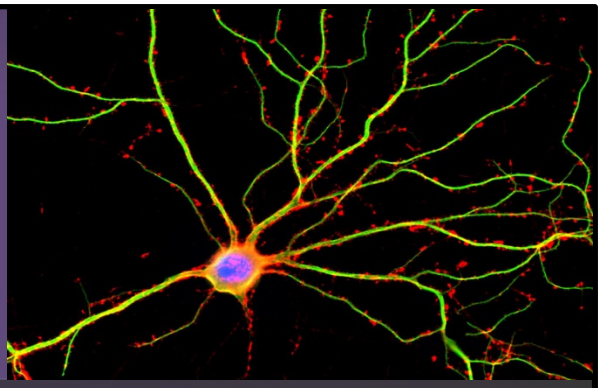
Eastern Carolina Chapter of the Society for Neuroscience Presents:



21st Annual Neuroscience Symposium
Catalyst for Collaboration

Featuring:

V. Reggie Edgerton, PhD

Distinguished Professor

University of California—Los Angeles

"Basis for a Much Higher Expectation for Greater Functional Recovery Following Chronic Spinal Injury"

Thursday, October 31st, 2019

East Carolina Heart Institute

www.ecu.edu/neurochapter

ECCSfN

Funding for this event was made possible by contributions from:

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SfN Society for Neuroscience — Advancing the Understanding of the Brain and Nervous System

The Eastern Carolina Chapter of the Society for Neuroscience would like to express our sincere gratitude to the following entities for their generous support of the 2019 Neuroscience Symposium:

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21st Neuroscience Symposium Schedule
October 31st, 2019 | East Carolina Heart Institute | 115 Heart Drive, Greenville, NC 27834

October 30th **5:00-7:00 Pre-Event Social at Pitt Street Brewing** with food (except alcohol) courtesy of the chapter and its sponsors. Please RSVP through the symposium registration link: https://ecu.az1.qualtrics.com/jfe/form/SV_3n5ztQafss3N1hb.

October 31st

8:00-3:35 **Registration**

8:30-10:15 Atrium **Poster Session 1 / Coffee**

10:15-11:30 Conference Room **Graduate Student and Postdoc Presentations**

10:15-10:40 Taylor Landry, PhD Candidate, Dr. Hu Huang's lab
Central α-klotho is a Novel Regulator of Arcuate Neuron Populations and Energy Metabolism in Mice

10:40-11:05 Helen Rodgers, Postdoctoral Fellow, Dr. Brewer and Clemens labs
Dopamine Receptor Modulators as Adjunct Therapy for Chronic Neuropathic Pain

11:05-11:30 Heath Partington, PhD Candidate, Dr. Jeff Eell's lab
The Role of Nurr1 in Environmental Regulation of the Dopaminergic Phenotype

11:40-11:45 **Welcome from Outgoing ECCSfN President:** Alexander Murashov, MD, PhD

11:45-12:00 **Opening Remarks**: S. Russ Price, PhD, Associate Dean for Research and Graduate Studies, BSOM, ECU

12:00-1:00 **Keynote Address:** V. Reggie Edgerton, PhD, Distinguished Professor, UCLA
Basis for a Much Higher Expectation for Greater Functional Recovery Following Chronic Spinal Injury

1:00-2:45 Atrium **Poster Session 2 / Hors d'oeuvres**

2:45-4:00 Conference Room **Faculty Presentations**

2:45 – 3:10 Jessica Ellis, PhD, Department of Physiology
Acyl-CoA Synthetase 6 Mediates Docosahexaenoic Acid (DHA) Enrichment and Neuroprotection

3:10 – 3:35 Tonya Zeczycki, PhD, Department of Biochemistry
Dancing with Myself: Alterations in Alpha-Synuclein Dynamics Increases Self-Oligomerization Tendencies

3:35 – 4:00 Stefan Clemens, PhD, Department of Physiology
D3 and D1 Receptors: The Yin and Yang in the Treatment of Restless Legs Syndrome with Dopaminergics

4:00-4:15 **Closing Remarks and Awards from Incoming ECCSfN President:** Fadi Issa, PhD

Podium Presentations

(in order of appearance)

Central α-klotho is a Novel Regulator of Arcuate Neuron Populations and Energy Metabolism in Mice

Taylor Landry, PhD Candidate

Department of Kinesiology
East Carolina Heart Institute
East Carolina University

B*ackground/Aims:* α-Klotho is a circulating factor with well-documented anti-aging properties; however, the central role of α-klotho in metabolism remains largely unexplored. In this study, we aimed to investigate the potential novel role of central α-klotho to regulate energy and glucose homeostasis via arcuate hypothalamic neurons.

Methods: Human cerebrospinal fluid (CSF) concentrations of α-klotho were determined by ELISA kit. Central administration of α-klotho was performed by using intracerebroventricular (ICV) injection for seven days in healthy, diet-induced obesity (DIO), and streptozotocin-injected (STZ) mice. Central inhibition of α-klotho was performed by ICV anti-α-klotho antibody administration for seven days in healthy mice. Electrophysiology and immunofluorescence experiments were performed to investigate the effects of α-klotho on ARC neurons. hrNPY-GFP reporter mice were used to investigate NPY/AgRP neuron activity, and immunohistochemistry was used to investigate POMC neuron activity and markers of cell proliferation. To determine the molecular mechanisms of hypothalamic α-klotho, we used the immortal hypothalamic GT1-7 cell line *in vitro* and ICV administration of fibroblast growth factor receptor (FGFR) antagonist *in vivo*.

Results: A strong negative correlation was observed between human CSF α-klotho concentrations and body weight (10 male, 12 female, 18-83 years old, BMI's: 19-32). Central α-klotho administration decreased food intake, reduced body weight, improved glucose clearance, and increased insulin sensitivity in mouse models of metabolic disease, but only suppressed food intake in healthy mice. Conversely, central anti-α-klotho administration impaired glucose clearance, but had no effects on food intake. Electrophysiology and immunofluorescent staining revealed α-klotho suppresses NPY neuron activity, increases POMC neuron activity, and promotes ARC cell proliferation. The effects of α-klotho on NPY/AgRP neurons were, at least partially, due to increased magnitude of mIPSC's. Mechanistically, α-klotho blunted serum-starvation-induced AgRP gene expression and increased phosphorylation of $ERK^{44/42}$, AKT^{ser473}, and $Foxo1^{ser256}$ in GT1-7 cells. These downstream effects were abolished by pretreatment with inhibitors of either FGFR1 or PI3kinase. Furthermore, α-klotho-mediated suppression of food intake and improvements in glucose clearance were blunted in response to central inhibition of FGFR signaling, while effects on NPY/AgRP neurons were completely abolished.

Conclusion: These results indicate a prominent role of the hypothalamic α-klotho-FGFR-PI3K signaling axis in the regulation of ARC neuron activity, energy balance, and glucose homeostasis, thus providing new insight into homeostatic and disordered control of metabolism.

Dopamine Receptor Modulators as Adjunct Therapy for Chronic Neuropathic Pain

Helen Rodgers, PhD
Postdoctoral Scholar

Emergency Medicine
Brody School of Medicine
East Carolina University

Pain management is a global health crisis. Current pharmaceutical options have side effects and major safety concerns including tolerance, dependence and addiction. Additionally, these pharmaceuticals are not effective in treating all types of pain. Two thirds of neuropathic pain patients receive inadequate pain relief drastically reducing their quality of life. Opioids represent a powerful and effective analgesic class of drugs for acute pain but are often ineffective for neuropathic pain and have major long-term use issues. The endogenous opioid system and the dopaminergic system have significant overlap and functional links. Therefore, we sought to leverage this connection by using dopaminergic receptor modulators as adjunct therapy to improve opioid analgesia and attenuate the undesirable safety concerns. Using a rat spinal cord injury model of neuropathic pain, we show restored morphine analgesia when using a dopamine D1R antagonist or D3R agonist as an adjuvant in animals that were morphine resistant. In addition, both combinations were capable of reducing the rewarding effects of morphine. Assessing these dopamine receptor modulators in healthy animals showed doses of morphine that did not provide analgesia on their own when given in combination with the dopamine receptor modulators produced a significant analgesic benefit. Chronic administration studies revealed the D1R antagonist and D3R agonist prevented morphine tolerance and reduced the duration of withdrawal symptoms with drug cessation. These preclinical results suggest that using dopamine receptor modulators as an adjunct with morphine may be significantly beneficial for clinical pain management of chronic pain including treatment resistant neuropathic pain.

The Role of Nurr1 in Environmental Regulation of the Dopaminergic Phenotype

Heath Partington, PhD Candidate

Department of Anatomy & Cell Biology
Brody School of Medicine
East Carolina University

The nuclear receptor Nurr1 is necessary, in ventral midbrain dopamine neurons, for development and maintenance of dopamine neurotransmission. Mutations in Nurr1 have been implicated in various dopamine related neurologic and neuropsychiatric disorders including addiction, depression, schizophrenia and Parkinson's disease. Based on previous research, environmental stimuli in adult mice, such as stress, mating, exercise, environmental enrichment, and changes in circadian cycle, can increase or decrease the number of dopamine neurons. For example, 14 days of exercise and environmental enrichment increases the number of midbrain dopamine neurons by ~15%. Additionally, 7 days in a male-female pair increases dopamine neurons in males but decreases them in females. Based on this data, our hypothesis is that Nurr1 will be important in this process of changing the dopamine neuron phenotype, with reduced Nurr1 attenuating induction of the dopamine neuron phenotype and exacerbating the loss of the dopamine neuron phenotype. To test this hypothesis, Nurr1-null heterozygous and wild-type mice were divided into treatment groups consisting of exercise (access to running wheel and running for 14d) and control mice and another treatment group consisting of mice placed in male/female pairs or control mice in same sex pairs for 7 d. Tissue was collected and the number of dopamine neurons, based on tyrosine hydroxylase immunoreactivity, will be measured. Using images acquired with the Celldiscover7, the number of labeled neurons across treatment groups will be counted. For accurate, unbiased cell counts, images will be collected in Z-stacks of the middle 80% of the measured thickness of each section in order to avoid overlap. Using Zeiss image deconvolution and extended depth of focus, we can obtain near-confocal quality images for cell counts. Based on preliminary data, we have identified a subpopulation of neurons in the ventral midbrain in wild-type mice that are Nurr1 immunoreactive ($Nurr1^{IR}$) but lack tyrosine hydroxylase immunoreactivity ($TH^{negative}$). Current studies are ongoing, but this data suggests that these $Nurr1^{IR}/TH^{negative}$ neurons may represent a latent population of dopamine neurons that have the potential to be induced to a dopamine phenotype. Understanding the molecular mechanisms associated with how dopamine neurons change their phenotype could be useful for treatment of diseases associated with dopamine neurotransmission.

