggressive encounters that ultimately lead to the formation of stable hierarchies of either socially dominant or subordinate animals. Although it has been shown that social status leads to neurophysiological changes in brain structure and function, our understanding of how identified brain circuits are modulated by social status in vertebrate model systems is limited. The activation pattern of the Mauthner neural circuit, which mediates the startle escape response in zebrafish, is likely affected by social experience through the regulation of dopamine synthesis and receptor activity. Injection of exogenous L-Dopa modulates the Mauthner escape circuit in a social status dependent manner, with an enhancement of response in dominants and an inhibition of response in subordinates. The dopaminergic system is also modulated on a transcriptional level, with social status-dependent regulation of dopamine reuptake (dat) and receptor expression (drd1b receptor). In addition, threshold for an animal's swimming behavior is influenced by social status. Taken together, this implies that the balance in the activation of the underlying neural circuits mediating escape and swimming behaviors is modulated in a social-status dependent manner.

GMCSF-MOG Induces Myelin-Specific Regulatory T-Cells that Inhibit Experimental Autoimmune Encephalomyelitis (EAE) in Mice

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FoxP3^{+} CD25^{high} regulatory T-cells (Tregs) play a crucial role in maintaining peripheral tolerance by suppressing auto-reactive T-cells. Developing a safe therapeutic approach to induce autoantigen-specific Tregs could provide an effective treatment for Multiple Sclerosis (MS), as well as for other autoimmune diseases. Previous studies have shown that the fusion protein GMCSF-MOG has tolerogenic activity that inhibits experimental autoimmune encephalomyelitis (EAE) in mice. In an effort to elucidate the mechanism by which GMCSF-MOG is inhibiting EAE, we investigated the ability of GMCSF-MOG to induce Tregs in vivo. In this study, we provide evidence that GMCSF-MOG can induce MOG-specific FOXP3^{+} Tregs. Treatment of 2D2-FIG mice with GMCSF-MOG resulted in an increase of FOXP3^{+} Tregs in the blood, with an additional increase observed after multiple immunizations. Furthermore, the depletion of GMCSF-MOG-induced Tregs by use of the anti-CD25 mAb PC61 restores susceptibility to EAE in C57BL/6 mice. In conclusion, subcutaneous immunization with GMCSF-MOG induces antigen- specific Tregs, which play a role in the inhibition of EAE in mice.

Efficacy of a Virtual Reality-Based Balance Perturbation System

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Postural stability requires the successful integration of multiple senses including vision, proprioception and vestibular inputs. Mild traumatic brain injuries (mTBI) are associated with postural instability and studies have shown that balance following an mTBI remains disturbed up to 6 months later in quiet standing and up to 1 year in dynamic tasks such as walking. It has been proposed that these balance dysfunctions are the manifestation of poor integration of the sensory system in determining appropriate motor output to maintain postural stability. Few methods of sensorimotor integration training exist and each of these methods must be done in a clinic, limiting the amount of rehabilitation training that can take place as no in-home solution currently exists. The purpose of this study was to examine a virtual reality-based system for training sensorimotor integration through a balance perturbation paradigm. It was hypothesized that the virtual reality training modality would alter center of pressure patterns in healthy young adults.

METHODS: Center of pressure (CoP) of 30 participants in four different visual conditions (eyes open, eyes closed, moving dot with no sound, and moving dot with sound) was measured with a modified Wii Balance Board. IKKOS goggles that displayed the dot and emitted an auditory clicking sound were worn during these two conditions. Mediolateral, anteroposterior, and total excursions were calculated.

RESULTS: There were significant differences between the mean anteriorposterior ($p < .001$) and mean total CoP ($p < .001$), but not for mean mediolateral CoP ($p = .469$). Post-hoc analyses revealed that the eyes open condition exhibited smaller mean CoP excursions than the other conditions ($p < .05$). The largest CoP excursions occurred during the eyes closed condition.

DISCUSSION: The current findings demonstrated that a virtual reality-based system is capable of perturbing balance in healthy young adults. Specifically, our data show that balance is enhanced with vision, regardless of the focal visual input. The auditory stimulus was not associated with any changes in CoP excursions suggesting that auditory perturbation does not affect postural stability. The findings of this study suggest that the IKKOS virtual reality-based system may be a successful home balance training modality.

Prevention of Synaptic Loss in Alzheimer's Triple-Transgenic Mouse Model with miRNA-431

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Alzheimer's Disease (AD) is currently the most prevalent form of dementia. With synaptic loss as the primary characteristic, AD not only results in memory loss, but in vital function degradation and eventually fatality. Ongoing research continues to accumulate evidence of excessive accrual of amyloid-β (Aβ) peptides in brain tissue as the probable source. Recent research in mouse models has indicated that the buildup of Aβ initiates the increased expression of Dickkopf-1 (DKK1) protein, an antagonist of Wnt signaling pathway. The intent of this study was to test the application of microRNA-431(miR-431) as a means of preventing further synaptic loss by targeting Kremen1, the DKK1 receptor. The Kremen1 receptor site targeting miR-431, as well as DKK1, and AβDDL (an amyloid-β derived diffusive ligand) were administered to cortico-hippocampal cultures isolated from 3x transgenic AD (3xTg) and wild type (WT) mice (control) of varying ages (3-9 months). Each cortico-hippocampal culture was then prepared for immunofluorescent imaging. 15-20 images were taken of independent neurons in each coverslip and analyzed. The number of pre- and postsynaptic puncta as well as length of axons and number of branches was then recorded and compared. Exposure to DKK1 and AbDDL reduced the number of pre- and postsynaptic puncta in 3x Tg as well as in WT cultures of the 3 month old mice .Treatment with miR-431 showed significant rescue of the synaptic sites (Synapsin1:control- 1.8±0.27, DKK1- 1.33±0.23, DKK1+miR431- 2.53±0.25 puncta/100um, $p<0.005$. PSD95: control- 1.43±0.0.41, DKK1- 0.78±0.11, DKK1+miR431- 1.78±0.28,puncta/100um, $p<0.01$). Similar results were observed on 6 and 9 month old mice. Analysis of the axonal length and number of branches showed that application of DKK1 or AbDDL significantly reduced the length of the axons and number of the branches. Treatment with miR-431 reversed the effect for the axonal length but did not affect the amount of branches (Axon's length: control-99,97±4.97um, AbDDL-63.97±5.64, AbDDL=miR-431-97.87±5.34 um,$p<0.0005$). These findings demonstrate that miR-431 can protect synaptic sites and axons from Aβ-toxicity in AD mouse model.

Behavioral Inhibition and Resting Frontal Alpha Asymmetry Are Related to Sleep Quality

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Approximately 50-70 million adults in the U.S. are living with a sleep-wake disorder, resulting in neurocognitive dysfunction, impaired daily functioning, and morbidity. Previous research has employed the Reinforcement Sensitivity Theory (RST) to explore the individual differences associated with sleep-related disorders. RST consists of neurophysiological systems with distinct neural pathways that direct approach and withdrawal behaviors, including the Behavioral Activation (BAS) and Behavioral Inhibition (BIS) Systems. BAS is associated with left frontal alpha activity (measured through EEG) and approach behavior, while BIS is associated with right frontal activity and withdrawal behavior. Prior research in our laboratory has related elevated BIS to non-adherence to CPAP treatment in an obstructive sleep apnea patient population. Moving forward, the relationships between BIS, BAS, baseline resting frontal alpha asymmetry, and self-reported sleep quality using the Pittsburgh Sleep Quality Index (PSQI) were examined in 75 university students. It was hypothesized that greater right than left baseline resting frontal asymmetry scores would be significantly related to poorer sleep quality. Further, it was predicted that BIS would be positively associated with the PSQI, while the opposite relationship would be observed for BAS. While associations were observed, they were opposite as hypothesized. Greater left activity was associated with the PSQI for FP1, $r(52) = .408$, $p < .01$), F7, $r(53) = .477$, $p < .01$), FT7, $r(52) = .362$, $p < .01$), and FC3, $r(50) = .328$, $p < .05$) when compared to homologous right hemisphere scalp sites. Further, BIS was weakly associated with the PSQI, $r(75) = -.246$, $p < .05$), while no association was observed for BAS. These results, however, do indicate that sleep quality is associated with self-reported behavioral inhibition and frontal EEG asymmetry. Implications for these findings are discussed.