Acyl-CoA Synthetase 6 Mediates Docosahexaenoic Acid (DHA) Enrichment and Neuroprotection

Jessica M. Ellis, PhD

Assistant Professor
Department of Physiology
Brody School of Medicine
East Carolina University

The omega-3 fatty acid, docosahexaenoic acid (DHA), is enriched in the central nervous system and thought to protect against neurological dysfunction. Yet, understanding the role(s) of DHA metabolism have remained unclear because of a lack of defined biochemical mechanisms regulating DHA enrichment. We recently discovered a critical regulatory node in DHA biochemistry by targeting long chain acyl-CoA synthetase 6 (*Acsl6*). ACSL6 is an enzyme that initiates cellular fatty acid metabolism and is enriched in the CNS. Genetically deleting *Acsl6* in mice resulted in large and specific reductions (35-72%) in DHA-containing phospholipids across the central nervous system. In the eye, we find slow, progressive retinal degeneration which presents early with increased gliosis then age-dependent thinning of the photoreceptor layer and reduced rhodopsin protein abundance. In the brain, Acsl6 deficiency manifests in early-onset aging-like neuropathology with increased gliosis accompanied by behavior abnormalities that include hyperactivity and reduced response to sensory stimuli. To test the role of Acsl6 in neuroinflammation, an acute LPS inflammatory challenge was employed. Acsl6 deficiency mice had increased gliosis at baseline and this increase is maintained over the time course after LPS exposure. Unlike reported DHA diet studies, we were surprised to discover that LPS-induced hippocampal pro-inflammatory cytokine expression and abundance of lipid inflammatory-mediators did not differ from controls. Taken together, these data suggest that loss of Acsl6-mediated fatty acid metabolism results in chronic neurological stress, subsequent adaptations, retinal degeneration, and impairments in motor function.

Dancing with Myself: Alterations in Alpha-Synuclein Dynamics Increases Self-Oligomerization Tendencies

Tonya Zeczycki, PhD

Assistant Professor
Department of Biochemistry and Molecular Biology
Brody School of Medicine
East Carolina University

A hallmark of Parkinson's disease (PD) is the formation of protein oligomers in the brain comprised almost entirely of the intrinsically disordered protein, α-synuclein. Toxic α-synuclein oligomers lead to neuronal death and contribute to cognitive and mental decline in PD. The molecular pathways leading to toxic α-synuclein oligomer formation, however, are not well defined. Little is currently known as to how this natively disordered protein adopts and maintains the highly structured, organized oligomeric species observed in the early stages of the disease; however, the conformational plasticity and underlying biophysical characteristics of this enigmatic protein are most likely key to its pathogenic activities. Contributing to α-synuclein oligomerization are numerous environmental conditions and both genetic and cellular processes. For example, the propensity for α-synuclein to form soluble and insoluble oligomers significantly increases after Transglutaminase 2 (TG2)-mediated post-translational modification. TG2-mediated inter- and intramolecular crosslinking, and polyamidation of α-synuclein results in increased amounts of α-synuclein oligomers in vivo, effectively correlating increased TG2 activity with increased PD pathology; however, in the context of PD, the biophysical consequences of both these modifications and subsequent modification-induced α-synuclein misfolding on oligomerization remains poorly understood. Using recombinantly expressed α-synuclein and a battery of biophysical and mass spectrometry techniques, we show that alterations in α-synuclein's inherent biophysical characteristics due to TG2-mediated post-translation modifications ultimately leads to increased oligomerization. This research is not only important to unraveling the complex molecular underpinnings of toxic protein oligomer formation in PD and other related synucleinopathies but may also open new avenues of therapeutic design specifically targeting toxic α-synuclein conformations.

D3 and D1 Receptors: The Yin and Yang in the Treatment of Restless Legs Syndrome with Dopaminergics

Stefan Clemens, PhD, HdR

Associate Professor
Department of Physiology
Brody School of Medicine
East Carolina University

Dopaminergic treatments targeting the D3 receptor subtype to reduce the symptoms of Restless Legs syndrome (RLS) show substantial initial clinical benefits but fail to maintain their efficacy over time. Sensorimotor circuits in the spinal cord are the gateway for the sensory processing of the symptoms and critical for the associated leg movements that relieve the symptoms and the periodic limb movements that often develop during sleep. There is a high preponderance of the inhibitory D3 receptor in the sensory-processing areas of the spinal cord (dorsal horn), whereas the motor areas in the ventral horn more strongly express the excitatory D1 receptor subtype. D3 and D1 receptors can form functional heteromeric ensembles that influence each other. In the spinal cord, long-term treatment with D3 receptor agonists is associated with the upregulation of the D1 receptor subtype and block of D1 receptor function at this stage can restore the D3 receptor effect. A model emerges that proposes that the behavioral changes in RLS, while responsive to D3 receptor agonists, may be ultimately be the result of unmasked increased D1-like receptor activities.

Recognition of Student Awards

In honor of Dr. Larry Means and Dr. Edward Lieberman, ECCSfN proudly recognizes the valuable service and efforts of both former ECU faculty in championing neuroscience at East Carolina University. For many years, student presenters at the Annual Neuroscience Symposium have competed for Best Undergraduate and Graduate Poster awards. In 2018, we were delighted to officially name these awards after each scholar. We hope that you are able to enjoy the accompanying biographies written about them.

Larry Means, PhD
Author: Tuan Tran, PhD

Dr. Larry Means grew up in Portland, Oregon and shortly after he retired from ECU in 2005, returned to his home state to live a life enriched with the splendor of the mountainous outdoors.

Larry earned his PhD from Claremont Graduate University, the oldest all-graduate institution in the United States, in 1968. He then completed a two-year postdoctoral fellowship under the mentorship of Bob Isaacson, specializing on hippocampal function, at the University of Florida Medical School.

He would then spend the rest of his career at ECU (1970– 2005), where he landed in the Department of Psychology, promoting research and instruction that emphasized the neurobiological underpinnings of behavior. In the early 1990s, he collaborated with Dr. Edward Lieberman to establish both an undergraduate and graduate neuroscience program at ECU. The undergraduate neuroscience program was housed in the Department of Psychology and the graduate program was housed in the Department of Physiology.

Larry was the impetus behind an undergraduate neuroscience program with very humble beginnings. The program experienced an admission rate of 8-10 students per year, offering courses across the scientific disciplines and humanities, involving students in a journal club, and securing honoraria for renowned speakers. He envisioned a program that provided undergraduate students a pipeline towards graduate school and medical school, by attracting ambitious and talented students that desired a challenging and multidisciplinary approach to learning. His legacy lives on today, as the undergraduate neuroscience program experiences an admission rate of 20-25 students per year, has a student body comprised of 90+ majors and 25+ minors, shows strong leadership with the Neuroscience Student Association, offers a well-organized Neuroscience Seminar Series, and engages intellectual discussion in a weekly Journal Café.

Dr. Larry Means was an indeed a stalwart of neuroscience and his work lives on today as reflected in student learning and research, the annual symposium, and interdisciplinary collaboration at ECU.

Edward Lieberman, PhD
Author: Kori Brewer, PhD

Dr. Edward Lieberman represented the first generation of neuroscientists at East Carolina, helping to establish the medical school's Department of Physiology as a founding member in 1976. A native of Lowell, MA, Dr. Lieberman obtained his B.S. from Tufts University and his M.S. from University of Massachusetts before heading south to complete his Ph.D. training at the University of Florida. He then spent 2 years at the University of Uppsala as a Swedish Medical Research Council Postdoctoral Fellow.

Dr. Lieberman was a pioneer in the field of neuron-glia interactions, using the crayfish medial giant nerve fiber as his preferred model system of study. His earliest publication on the topic appeared in *Science* in 1967 with his final manuscript appearing the year of his retirement in 2006.

Throughout his long career at ECU, Dr. Lieberman was a champion for the neurosciences. His desire to unite neuroscientists and drive collaboration drove him to develop and direct ECU's first Annual Neuroscience Symposium in 1998, an event that he continued to attend well beyond his retirement and that serves as the basis for today's ECCSFN symposium. Prior to retiring, Dr. Lieberman dedicated much of his time to constructing a proposal to establish a Center for the Neurosciences at ECU. While never fully realized, the results of his efforts are evident today in the ever-expanding, interdepartmental, neuroscience community at ECU.

Poster Session 1 Research

(presenters are in alphabetical order)

Vinclozolin and Neurodevelopmental Disorders

Ameera, Afifi, Ibrahim, Reenad, McCoy, Krista

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

The increase in neurodevelopmental disorders, such as autism, has in some cases been attributed to embryonic exposure to pesticides and other pollutants. Currently, there are no approved prenatal supplement that can protect the developing fetus from environmental contaminants. However, sulforaphane derived from cruciferous vegetables such as broccoli, augments naturally occurring cytoprotective mechanisms and has been proposed as an ideal prenatal supplement to protect the fetus from containments. Therefore, sulforaphane should protect the developing brain from toxicant-induced neurodevelopmental disorders. To evaluate this idea, we tested whether the pesticide vinclozolin induces in autism-like brain morphologies and whether sulforaphane can protect vinclozolin exposed fetuses from these neuronal defects. To test these hypotheses, we exposed pregnant mice to vinclozolin, vinclozolin and sulforaphane, or to the corn oil control. The pregnant dams were exposed at embryonic day (E) 13.5 to E16.5 and were humanely sacrificed at E18.5. Heads from the embryonic male were decalcified in formic acid, dehydrated through a series of alcohol baths, and embedded in paraffin. Heads were coronally sectioned at a section thickness of 15 micrometers. Brain tissue were then stained with Hematoxylin and Eosin. We then evaluated the number of cells within the hippocampus, a brain structure that is significantly involved in memory that defines appropriate “scripts” used in social interactions. The number of pyramidal cells within the CA1 region of the hippocampus, was counted using Stereologer by Microbrightfield Bioscience. In this poster presentation we will present the validated methodological approach that we will use to evaluate our specimens.

Pursuing Parkinson's: Characterization of the Newborn Mouse Dopamine Neuron Phenotype

Samantha Barker, J. Makenzie Nutter, Heath Partington, Jeffrey B. Eells

Department of Anatomy and Cell Biology, Brody School of Medicine, East Carolina University, Greenville, NC

Loss of dopamine (DA) in the midbrain has been associated with Parkinson's Disease and further investigation is needed to understand how the changing dopamine phenotype is implicated in this disorder. Based on previous research in adult mice, DA neurons may be able to switch on and off the dopamine phenotype in response to environmental stimuli. Using markers essential to the dopamine phenotype, such as dopamine transporter (DAT) and tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, we can differentiate between current and former dopamine neurons in the midbrain. Prior research in our lab has revealed that in adult transgenic DATCre/YFP mice, current dopamine neurons are characterized as neurons that express both YFP and TH. Former dopamine neurons are YFP positive and TH negative, which indicates that the neuron is not actively producing dopamine but did at one point. In this study, newborn mice transgenic for DATCre/YFP were used to further demonstrate the nature of this changing phenotype. Since the dopamine neuron phenotype in adult mice is regulated by environmental stimuli, we hypothesized that all neurons in a newborn mouse positive for YFP will also be positive for TH, indicating that all neurons expressing DAT are also actively producing dopamine, since newborn mice have not yet been exposed to environmental stimuli (blind and deaf at time of birth). Our results show that newborn mice have a densely packed ventral tegmental area (VTA) and substantia nigra (SN) compared to adult male controls. As expected, nearly all YFP positive neurons are also TH positive (~95%), with only a few YFP positive, TH negative neurons. In adult mice, however, approximately 53% of the YFP positive neurons are also TH positive. This supports our hypothesis that environmental stimuli influence the dopamine phenotype and further investigation is essential to understand the mechanisms behind how DA neurons change in neurological disorders such as Parkinson's disease.

The Effects of Social Status on the Morphology of Hypothalamic Dopaminergic Neurons

Heagy, Faith, Blain, Elena, Clements, Katie, Issa, Fadi A.

Department of Biology, East Carolina University

Social interactions have profound effect on the physiology and behavior of many animals. However, the cellular mechanisms of how social dominance is reflected cellularly remains unknown. Our aim is to investigate how social dominance influences the morphological architecture of hypothalamic dopaminergic (DA) nuclei known to regulate locomotor behaviors using zebrafish as a model organism. When paired, zebrafish establish social relationships that consist of a dominant and submissive fish. Many of the sensory social cues (i.e. visual, tactile, and olfactory) are relayed and integrated in the hypothalamic dopaminergic posterior tuberculum nucleus (PTN) making it a prime target to examine the effects of social dominance on its morphological plasticity during social contests. Moreover, the PTN is known to project caudally into the spinal cord and innervate spinal circuits involved in locomotor behavior. We tested the hypothesis that Dominant zebrafish have higher expression of DA neurons compared to Subordinates. To test this hypothesis, we used the transgenic zebrafish line *Tg(dat:eGFP)* to examine whether social dominance affects the number of DA neurons within the PTN. Adult male zebrafish were isolated for one week, then paired for two weeks in order to establish stable social dominance relationships. After pairing, the brains were extracted, fixed, imbedded in O.C.T compound and frozen. This was followed by slicing of brain tissue at a 30 um slice thickness. As a control, we sampled brain tissue from animals that were housed communally. The slides were then imaged using confocal microscopy. The Imaris software was used to quantify the number of dopaminergic cells in the hypothalamic regions: PTN, A10, PVOPB, and PVOPA. The Results show that Dominant fish have a higher number of DA neurons in the PTN region compared to Subordinate fish but there were no significant differences compared to communal animals. Further, the number of DA neurons in Subordinates were significantly lower compared to communal animals. The results suggest that the number of DA neurons with the PTN is socially regulated. Our results suggest that the PTN is prone to morphological plasticity during competition for social dominance and these morphological adaptions may underlie behavioral differences in locomotor activity.

Endocannabinoid Regulation of Excitatory Synapses in IPSC Derived Cortical Spheroids

Alexis Papariello[1], David Taylor[1], Ken Soderstrom[1], and Karen Litwa[2]

[1]Department of Pharmacology and Toxicology, Brody School of Medicine at East Carolina University, Greenville, NC
[2] Department of Anatomy and Cell Biology, Brody School of Medicine, Brody School of Medicine at East Carolina University, Greenville, NC

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder that is characterized by social deficits, behavioral abnormalities, and disrupted stimuli processing. At the cellular level, there is often an imbalance of excitatory and inhibitory neuronal synapses as well as defective synaptic pruning. Induced pluripotent stem cell (IPSC) derived neurons from ASD patient fibroblasts exhibit altered an increased ratio of excitatory to inhibitory synapses. Changes to the endocannabinoid system (ECS), a global regulator of synaptic plasticity, may play a role in producing ASD during early fetal development. The ECS decreases presynaptic neurotransmitter release thru the activation of Gi-coupled CB1 receptors by the endocannabinoids 2-AG and AEA among other cannabinoid agonists. Preliminary RT-PCR results indicate that MAGL, the enzyme that metabolizes 2-AG, has increased mRNA levels in ASD patient-derived neurons relative to controls. We are thus investigating MAGL to determine if it is a mediator of excitatory dysregulation in ASD patients.

Preliminary mRNA evidence suggests comparable levels of CB1 receptor expression in control and ASD patient-derived neurons. Because of this, we believe there is less 2-AG-mediated agonist effect on CB1 and thus a decrease in the amount of Gi-coupled inhibitory effect on the neuron. **Our overall hypothesis is that increased expression of MAGL in ASD patient-derived cortical organoids is associated with the dysregulation of normal excitatory-inhibitory tone.** In our experiments we will determine if there is an increase in MAGL expression in ASD patient derived brain organoids compared to control organoids and we will also determine 2-AG levels. Next we will validate our proposed model of ECS dysfunction using CB1R modulators in control neurons. We expect that increased MAGL is associated with an increase in excitatory synapses. We also expect that CB1R agonism in ASD patient derived neurons will reduce neurite complexity and restore the excitatory-inhibitory balance while CB1 inverse agonism will cause an ASD-like phenotype in control neurons.

Kinin B1 Receptor Blockade Prevents Angiotensin II-Induced Neuroinflammation and Oxidative Stress

Rohan Parekh, Srinivas Sriramula

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

Hypertension remains an important medical and public health issue in the United States. Evidence suggest that a bidirectional interaction between the nervous and immune systems ultimately promotes the development of the neurogenic hypertension. Our lab previously reported that kinin B1 receptor (B1R) expression is increased in the hypothalamic paraventricular neurons of hypertensive mice with elevated angiotensin II (Ang II) levels in the brain. However, the role of B1R in neurogenic hypertension and its interaction with Ang II type 1 receptor (AT1R), the major receptor of Ang II, has not been studied. In the present study, we tested the hypothesis that B1R knockdown prevents the development of Ang II-induced neurogenic hypertension. To test this hypothesis, wild-type (WT) and B1R knockout (B1RKO) mice were implanted with telemetry probes for conscious blood pressure (BP) monitoring and infused with saline or Ang II (1 μg/kg/min, SC) for 2 weeks. Ang II infusion for 2 weeks significantly increased BP in WT mice but this increase in BP was attenuated in B1RKO mice. To further understand the neuron specific B1R mediated signaling, we cultured mouse neonatal primary hypothalamic neuronal cultures and investigated the interaction between Ang II and B1R activation. Ang II stimulation of primary neurons significantly increased the expression of B1R mRNA (4-fold vs. control; $n=6$; $p<0.05$) and resulted in neuroinflammation as indicated by upregulated expression of inflammatory markers interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF), and monocyte chemoattractant protein 1 (MCP-1). These changes were attenuated by pretreatment of R715 (a B1R specific peptide antagonist). In addition, DHE staining showed that Ang II treatment increased reactive oxygen species production in neurons, indicating increased oxidative stress, which was prevented by pre-treatment with B1R antagonist. Ang II-induced increase in the key isoforms of NADPH oxidase, Nox2 and Nox4 expression was also blunted by pretreatment with R715. These data suggest that B1R knockdown decreases Ang II-induced hypertension by reducing inflammation and oxidative stress. Our study demonstrates a causal relationship between B1R expression after Ang II stimulation, suggesting a possible crosstalk between AT1R and B1R in neuroinflammation and hypertension.

A Role for Dopaminergic Signaling in Opioid Resistance Following Spinal Cord Injury

Ryan Patton[1], Helen Rodgers[1], Jacob Yow[1], Szu-Aun Lim[1], Stefan Clemens[2], Kori Brewer[1,2]

[1]Brody School of Medicine Department of Emergency Medicine, East Carolina University, Greenville, NC, United States
[2]Brody School of Medicine Department of Physiology, East Carolina University, Greenville, NC, United States

Opioids are not universally effective in treating neuropathic pain following spinal cord injury (SCI). We have shown in a rat model of SCI that 2/3 of the animals do not respond to morphine, but adjuvant addition of a dopamine (DA) D1R antagonist or D3R agonist was able to restore the lost morphine response, suggesting that morphine resistance after SCI involves DA-ergic signaling. The aim of this study was to determine the analgesic response of morphine-responsive and nonresponsive SCI rats to DA modulators alone and in combination with morphine and determine if changes in CNS DA levels were associated with the altered morphine response. Baseline nociceptive thresholds were measured in uninjured (n = 8) and SCI (n = 25) adult female rats before and after injection of morphine (2mg/kg) or saline (control). Rats then had thresholds re-assessed after injection of morphine + pramipexole (D3 agonist; 0.1mg/kg), morphine + SCH 39166 (D1 antagonist; 0.1mg/kg), pramipexole, or SCH 39166. Lumbar spinal cord and striatum samples were collected from a subset of rats in all groups and processed for metabolomics and targeted mass spectrometry to identify metabolite differences and quantify levels of DA. Morphine alone increased sensory thresholds in all uninjured rats but only 1/3 of SCI rats responded to morphine. SCI animals, regardless of response to morphine, showed improved analgesia with morphine + D3R agonist. However, only SCI nonresponsive rats showed improved analgesia with morphine + D1R antagonist compared to morphine alone. Metabolomics principal component analysis identified three clusters that corresponded to uninjured, SCI morphine-responsive and SCI morphine-nonresponsive groups. Preliminary analysis suggests differences in DA, prostaglandin and endogenous opioids pathways. Striatal DA levels were decreased in SCI morphine-nonresponsive rats compared to SCI morphine-responsive animals. In the lumbar spinal cord, DA levels were decreased in both morphine-responsive and nonresponsive SCI rats compared to uninjured rats. These data suggest that differences in DA pathways may affect morphine responsiveness following SCI.

Different Neurobehavioral Strategies in Motor Learning Between Left- and Right-Hand Dominant Individuals

Sydney Rossback, J. C. Mizelle

Department of Kinesiology, East Carolina University

Sequential motor actions are necessary for both complex behaviors and everyday activities. Researchers have assumed that left-hand-dominant (LHD) and right-hand-dominant (RHD) individuals have mirrored patterns of brain activation. Recent work, however, has shown this is not accurate and that left and right dominant individuals show different neurobiological patterns during cognitive motor tasks. Because of observed differences in LHD versus RHD, it is possible that the underlying processes for acquiring new skills is different for the two groups. It is important that these differences are understood because LHD may benefit from different instruction strategies in motor learning. The focus of this work was to determine differences in brain activation in LHD and RHD in motor learning. Twenty healthy adult volunteers (12 RHD, 8 LHD) participated. All participants performed an implicit motor learning task with their dominant hand. Participants performed a fixed series of button-presses that were hidden in a longer series of button presses. Brain activity (EEG) and behavioral outcomes (response time) were recorded throughout the experiment. EEG data were evaluated for corticocortical connectivity in theta band (4-8 Hz) using imaginary coherence. LHD and RHD both showed a similar decrease in response time. However, they encoded the information through different neurobiological mechanisms. EEG connectivity patterns in the theta band showed that LHD rely on bilateral neural circuits, while RHD use anterior-posterior networks. The results of this study may have implications for several fields including rehabilitation, coaching, and military training techniques.

No Differences Between Gamma and Beta Corticomuscular Coherence Coefficients During Static Balance Tasks

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Electroencephalography (EEG) signal can be analyzed in different manners. Frequency-domain, or power-based analyses, target certain frequency bands (i.e., delta = .5-4 Hz; theta = 4-8 Hz; alpha = 8-13 Hz; beta 13-30 Hz; gamma = 30+ Hz) that can be associated with activity in certain areas of the brain. Corticomuscular coherence is the temporal correlation between brain activity, and muscle activity, measured by surface electromyography (EMG). A few studies have identified beta- and gamma-band corticomuscular coherence between the brain and lower-extremity muscles, but studies do not typically investigate brain waves in frequencies above 50 Hz (i.e., low- and high-gamma). A study shows a significant shift from beta- to gamma-range synchronization occurs when the force output is dynamic. This phenomenon has also been demonstrated during corticomuscular synchronization of the lower-limbs, with isometric contractions favoring beta-range oscillations, while isotonic contractions favor gamma-range oscillations. Several studies have demonstrated that VR perturbation paradigms challenge balance control. In this study we sought to investigate the presence and magnitude of low- and high-gamma band corticomuscular coherence between the left (LGL) and right (RGL) gastrocnemii lateralis, and the respective contralateral primary motor cortex (M1) during static balance tasks: eyes open (EO), eyes closed (EC), virtual reality baseline (VB), and virtual reality perturbation (V2). Eleven (N = 11) healthy young adults participated in the study. Subjects were instrumented with a 64-channel EEG cap. Bipolar Ag/AgCl EME electrodes were placed on the belly of left and right gastrocnemii lateralis. Participants then stood on a force platform for 30 seconds each trial. Three trials were performed per condition. Three frequencies of interest were chosen for this study: beta, low- and high-gamma. A (frequency) 3 x 4 (condition) ANOVA was used to compare the means of corticomuscular coherence within each muscle. There were no significant main effects ($p_{\text{-Freq}} = .53$, $p_{\text{-Cond}} = .78$) or interactions ($p = .99$) for LGL. Similarly, there were no significant main effects ($p_{\text{-Freq}} = .75$, $p_{\text{-Cond}} = .80$) or interactions ($p = .95$) for RGL. These results indicate that gamma and beta bands are similar in corticomuscular synchronization magnitude.

The Neural Correlates of Patellar Apprehension and Perceived Pain in Patients with Patellofemoral Pain

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Patellofemoral pain (PFP) is a common musculoskeletal disorder, historically, attributed to deficits in neuromuscular control. Recent data indicate that PFP may also elicit distinct central nervous system changes during active knee movements, but the neural correlates of clinician-based apprehension tests and perception of pain/fear during testing is unknown. The purposes of this study were to determine the neural correlates of a commonly used clinical test for patients with PFP—a patella apprehension test—and to identify how brain activity during testing is associated with patients' perception of pain. Females with PFP (n=14; 14.3 ± 3.2yrs.) completed a left leg patella apprehension test (apprehension test) during functional magnetic resonance imaging (fMRI). The test included 18 s of interspersed rest/test blocks during which an experimenter manually applied intermittent medial stress to the patella during test blocks. Patients' rated their pain unpleasantness immediately after using a VAS. fMRI preprocessing and analyses were completed within FSL using standard cluster-wise corrections and significance thresholds. Brain activation during test blocks and neural correlates of pain unpleasantness were examined. During the apprehension test, there was increased activation in the left precentral gyrus and cerebellum crus I; right parietal operculum, superior frontal gyrus, postcentral gyrus; and bilateral middle temporal gyrus (*ps* < .04). Greater activation in the left cerebellum VIIa/VIIb during testing was associated with a higher level of rated pain unpleasantness (r = 0.78, $p < .001$). The present study revealed that during a common clinical test for PFP there is increased neural activation in regions associated with pain and sensorimotor control in patients with PFP, indicating that apprehension of subluxation or pain may assay distinct neural processes. Specifically, patients with higher ratings of pain unpleasantness following the test had greater activation in the cerebellum VIIa/VIIb during testing. This region of the cerebellum is important for sensorimotor processing, as well as cognitive and affective regulation. Negative emotions can bias attention towards pain and the observed greater activation in these cerebellar regions may indicate dysfunctional neural integration in response to perceived unpleasant noxious stimulation for patients with PFP.

Prostatic Radiation Increases Bladder Cholinergic Nerve Density Leading to Enhanced Nerve-Mediated Contractions

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Introduction: In the US, prostate cancer affects 1 in 9 men. Though prostatic radiation therapy (RT) does not directly irradiate the bladder, the nerves supplying the bladder are located on the prostate's posterolateral surface and often receive a direct dose. Radiation-induced damage to these nerves can cause bladder dysfunctions. This study examines the impact of prostatic RT on bladder smooth muscle contractility and innervation.

Methods: Male Sprague-Dawley rats (8 weeks) received a single dose of prostatic radiation (0 or 22 Gy). The bladders and the major pelvic ganglia (MPG) were collected 2- and 10-weeks post-RT. The bladder was separated into strips for contractility experiments and the top portion was fixed for histology. Contractile responses to electrical field stimulation (EFS) and carbachol were measured. Bladder sections were stained with Masson's trichrome for smooth muscle content. Additional sections underwent immunofluorescent staining for neuron-specific class III beta-tubulin (non-specific neuronal marker), choline acetyltransferase (ChAT; cholinergic nerve marker), α-smooth muscle actin, and 4′,6-diamidino-2-phenylindole (DAPI). Gene expression was assessed in MPGs by qPCR for ubiquitin carboxy-terminal hydrolase L1 (Uchl1;) and ChAT.

Results: Bladder smooth muscle content was significantly increased at 2 weeks post-RT and was not different from controls at 10 weeks post-RT. Both EFS-mediated and carbachol stimulated bladder contractions were significantly decreased at 2 weeks post-RT. Following 10 weeks of RT, nerve-mediated contractions were markedly increased while carbachol contractions were unchanged. No change was found in the number of non-specific neurons within the bladder smooth muscle layer 2- or 10-weeks post-RT. However, 2 weeks post-RT showed a significant increase in the number of cholinergic neurons compared to controls. By 10 weeks post-RT, detrusor cholinergic neuron density was unchanged from controls. MPG gene expression of ChAT was increased at 2 weeks and decreased at 10 weeks post-RT. In contrast, there was no change in Uchl1 at 2- or 10-weeks post-RT.

Conclusions: At early time points post-RT, both carbachol and nerve-mediated contractions are decreased, and ChAT MPG gene expression is increased. EFS bladder contractions become elevated by 10 weeks post-RT. In parallel to the amplified bladder contraction, the number of cholinergic nerve endings was also increased at 10 weeks post-RT. Augmented innervation and bladder contractility post-RT may contribute to long-term bladder dysfunction following prostatic RT in cancer survivors.

The *lhfpl5* ohnologs *lhfpl5a* and *lhfpl5b* Are Required for Mechanotransduction in Distinct Populations of Sensory Hair Cells in Zebrafish

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Hair cells sense and transmit auditory, vestibular, and hydrodynamic information by converting mechanical stimuli into electrical signals. This process of mechano-electrical transduction (MET) requires a mechanically-gated channel localized in the apical stereocilia of hair cells. In mice, lipoma HMGIC fusion partner-like 5 (LHFPL5) acts as an auxiliary subunit of the MET channel whose primary role is to correctly localize PCDH15 and TMC1 to the mechanotransduction complex. Zebrafish have two lhfpl5 genes (lhfpl5a and lhfpl5b), but their individual contributions to MET channel assembly and function have not been analyzed.

Here we show that the zebrafish lhfpl5 genes are expressed in discrete populations of hair cells: lhfpl5a expression is restricted to auditory and vestibular hair cells in the inner ear, while lhfpl5b expression is specific to hair cells of the lateral line organ. Consequently, lhfpl5a mutants exhibit defects in auditory and vestibular function, while disruption of lhfpl5b affects hair cells only in the lateral line neuromasts. In contrast to previous reports in mice, localization of Tmc1 does not depend upon Lhfpl5 function in either the inner ear or lateral line organ. In both lhfpl5a and lhfpl5b mutants, GFP-tagged Tmc1 and Tmc2b proteins still localize to the stereocilia of hair cells. Using a stably integrated GFP-Lhfpl5a transgene, we show that the tip link cadherins Pcdh15a and Cdh23, along with the Myo7aa motor protein, are required for correct Lhfpl5a localization at the tips of stereocilia. Our work corroborates the evolutionarily conserved co-dependence between Lhfpl5 and Pcdh15, but also reveals novel requirements for Cdh23 and Myo7aa to correctly localize Lhfpl5a. In addition, our data suggest that targeting of Tmc1 and Tmc2b proteins to stereocilia in zebrafish hair cells occurs independently of Lhfpl5 proteins.

Dopamine Receptor and Dopamine Transporter Expression in the Human Spinal Cord

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Introduction: Understanding the role of the neuromodulator dopamine (DA) in the spinal cord is pivotal to research in neurological disorders such as Restless Leg Syndrome (RLS), opioid tolerance, and Parkinson's Disease. The actions of DA are mediated by two classes of dopamine receptors, D1-like (D1 and D5) and D2-like (D2, D3, and D4). In general, D1-like receptors mediate excitatory actions, while D2-like receptors have inhibitory functions. All five DA receptors are expressed in the mouse spinal cord, but not in nonhuman primates. Receptor expression in the primate spinal cord has been detected for D2, D3 and D5, but not D1, and there was inconclusive evidence for the D4 receptor. There are not data available concerning DA receptor expression in the human spinal cord. The goal of our study is to establish the presence of DA receptors in human spinal cord tissue through the use of immunohistochemistry (IHC).

Methods: Fixed spinal cord segments (lumbosacral) were obtained from the Brody Medical School Anatomy Laboratory and were further sliced and mounted to slides. Tissues were embedded, sectioned at 5 um, and subject to de-paraffinization, antigen retrieval, blocking and antibody exposure, including negative and peptide controls. After staining, the slides were imaged using the Olympus BX51 microscope, and Adobe Illustrator was used to assemble the images. Low power 2X images were used to create a full cross-section view of the tissues. Higher power (20X or 40X) were used to show the distinguishing features of DA receptor expression in motoneurons (MNs).

Results: Contrary to the data from nonhuman primates, and in line with mouse findings, we found that all five DA receptors are expressed in the human spinal cord. The results from our experiments showed presence of dopamine receptors D1, D2, D3, D4, and D5, as well as the DA transporter (DAT). D1 expression, which was notably absent in monkeys, was highly expressed in the ventral horn and in MNs. Similarly, D2 and D3 were also highly expressed in the ventral horn with slight spatial differences than D1. D5 was highly expressed in the dorsolateral region. D4 and DAT appeared to be evenly distributed throughout the spinal gray matter, but this may be due to weak staining.

Conclusion: This study is the first to show the presence of all five DA receptors in the human spinal cord, as well as the presence of DAT. The presence of these receptors can help give us a sense of DA's function in the spinal cord. This knowledge can be further used to develop clinical drugs that target DA receptors.

Poster Session 1
Clinical Case Studies

(presenters are in alphabetical order)

Acute Spinal Cord Injury INSPIRE 2.0 Study: Patient Four of Two-Arm Study of Surgical Implantation of a Biodegradable Scaffold

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Background: Implantation of a biodegradable scaffold shortly following spinal cord injury (SCI) has been shown in animal models to reduce chronic cavitation, increase sparing of uninjured white matter proximal and distal to the injury, and increase the deposition of neuropermissive remodeled tissue. The INSPIRE 1.0 and 2.0 studies enroll(ed) subjects with neurologically complete SCI T2-T12/L1 injury, Abbreviated Injury Scale (AIS) A, within 96 hours of injury. The 1.0 study was open and consisted of durotomy and sometimes myelotomy with removal of acutely necrotic tissue and insertion of a scaffold. The 2.0 study is a two-arm, blinded study with (1) a scaffold arm (10 patients) and (2) a comparison arm in which no scaffold is implanted (10 patients).

Case Report: The patient is a 25-year-old man who sustained a traumatic spinal injury in a motor vehicle collision. He presented with loss of motor and sensory modalities in his bilateral legs. Imaging identified (1) a T4-T5 fracture dislocation with (2) spinal cord contusion and small amounts of epidural blood. The patient successfully underwent posterior cervical thoracic fusion at C4-T6. Results regarding insertion or lack of insertion of the scaffold are blinded. The definition of study success will be based on improvement of at least one grade on the AIS assessment scale at the six-month clinical visit.

Conclusions: Severe SCI leads to the rapid formation of irreversibly damaged parenchyma. This report documents the entry of patient four into the INSPIRE 2.0 acute spinal cord injury. Followup regarding the arm into which this patient was randomized and his clinical outcome will be reported after the trial closes and data are made available for evaluation. This patient will add additional data to continue to build upon initial observations from the 1.0 study in which 25% AIS conversion rate.

Neuromyelitis Optica Spectrum: Three Cases and Review of Current Diagnostic Criteria

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Background: Central nervous system (CNS) demyelination can result from inflammatory, infectious, and toxic-metabolic disease processes. Inflammatory / immune-mediated diseases of the CNS include, most commonly, multiple sclerosis (MS) and, less commonly, acute disseminated encephalomyelitis (ADEM), and neuromyelitis optica (NMO). NMO can be distinguished from MS and ADEM by relative clinical and radiologic restriction of disease to the optic system and the spinal cord with laboratory identification of anti-aquaporin-4 in over 80% of cases (seropositive) or anti-myelin oligodendrocyte glycoprotein antibodies in a minority of cases but absence of antibodies in a proportion of cases (seronegative). Diagnosis of NMO can be challenging and patients can be misdiagnosed with MS. We present three cases that illustrate the spectrum of NMO.

Methods and Materials: The clinical, radiologic, and pathologic data from three patients with the working diagnosis of NMO are compiled and contrasted. Literature searches are conducted.

Results: Three cases are compared and contrasted: (1) an autopsy case of a 45-year-old woman with a clinical history of MS, severely debilitated, with demyelination limited to the optic nerves, chiasm, and tracts and the spinal cord; (2) a clinical case of a 29-year-old woman who presented with ascending numbness to the abdomen with optic nerve and spinal cord signal abnormality consistent with demyelination and a positive anti-aquiporin-4 antibody evaluation, diagnosed as NMO, and (3) a clinical case of a 27-year-old woman who presented with lower extremity weakness and progressive blindness with abnormal spinal cord signal on MRI but negative for anti-aquaporin-4 antibody, diagnosed as seronegative NMO.

Conclusions: These three cases illustrate the clinical, laboratory, radiologic, and histopathologic spectrum of neuromyelitis optica. Correct diagnosis and then appropriate therapy of a demyelinating disease requires knowledge of the differential diagnosis and diagnostic testing.

Gamma Knife Radiosurgery

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Background: Pseudoprogression is a subacute therapy-related brain injury usually occurring within 3-4 months following completion of radiotherapy, occasionally associated with chemotherapy or/and immunotherapy, with a transient increase in contrast enhancement on imaging studies. It generally resolves spontaneously over time. However, distinction between recurrent neoplasm vs. pseudoprogression is difficult. Most patients are clinically asymptomatic and intervention is unnecessary. Although it is well-documented in glioblastoma, pseudoprogression is often underreported or recognized in metastatic diseases. We present the case of a patient with metastatic melanoma status-post resection and gamma-knife radiosurgery who developed pseudoprogression.

Methods and Materials: The clinical, radiologic, and pathologic data from the patient are compiled. Literature searches are conducted.

Result: The patient is a 63-year-old woman with a history of metastatic melanoma of unknown primary to the left medial frontal lobe, status-post resection and gamma knife radiosurgery to the resection bed. Serial imaging studied identified initial good response but subsequent interval increase in size in the area of the lesion with heterogenous contrast enhancement suggestive of progression of disease. Resection of the abnormal tissue was identified. Histopathologic evaluation identified necrotic neoplasm and marked reactive changes consistent with pseudoprogression.

Discussion: Contrast-enhancing lesions on MRI may indicate either true tumor progression or postradiotherapy, chemotherapy, and/or immunotherapy changes, thus interpretation must be made with caution. Early recognition and differentiation of these situations is crucial to avoid unnecessary reoperation or therapeutic regimen change. Pseudoprogression is a phenomenon attributing to vasodilation, focal inflammation with demyelination, blood–brain-barrier impairment, and changes in vascular permeability. Some studies suggest a survival benefit in patients exhibiting early pseudoprogression, potentially indicating higher treatment efficacy. Unfortunately, conventional MRI is insufficient to distinguish true tumor progress from pseudoprogression since increased enhancement can be induced by a variety of non-neoplastic processes. Asymptomatic patients with contrast-enhancing lesions will need close surveillance with serial imaging.

Variably Symptomatic Metastasis to the Pituitary Gland: A Case Series

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Background: Pituitary metastasis (PM) from a systemic organ neoplasm is uncommon, representing approximately 0.4 - 0.9% of all intracranial metastatic diseases, with breast carcinoma most frequent. In contrast to pituitary adenomas, the posterior lobe is more susceptible to metastasis than the anterior lobe, usually as micro-metastasis, with only a small proportion of symptomatic cases (2.5-18.2%). Clinical manifestations can include diabetes insipidus, visual defect, hypopituitarism, and/or headache.

Methods: A 10-year retrospective laboratory information system query was undertaken. Patient medical records and glass slides were concurrently reviewed and a literature search was performed.

Results: Seven cases of PM were identified, 5 surgical resection and 2 autopsy cases. The male-to-female ratio was 6:1. The average age was 63-years-old (range 49 to 76). Five patients had a documented history of malignancy. Lung was the most common primary site (2 adenocarcinomas and 1 neuroendocrine carcinoma), followed by breast (1), colon (1), kidney (1) and prostate (1). Radiologically, the lesions were often described as “enlarged pituitary gland” or “sellar and suprasellar mass lesion” and pituitary adenoma was the favored clinical diagnosis. Notably, in 2 cases, the metastasis involved an existing pituitary adenoma (“tumor-to-tumor metastasis”): 1) a breast carcinoma involving a corticotroph adenoma and 2) a lung adenocarcinoma involving a mammotroph adenoma. Tumor metastasis to a pre-existing pituitary adenoma is extremely rare with fewer than 30 published cases to date (lung cancer: 6 cases; breast cancer: 4 cases).

Conclusion: It is not possible to differentiate PM and a pituitary adenoma, or PM to an adenoma, without pathologic diagnosis. When a patient presents with a pituitary mass and DI, PM should be considered in the differential, especially in those with a history of malignancy. A better understanding of this uncommon phenomenon and high clinical awareness will attribute to earlier diagnosis, appropriate treatment, and better outcome.

Supratentorial Cortical Anaplastic Ependymoma in a 17-Month-Old Girl

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Background: Ependymomas are neuroepithelial tumors which originate from glial progenitor stem cells, usually near the ventricles or the central canal, constituting 7% of glial neoplasms. In pediatric patients, infratentorial location is more common than supratentorial. Cortical ependymoma is a rare subset of supratentorial ependymoma with no connection to the ventricular system.

Case Report: We report a case of a supratentorial cortical anaplastic ependymoma in a 17-month-old girl who presented with epileptic seizures and paralysis of upward gaze. Imaging of her brain identified a 10 cm, circumscribed, complex solid-cystic mass in the left frontotemporal region; no connection to the ventricles was appreciated. Histological examination revealed a high-grade, mitotically active glial neoplasm with (1) a small round blue cell component and (2) a more differentiated-appearing component with occasional true rosettes and pseudorosettes. Most neoplastic cells expressed L1CAM, glial fibrillary acidic protein, and CD56; EMA immunoreactivity was seen in some cells. By fluorescence in situ hybridization there was rearrangement of RELA and C11orf95, suggesting the presence of C11orf95-RELA fusion, the oncogenic driver of supratentorial ependymoma. The tumor was categorized as cortical anaplastic ependymoma, RELA fusion-positive, WHO grade III.

Literature Review: Fewer than 140 cases of cortical ependymoma were identified in the English literature, with higher incidence in younger population, including 33 pediatric anaplastic ependymomas (16 months- to 18 years-old). Seizure (51.5%) and headache (42.4%) were the most frequently reported clinical findings. Imaging identified large, well-demarcated masses, usually involving the frontal lobe, with a solid-cystic appearance and frequent calcification. Recurrence was documented in 30% of cases, greatest in high-grade tumors (46%).

Conclusion: Due to its rarity, atypical location, radiological findings and clinical manifestations, cortical ependymoma is often misdiagnosed. Unlike its ventricular counterpart with grade II predominance, approximately half of the cortical ependymomas demonstrate anaplastic features, requiring timely diagnosis, treatment, and long-term follow-up.

Cerebrospinal Fluid Cytology, Imaging, and Laboratory Findings in Patients with Neoplastic Meningitis

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Background: Neoplastic meningitis (NM) is a late and often fatal central nervous system complication of advanced neoplasms, primary or metastatic, with dissemination of malignant cells to the meninges, subarachnoid space, and cerebrospinal fluid (CSF). Diagnosis is based on a combination of clinical findings, radiographic findings, and CSF analysis. This study correlates clinical and pathologic findings in a series of abnormal CSF cytology specimens.

Methods and Materials: We retrospectively identified 142 abnormal CSF cytology specimens in 72 patients (male: 32; average age: 54-years-old) from 2008-2018 at our institution. Clinical, radiologic findings, and CSF biochemical data were reviewed. Cytology glass slides were re-evaluated by a board certified cytopathologist.

Results: Most patients with abnormal CSF cytologic findings (94.4%) had metastatic malignancies with brain, spinal cord, and/or leptomeningeal involvement, including 37 solid tumors and 31 hematopoietic malignancies. In 67 cases the clinical diagnosis of NM was supported by cytologic, flow cytometric, and/or radiographic evidence. MRI was obtained in 40 patients with solid tumors and 28 patients with hematopoietic malignancies; 38 (55.9%) revealed possible leptomeningeal spread, but less commonly abnormal in hematopoietic malignancies (35.7%). Flow cytometry, performed in 20/31 hematopoietic cases, demonstrated 100% sensitivity. Elevated CSF white cell counts and protein concentrations were noted in 69.5% and 65.9% of cases, respectively. CSF glucose showed no strong correlation with NM. The overall prognosis of NM was devastating, with a median survival of 1.5 months (3 days-27 months). Of note, patients with solid tumors had significantly shorter survival than those with hematopoietic malignancies ($P = 0.0054$).

Conclusion: Notwithstanding the high sensitivity of MRI, in early stage of NM, leptomeningeal involvement can be difficult to detect by imaging studies alone if only microscopic metastases are present. Negative cytologic and radiographic findings do not exclude NM if the clinical presentation and laboratory tests are strongly suggestive of the diagnosis.

Ocular Findings in Infants and Young Children with Fatal Abusive Head Trauma

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Background: Pediatric abusive head trauma (AHT), as defined by the Centers for Disease Control, is an injury to the skull or intracranial contents of an infant or young child (< 5 years of age) due to inflicted blunt impact and/or violent shaking. This study sought to evaluate the contribution of eye examination in AHT.

Methods: Sixty-two eyes from 31 unrestricted pediatric autopsies (1 month - 5 years-old, M:F = 19:12) over a 10-year period were analyzed. Complete gross and histopathologic examinations were performed.

Results: Among the 31 decedents, traumatic head injuries (THI) were confirmed in 21 cases, including 19 AHTs and 2 accidental falls and 3 were "other traumatic injury" (1 traumatic neck injury, 2 ligature strangulations); 7 cases were diagnosed as "non-traumatic injury" (1 positional asphyxia, 1 psychogenic water intoxication, 1 chloroform toxicity, 1 status epilepticus, 3 undetermined). Retinal hemorrhages (RHs) and optic nerve sheath hemorrhage (ONSH) were the most common ocular findings in THI (67%), followed by orbital/perineural soft tissue hemorrhage (57%). However, RHs were also seen in 2 non-traumatic cases (positional asphyxia and undetermined) whereas ONSH was only seen in THI. Perimacular folds were observed in a small portion of AHTs but were only seen in AHT. Higher diagnostic accuracy was noted in subdural hemorrhage (SDH) (90.32%), subarachnoid hemorrhage (SAH) (87.10%), and ONSH (77.42%).

Conclusion: RHs have been reported in approximately 75-85% of children suffering AHT and up to 10% of children with unintentional head injury. This study found that RHs are neither sensitive nor specific for AHT. While postmortem eye examination should be performed in cases with unexplained death or suspected abuse, findings must be interpreted in the context of other autopsy findings and case data. The combination of ONSH and SDH/SAH are associated with traumatic head injury, with or without RH and/or skull fractures.

Extraneural Soft Tissue Perineurioma Involving Epidural Space and Pleura Presenting with Lower Extremity Weakness: A Novel Presentation of a Rare Soft Tissue Neoplasm

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Background: Extraneural soft tissue perineuriomas (ESTPs) arising in an extraneural location are a rare soft tissue neuroplasm. We present, to our knowledge, the first case of a combined pleural and extradural ESTP presenting with neurologic dysfunction.

Methods and Materials: Clinical and histopathologic features of the case were compiled summarized. A PubMed.gov literature search was undertaken.

Results:
Case Report: A 37-year-old man presented with progressive weakness and numbness of his legs. Imaging of his vertebral column identified a T4-T5 epidural space mass with significant spinal cord compression in continuity with a pleura-based paraspinous mass, 3.2 X 2.7 cm. The clinical differential diagnosis include solitary fibrous tumor, Schwann cell neoplasm, and meningioma. The pleura-based mass was biopsied first at an outside hospital and, based on smooth muscle actin (SMA) expression in a spindle cell neoplasm, misdiagnosed as schwannoma. Resection of the epidural mass reveled a spindle cell tumor with expression of epithelial membrane antigen, smooth muscle actin, and CD99 with no expression of CDD34 or SOX-6 ruling out solitary fibrous tumor. The final diagnosis was extraneural soft tissue perineurioma. The pleural neoplasm was subsequently resected and showed similar findings. The patient experienced gradual improvement of his symptoms.

Literature Review: Extraneural soft tissue perineuriomas are rare soft tissue neoplasm with a characteristic immunohistochemical phenotype that allows for definitive diagnosis. We identified no cases which reported pleura-based or extradural-based lesions.

Conclusions: ESTPs are rare soft tissue tumors presumably arising from perineurial cells or associated stems cells from nerves. Most cases are benign and show little or no tendency for local recurrence.

Acknowledgement: This case was reviewed in consultation by Christopher Fletcher, M.D., a soft tissue expert at Brigham and Woman's Hospital.

Association of Demographics and Test Categories Obtained from Concussion Vital Signs (CVS) Prolong Sports-Related Concussion Recovery in High School Athletes

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Background: The CVS is a neurocognitive test used to evaluate concussed high school athletes for symptoms overlooked during Return-To-Play (RTP), a progressive recovery protocol recommended by established concussion guidelines. Since the CVS has limited evidence and clinical utility in adolescent concussion management, there is a need to measure its time delay on RTP and identify clinical associations that can assist in the interpretation of the test.

Hypothesis: The CVS prolongs RTP completion in high school athletes diagnosed with a concussion, who would otherwise be approved to advance according to published guidelines; this delay is associated with demographics, CVS test results, and defined time-points within the recovery process.

Methods: This is a retrospective cohort study (UMC-IRB 18-002973 approved) of patients who were (1) diagnosed with a concussion at ECU Family Medicine, (2) injured from January 2016 to December 2018, (3) 13-19 years old, (4) Pitt County, NC high school athletes, and (5) provided a complete patient chart with all of the desired outcome variables. The following clinical criteria were assessed for a delay on RTP due to CVS testing: (1) demographic variables, (2) CVS test categories (Memory, Reaction Time, Cognitive Functioning), and (3) concussion recovery timelines.

Results: 70% of athletes sustained a delay on RTP due to CVS testing, and approximately 1-in-5 were delayed by at least 10 days. CVS testing resulted in a significant delay on RTP in athletes with documented learning disabilities ($p < 0.017$). Both female athletes ($p < 0.036$) and athletes who did not play football ($p < 0.002$) had a significantly longer overall recovery time. Visual Memory, Simple Reaction time, and Verbal Memory were the CVS test categories that most commonly lead to abnormal testing (57%). 44% of athletes incurred a prolonged RTP due to a single failed CVS test category.

Conclusion: Despite being an easily-accessible test which has potential to detect missed symptoms, the application of the CVS may unjustifiably extend the RTP of concussed athletes. The proposed associations are a framework to better gauge the performance of an athlete's CVS testing and warrant future studies to improve our knowledge within adolescent concussion care.

Poster Session 2

Research

(presenters are in alphabetical order)

Impact of Brain Iron Deficiency on Sleep: A Possible Animal Model for Restless Legs Syndrome

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Introduction: Restless Legs Syndrome (RLS) is a sensorimotor disorder that follows a circadian cycle and strongly affects sleep. While the disorder is defined by a patient self-reported "urge to move", RLS patients often also manifest with periodic limb movements during sleep (PLMS), which are clinically-objective outcome measurements. Brain iron deficiency (BID) is associated with RLS and PLMS, but the effect of inducible BID on sleep has not yet been evaluated. We here present a new technical approach and the first data that assess the outcome of *inducible and reversible* BID in a rodent model.

Methods: Following a previously established protocol, C57Bl/6 mice were exposed upon weaning for a minimum of five weeks to either control (Ctrl, 48 ppm Fe, N=6) or iron-reduced diets (~5-6 ppm Fe, N=10), which did not lead to an overall anemic phenotype. For sleep recordings, animals were placed in a modified home-cage fitted with electric field sensors (Plessey Semiconductors Inc.) that detects respiratory movements during sleep, and a video system was used to confirm sleep. A subset of BID-induced mice (N=4) was subsequently fed with control food for 5 weeks, to assess the potential recovery from BID. For the recording sessions, animals were observed between three to five hours/day, for up to five consecutive days.

Results: BID animals showed markedly different sleep patterns compared to controls but regained normal sleep patterns when brain iron levels were restored. The average hourly sleep duration of control animals was 13m 59s / hr (+/- 2m 40s SE), while sleep duration in BID animals was significantly decreased to 7m 05s / hr (+/- 1m 05s SE). Similarly, the average number of sleep episodes per hour was 2.3 (+/- 0.3 SE) in control and 1.6 (+/- 0.2 SE) in BID animals. After recovery from BID, average sleep time increased back to 12m 42s / hr (+/- 1m 55s SE, p=0.012, One-Way ANOVA) and hourly number of sleep episodes returned to 2.3 (+/- 0.3 SE, p=0.075, One-Way ANOVA).

Conclusion: Our results suggest that BID induces an altered sleep phenotype that, in general, resembles that of RLS patients with PLMS. The inducible BID mouse model may provide a tool to understand the mechanisms that underlie PLMS and altered sleep, which is recoverable with normal chow.

Rho GTPase Modulators ZCL278 and ZCL279 Impact Associative Learning in a Mouse Model of Alzheimer's Disease

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Background and Purpose: This study was conducted to explore the impact of Rho GTPase modulators, ZCL278 and ZCL279, on neurocognitive function using a triple-transgenic mouse model (3xTg) of Alzheimer's disease (AD). ZCL278 inhibits Cdc-42 and the growth of tumor cells, while ZCL279 activates Cdc42, which in turn elicits dendritic growth and promotes cell communication. Dendritic growth during learning is key to neuroplasticity underlying proper memory storage. Growth-morphology is governed by actin-regulatory-proteins (ARPs), which are regulated by Rho-GTPases such as Cdc42. This study is very novel because ZCL's effects on modulating neurocognitive function in the 3xTg AD model are unexplored.

Methods: We hypothesize that ZCL279 enhances hippocampal-dependent associative learning in 3xTg mice by stimulating cellular activity through Cdc-42 activation, while ZCL278 reduces cellular activity, which may not be facilitative for new learning. Twelve-month-old 3xTg or wild-type mice received ZCL278 or ZCL279 (100 μg/g) or sesame-oil (130 microliters) every other day for 60 days (30 total injections). To examine hippocampal-dependent learning, they received trace eyeblink classical conditioning (TECC) for six consecutive days.

Results: We have preliminary results indicating that 3xTg mice treated with ZCL279 show improvements in acquiring TECC, while modest results for those that received ZCL278. 3xTg control mice were impaired in acquiring TECC.

Discussion: ZCL278 and 279 may produce synaptic changes in areas of the brain affected by Alzheimer's disease, particularly the hippocampus. Our results indicated that at minimum, non-spatial associative learning impairments typically observed in 3xTg mice were spared by chronic treatment with ZCL279. The findings from this study may help elucidate the link between cellular changes and AD pathology, by identifying treatment targets that positively alter synaptic function underlying learning and memory storage.

Long-chain Acyl-CoA Synthetase 6 Regulates Brain Docosahexaenoic Acid (DHA) Metabolism and is Required for Neuroprotection

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The omega-3 fatty acid, docosahexaenoic acid (DHA), is enriched in the central nervous system and thought to protect against neurological dysfunction, in part due to its anti-inflammatory properties. However, the role of DHA in the development of neurological dysfunction has remained unclear because of a dearth of understanding of the biochemical mechanisms that regulate DHA enrichment in the brain. To elucidate these mechanisms, we genetically targeted long-chain acyl-CoA synthetase isoform 6 (Acsl6) because this enzyme initiates cellular fatty acid metabolism and is enriched in the brain. We discovered that deleting Acsl6 in mice resulted in large and specific reductions (35-72%) in DHA-containing phospholipids across the central nervous system concomitant with an increase in the pro-inflammatory omega-6 fatty acid, arachidonic acid. Acsl6 deficient mice ($Acsl6^{-/-}$) develop age-related gliosis and are hyperactive and hyporesponsive to auditory and foot-shock sensory stimulation. Upon an acute LPS-induced inflammatory challenge, $Acsl6^{-/-}$ mice do not display changes in pro-inflammatory cytokine expression and pro- and anti-inflammatory lipid mediators compared to controls. However, while at baseline $Acsl6^{-/-}$ mice show increase gliosis and expression of microglia markers, 24 hours post-LPS $Acsl6^{-/-}$ mice show reduce expression of microglia markers and anti-inflammatory cytokines. Together our findings suggest that either Acsl6 loss results in gliosis which may prime mice for faster resolution after a pro-inflammatory insult or that there is an alternative mechanism compensating for the loss of the neuroprotective fatty acid, DHA.

Epigenetic Manipulation Factors of Drosophila Fly a Predictor in the Epigenetics of Obesity in Humans

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In recent years there has been an increase in obesity worldwide. Obesity is a risk factor for other diseases such as metabolic disorders and cardiovascular disease. The purpose of this study was to investigate the epigenetic effects of different exposures such as diet and exercise on metabolic phenotype in a fruit fly model. The drosophila and humans genes share 90% homology. Therefore, disease pathways and metabolic pathways of the two operate similarly. In this study we examined interaction between diet and exercise by subjecting fruit flies to control diet, western diet, exercise, and western diet with exercise. The western diet is rich in saturated fat, sugars and salt. The metabolic phenotype was assessed by measuring respirometry, climbing, ability, weights and mortality. The results indicate that western diet increases mortality rate, and negatively impacts reproduction. Interestingly exercise counterbalances negative effects of the western diet. Another test performed on the different groups of flies was climbing test. In climbing test, the exercise flies negated the effects of the western diet. The western diet shows a decrease in climbing ability. The weights of the flies were also calculated, the western diet weighed less than the other groups. The data from the tests show that exercise can counteract obesity and that we can use the drosophila as a model to understand the obesity mechanisms in humans.

Delta-9-Tetrahydrocannabinol Alters Cannabidiol Efficacy to Improve Vocal Recovery Following Brain Injury

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Cannabidiol (CBD) has been proven effective for the treatment of genetic childhood seizure syndromes and is shown to improve the recovery of complex learned behaviors, like vocal learning, following brain injury. However, reports have indicated that patients experienced varied efficacy of the drug. Cannabidiol, like Delta-9-Tetrahydrocannabinol (THC), is an extracted component of cannabis; thus, each CBD extraction contains a small, yet varied amount of THC. One possible hypothesis for the varied efficacy of CBD in treating these genetic syndromes could be the different concentrations of THC in the CBD extract. Therefore, we aim to investigate the effects of 10 mg/kg (positive control) and 3 mg/kg CBD with either 0.02 or 0.08% THC, the euphorigenic component of cannabis, in our zebra finch model of vocal learning. We employed a microlesion model to mimic a brain injury involving a cortical-like brain region to assess if THC influences the recovery of learned zebra finch vocalizations. After analyzing various acoustic features and syllable transitions for each treatment group, we found that both 3 mg/kg CBD with either THC content improves syntax, whereas 0.02% THC content was dramatically more effective at improving phonology than 0.08% THC. Our results support the hypothesis that the concentration of THC within a CBD extract influences the efficacy of CBD in the recovery of vocal learning following loss of about 10% of a motor cortical brain region.

Exercise and Diet Programs Drosophila simulans Offspring Metabolic Phenotype

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Obesity in pediatric patients has risen dramatically in recent decades, along with susceptibility to Type 2 Diabetes (T2D), cardiovascular disease, cancer, and mortality. The relative contribution of genetic and environmental conditions is unclear in these cases. Drosophila simulans contain an insulin-like growth pathway (IGF-1), which is a large contributing factor in anabolic stimulation and growth in children. The fat body of Drosophila is the equivalent of the vertebrate adipose tissue and liver in regards to its storage and major metabolic functions. Thus, Drosophila is an ideal model to study the effects of environmental conditions, namely exercise and diet, on obesity phenotype. The purpose of this experiment was to characterize the model of obesity in Drosophila, and establish Drosophila as a model to study detrimental effects of western diet interplayed with exercise. A variety of tests were used to explore this relationship. Some attention was also given to how diet/exercise act as epigenetic factors that affect metabolic function of parents and reprogram offspring phenotype transgenerationally. F0 male flies were broken into four groups, control (C), exercise (E), western diet (WD), and western diet with exercise (WDE), and exposed for 5 days. The F0 flies were then evaluated for behavioral and physical changes through monitoring locomotor activity, sleep-wake cycle, food preference, weights, and mortality. A subsection of each group was bred after exposure rather than tested, and their F1 offspring, in some instances, were tested. Western diet resulted in greater mortality and reduced activity in F0 and F1 flies. When flies were exposed to diets containing one of the three main western diet ingredients (NaCl, sugar, and palm oil), our data indicates that the combination of all three ingredients is most detrimental compared to the effect of each individual ingredient. The data also showed increased nighttime activity in western diet flies, suggesting a disrupted circadian rhythm and possible neurological issues. The effects of western diet appeared to be counteracted to a degree when combined with exercise. Our results indicate that obesity characteristics in Drosophila are comparable in many instances with that of humans, making them a good study model for obesity.

Cytoskeletal Remodeling Regulates Synaptogenesis in a Model of Human Fetal Brain Development

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Excitatory synapse formation occurs during mid-fetal gestation. However, due to our inability to image fetal synaptogenesis, the pre-natal period remains understudied. The recent development of human fetal brain spheroids provides access to this critical period. Using human neurons and brain spheroids, we address how altered actin regulation impacts the formation of excitatory synapses during fetal brain development. We demonstrate that inhibition of RhoA kinase (ROCK) signaling promotes neurite formation and elongation. In addition to increasing neural complexity, ROCK inhibition increases spine precursor length. These increases correspond with increased excitatory synapse formation in human brain spheroids. Rac-driven actin polymerization drives this increase in excitatory synaptogenesis and supports spontaneous action potential formation. Using STORM super-resolution microscopy, we localize key upstream RhoGTPase regulators (GEFs, GAPs) to nascent excitatory synapses, providing evidence for differential actin regulation at pre- and post-synaptic compartments of emerging synapses. These results demonstrate that coordinated RhoGTPase activities underlie fetal excitatory synaptogenesis and identify critical regulators of early synaptogenic events.

Focal Adhesion Characterization During Neuronal Differentiation of hIPSC-derived Neurons

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Neurons function in highly organized circuits; these circuits rely on neuronal localization and morphology. Cell morphology, motility, and signaling are all dependent on focal adhesions. Focal adhesions are points of contact between neurons and the extracellular matrix. In the process of maturation, focal adhesions increase in size, elongate, and alter their protein composition. These dynamic states alter cell function though localized signaling events. It is currently unknown how focal adhesions change in human neurons during neuronal differentiation. We use human induced pluripotent stem cells (hIPSCs) as a tractable model to study changes in focal adhesions within the first 24 hours of neuronal differentiation. In order to evaluate focal adhesion complexes, we use a novel neuronal progenitor cell line gene edited to express paxillin-GFP. Paxillin is a focal adhesion protein found throughout the stages of focal adhesion maturation. Additionally, we co-stained for markers of nascent focal adhesion complexes, α-actinin, and markers that are required later in focal adhesion maturation, including vinculin and zyxin. We use Total Internal Reflection Fluorescence (TIRF) microscopy to resolves the adhesion complexes into discrete structures. We then analyzed neuronal morphology and focal adhesion composition, size, area, and distribution. Our preliminary data shows that during neuronal differentiation paxillin remains constant, α-actinin decreases, vinculin increases, and zyxin redistributes. We conclude that while focal adhesions proteins change with neuronal differentiation the specific alterations are unique to each protein. Future studies will explore how the soft environment of the brain alters focal adhesion dynamics in neurons.

The Voltage-Gated Ca^{2+} Channel Cav1.3a Regulates Expression of *parathyroid hormone 2* in Zebrafish

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Excitation-transcriptional (E-T) coupling is a mechanism of gene regulation that involves calcium (Ca^{2+}) entering a cell through an ion channel in response to neural or sensory stimuli. These Ca^{2+} ions interact with intracellular signaling proteins to alter levels of mRNA expression in target cells. L-type voltage-gated calcium channels (L-VGCCs) are one family of ion channels responsible for E-T coupling. Cav1.3 (Cacna1d) is a L-VGCC that is expressed in the brain and the sensory hair cells of the auditory and vestibular organs. However, the specific role of Cav1.3 in E-T coupling in hair cells and other neural tissues during development has not been investigated.

To determine whether Cav1.3 channels regulate gene expression in zebrafish, we performed RNA-seq experiments comparing transcript abundance between wild type larvae and those where the Cav1.3 channel was inactivated either genetically (*cav1.3a* mutants) or pharmacologically (the L-type Ca^{2+} channel blocker isradipine). We detected a set of downregulated transcripts and have begun to confirm the RNA-seq results by mRNA *in situ* hybridization on *cav1.3a* mutant larval zebrafish and larvae treated with isradipine. One of those downregulated transcripts is *parathyroid hormone 2* (*pth2*), which codes for a peptide hormone implicated in several neuroendocrine-regulated responses such as fear, stress, and pain, and also in the control of pituitary hormone secretion and reproductive behavior. In wild type zebrafish, *pth2* is expressed exclusively in cells near the ventral part of the posterior tuberculum in the forebrain. Our *in situ* hybridization results show that *pth2* is not expressed in *cav1.3a* mutants, in agreement with our RNA-seq data. In isradipine-treated larvae, *pth2* transcripts are expressed to a much lower degree than in the control. Also, larvae that are treated with isradipine and subsequently washed out over the course of a day experience a partial recovery in *pth2* expression. These results indicate that Cav1.3a channel activity is critical for *pth2* expression during zebrafish development. Determining which genes are regulated by Cav1.3a channel activity will help us to understand how E-T coupling contributes to the development of vertebrate neurosensory systems.

Effects of Remote Limb Ischemic Conditioning on Muscle Power, Motor Learning, and Functional Mobility in Children with Cerebral Palsy: Study Protocol for a Randomized Controlled Trial

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Remote limb ischemic conditioning (RLIC) is a clinically feasible method in which brief, sub-lethal bouts of ischemia protect remote organs or tissues from subsequent ischemic injury. A single session of RLIC improves motor learning and exercise performance, and increases muscle activation in healthy young adults and in individuals with stroke due to multifactorial mechanisms of RLIC. The purpose of this study, therefore, is to assess if RLIC combined with training can improve muscle power, motor learning, and functional mobility in children with cerebral palsy (CP). In this prospective, double-blind, randomized controlled trial, we will recruit total 24 children with CP (RLIC=12, sham=12), age range 6-13 years. We will deliver conditioning via five cycles of alternating inflation and deflation of a pressure cuff to the thigh using a rapid inflation cuff pressure of 200 mmHg in the RLIC and 25 mmHg in sham group. Both groups will receive quadriceps power training (dose: 60-80% of 1 repetition maximum training load; 6 sets of 5-7 repetitions/set; 3 times/week for 4 weeks); 15 trials of balance training on an instrumented dynamic stability platform using motor learning principles; and 15-20 minutes of short-burst interval treadmill training. RLIC/sham conditioning + training will be delivered 3 times/week for 4 consecutive weeks. Our primary outcomes include quadriceps muscle power (peak knee extension power using HUMAC Norm isokinetic testing); quadriceps activation (electromyography (EMG) amplitude); average time on dynamic balance task; and self-selected and fast walking speeds using 10-meter walk test. Assessments will be performed pre- (baseline) and post-intervention (immediately after 4-week training). We hypothesize that as compared to sham conditioning + training, RLIC + training will significantly increase quadriceps muscle power and activation; balance score; and walking performance (functional mobility). The significance of this innovative investigation is that it may establish a novel intervention to improve walking performance of children with CP. Thus, we may build evidence for the efficacy of RLIC as an adjunct to improve the current rehabilitation strategies to enhance overall functional outcomes. The major advantages of RLIC as a promising priming agent are the non-invasive nature, low cost, simple technology, and clinical feasibility.

Hyaluronan-Rich Extracellular Matrix Regulates Synaptic Formation and Function in Cortical Brain Development

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Changes in the ratio of excitatory to inhibitory (E/I) synapses is a common underlying mechanism of many neurological diseases. Increased hyperexcitability of cortical neurons is characteristic of neurodevelopmental disorders, such as epilepsy and autism spectrum disorders. Currently, we know little about how this E/I ratio is established. However, changes in the extracellular matrix (ECM) during neurodevelopment may alter synapse formation. Hyaluronan (HA), the major component of the brain ECM, is a macromolecule that controls cellular spacing. Through interaction with its receptor, CD44, HA regulates RhoGTPase signaling pathways. RhoGTPases are master regulators of actin organization. Since the actin cytoskeleton is enriched in excitatory synapses, we hypothesize that HA restricts excitatory synapse formation through regulation of RhoGTPase signaling. Using human-derived cortical brain spheroids, we manipulated HA levels and observed the resulting effects on synapse formation and neurotransmission. Consistent with our hypothesis, enzymatic digestion of hyaluronan leads to increased excitatory synapses and decreased inhibitory synapses. The elevated excitatory synapse formation resulted in increased spontaneous neural activity. In contrast, the addition of high molecular weight hyaluronan into the environment has opposite effects, decreasing excitability and resulting in decreased spontaneous neural activity. Protein analysis of RhoGTPase activity confirms these results. These data support a regulatory role for the ECM in cytoskeleton remodeling at the synapse and establish a new model of ECM regulation of E/I imbalances associated with human neurodevelopmental disorders.

Poster Session 2
Clinical Case Studies

(presenters are in alphabetical order)

Source of Neuroembolic Material in Acute Stroke Evaluation: Papillary Fibroelastoma of Cardiac Valves is in the Differential Diagnosis of Neuro-embolic Mitral Valve Lesions

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Background: Evaluation for the etiology of an embolic stroke is critical for the prevention of future embolic events. Papillary fibroelastomas are rare developmental / neoplastic lesions which arise on cardiac valves and fragments can embolize to the brain and other organs. We report the case of a patient who presented with clinical and imaging findings consistent with a stroke with subsequent identification of an aortic valve lesion consistent with a papillary fibroelastoma.

Methods and Materials: Clinical, imaging, and pathology findings from the patient were summarized. This case was compared to another case where surgical resection of the lesion was undertaken. A literature search was undertaken.

Results:
Case Report: The patient is a 72-year-old woman who presented with aphasia and right hemiplegia. Imaging of her brain revealed a left middle cerebral artery (MCA) infarct and CT-angiography revealed a left M2 MCA occlusion. While an initial echocardiogram was negative, a followup study and then a cardiac magnetic resonance imaging study identified a 0.4 cm low signal intensity structure on the A2 portion of the anterior leaflet of the mitral valve. The presumptive diagnosis of papillary fibroelastoma was made. Cardiothoracic surgery deferred surgical intervention. The patient is being followed clinically.

Literature Search: Fibroelastomas are the most common benign valve neoplasm and, after atrial myxomas, the second most common primary cardiac neoplasm. This patient fit well within the typical age of presentation, most often in the age range of 60 +/- 14 years. The lesion most commonly arises on the aortic valve and second most commonly on the mitral valve. They come to clinical attention after embolism or incidentally during an ultrasound performed for another reason.

Conclusion: This case illustrates a classic presentation of a very uncommon cause of ischemic strokes. This lesion should be in the differential diagnosis of a cardiac valve lesion.

Visual Symptom Presentation of Intracranial Neoplasms: Spectrum of Lesions and Locations

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Background: Many possible explanations underlay changes in vision and evaluation by an optometrist or ophthalmologist is often undertaken. While an eye-related disease process is most often at fault, a significant subset of vision deficits is the result of an intracranial neoplastic disease process.

Methods and Materials: Cases seen or reviewed from 2010-2018 in which vision deficits constituted an important presenting feature were compiled. Cases were selected which illustrated the range of neoplastic processes seen in different intracranial locations which manifested with vision loss. Most cases were seen by an optometrist and/or an ophthalmologist prior to brain imaging studies.

Results: Twenty cases were reviewed. Lesions compressing the optic nerve(s) and/or optic chiasm included two meningiomas, four pituitary adenomas, one Rathke cleft cyst, and two craniopharyngiomas. Intraaxial or extraaxial neoplasms disrupting optic tract fibers or geniculocalcarine fibers included four primary neoplasms (glioblastomas, pleomorphic xanthoastrocytoma), two metastatic neoplasms (lung (adenocarcinoma and squamous cell carcinoma) and a meningioma. Intraventricular lesions leading to hydrocephalus and increased intracranial pressure manifesting with papilledema and optic nerve and disc compression included a medulloblastoma and a fourth ventricle hemangioblastoma arising from the medulla initially misinterpreted as a retinal degenerative disease.

Conclusions: The primary goal of this project was to provide a survey of intracranial neoplasms that can manifest with vision deficits initially misattributed to eye disease. In some cases, there was a significant delay prior to head imaging studies and then delay in the correct diagnosis and treatment. Given the irreversibility of the visual deficits when such a delay occurs, there should be a low threshold for obtaining a screening brain scan when a peripheral problem fails to explain all of a patient's symptoms. A separate study will summarize the proportion of patients ultimately diagnosed with an intracranial neoplasm who presented with vision deficits as a component of their symptoms.

Schwannoma Mimics: Neurofibroma and Myopericytoma Presenting as Painful Soft Tissue Extremity Lesions

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Background: Painful lesions in the soft tissue of the extremities evoke a differential diagnosis including peripheral nerve sheath tumor and, specifically, most commonly, Schwannoma. Diagnostic evaluation often includes ultrasound or/and magnetic resonance imaging (MRI) studies with in some cases resection with the goal of relieving the patient's pain. We present three cases in which the clinical diagnosis of a painful extremity soft tissue tumor errantly favored Schwannoma.

Methods and Materials: Clinical features of the three cases were summarized from the patients' electronic medical records. Histopathologic evaluation was undertaken.

Case Reports:
Case A: A 72-year-old woman presented with pain and numbness in the dorsum of her left lower extremity. Ultrasound identified a 3.8 cm mass associated with the common fibular nerve. Resection was undertaken. Histopathologic and molecular evaluation revealed a neurofibroma. The patient's pain resolved following resection of the lesion.

Case B: A 55-year-old man presented with right arm pain. MRI identified a 1.0 cm mass adjacent to the lateral epicondyle. Resection was undertaken. Histopathologic evaluation revealed a proliferation of spindle-shaped cells consistent a myopericytoma. The patient's pain resolved following resection of the lesion.

Case C: A 46-year-old woman presented with left lower extremity pain and paresthesias. Ultrasound revealed a 0.25 cm nodule in the upper calf; the lesion could not be identified by MRI. Histopathology revealed a blood vessel partially obliterated by a proliferation of spindle-shaped cells consistent with diagnosis as a myopericytoma. The patient's pain resolved following resection of the lesion.

Discussion: Definitive diagnosis of soft tissue lesions requires histopathologic evaluation. Clinical pitfalls in accurate diagnosis include ascribing too much certainty to radiologic modalities including ultrasound studies and lack of generation of a thorough differential diagnosis. Given the benign nature of each of the patient's, misdiagnosis favoring Schwannoma did not lead to any adverse outcome.

Sarcoidosis Presenting with Non-Pulmonary Symptoms: Symptoms of Hydrocephalus as An Uncommon Presentation of a Common Disease

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Background: Sarcoidosis is a multisystem, granulomatous disease of unknown etiology. Respiratory deficits are the most common presenting symptom in most patients due to pulmonary interstitial injury and hilar and mediastinal lymph node involvement. However, sarcoidosis can affect nearly every organ system and presentation with non-pulmonary symptoms is occasionally seen. We report a case presenting with symptoms of obstructive hydrocephalus.

Methods and Materials: Clinical features were summarized from the patient's medical record. Key histopathologic features were reviewed. A PubMed.gov literature search was undertaken using relevant key words.

Case Report: A 29-year-old woman presented to the emergency department a persistent headache associated with nausea, vomiting, and visual disturbances. Lumbar puncture to rule out meningitis identified an elevated opening pressure. Brain imaging identified non-communicating hydrocephalus with periependymal enhancement, including around the aqueduct of Sylvius and at the massa intermedia and anterior commissure. A broad differential diagnosis was considered (inflammatory, infectious, and neoplastic). CT scan of her chest identified bulky mediastinal and hilar lymphadenopathy.
Emergent endoscopic third ventriculostomy with biopsy of the ependymal lesion was performed. Pathological examination revealed non-caseating granulomatous inflammation. Acid fast bacillus and fungal stains were negative. Laboratory tests were significant for an elevated serum calcium level; angiotensin-converting enzyme level was normal. Aggregate findings were most consistent with sarcoidosis with central nervous system (CNS) involvement.

Discussion: Sarcoidosis with CNS involving poses diagnostic challenges due to non-specific clinical and radiological findings. Approximately 5% of patients with systematic sarcoidosis have CNS involvement. Hydrocephalus is an uncommon finding, observed in 6-9% of neurosarcoidosis cases. Clinical awareness, CT screening for other potentially affected organs and timely biopsy are crucial for the diagnosis. Neurosarcoidosis has an overall good prognosis, with 90% of cases improved over time. However, cases presenting with hydrocephalus have a worse long-term prognosis with a mortality rate of up to 75%.

Association Between Dementia Including Alzheimer Disease and Death Near Bodies of Water

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Background: Alzheimer disease is the most common neurodegenerative disease with an average age of onset of 65-years. Dementia with disorientation is a common symptom. We identified and investigated a possible association between dementia and death near bodies of water in eastern North Carolina. Limited studies have looked into wandering behaviors in Alzheimer patients including deaths near water and no study has provided histological correlation.

Methods and Materials: A retrospective study of drowning deaths seen at our medical examiner office between 2014 to 2019 was conducted. Special attention was paid to patients over 50-years and their associated medical conditions.

Results: Drowning as the cause of death was determined in 120 cases; with autopsies performed on 83 and external examinations on 37. A history of dementia was known in 8 individuals (Alzheimer disease: 6; Huntington disease: 1; neurosyphillis-associated dementia: 1). The average age of the Alzheimer patients was 83.6 (76 to 89 years-old; male-to-female ratio 4:2), constituting 33.3% of all autopsy cases over 65 years of age. All patients were last seen at home. None were living in a skilled nursing facility. All bodies were found within a body of water (ditch, canal, or pond). Histological examinations available on two cases identified advanced-stage Alzheimer disease. Contributing factors in non-dementia drowning deaths included alcohol intoxication, substance abuse, cardiovascular disease, motor vehicle collisions, psychiatric problems, seizure disorder, and autism.

Conclusions: Our findings indicate an association between individuals with dementia and accidental death in bodies of water close to their living environment. Despite the small number of cases, a positive correlation between drowning and Alzheimer dementia is noted. The incidence of Alzheimer disease in our series was significantly higher than the national average of the same age group (17%). Close attention and supervision should be provided to patients with dementia.

Combining EMG with Ultrasound Can Help Diagnose a Soft Tissue Lesion That Presents as Median Nerve Mononeuropathy: A Case Study

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A 25 year-old female with no significant medical history presented to the outpatient clinic for electrodiagnostic assessment of the left upper extremity. She reported slowly progressive symptoms consisting of left-hand numbness, weakness, and "popping" sensation that is present day and night. Physical examination of the left upper extremity revealed 4/5 strength in finger flexors, and a positive Tinel's sign at the wrist. Active range of motion of left finger flexion revealed a dynamic mass in the left wrist, which disappeared with relaxation. Initial nerve conduction studies to the left ulnar and median motor and sensory nerves revealed a prolonged left median sensory latency. This indicated a mild abnormality in myelin function of the left median nerve between the wrist and the second digit. An ultrasound was then conducted which revealed a median nerve sheath mass in the left hand. Patient was referred to orthopedic surgery and had this mass removed. The surgeons removed a 1.5 cm x 1 cm mass adherent to the volar aspect of the FDS tendon of the second digit. The specimen was sent to the pathology lab, which diagnosed a fibroma of the tendon sheath. A fibroma of the tendon sheath is an uncommon tumor. Maeda et al. (2017) reported a similar case, citing that 98% of these fibromas occur in extremities, with 81.8% involving fingers, wrists, and hands. This study also reported the "trigger wrist" finding as noted in our case. When this tumor is present in the hand or wrist, a patient can present with symptoms of median mononeuropathy, as in this case. This case supports Maeda et al. findings, including location and the trigger wrist noted by patient. It is a great example of how a dynamic evaluation, incorporating electrodiagnostics and ultrasound, can lead to a surprising diagnosis. Providers should consider a fibroma of the tendon sheath when investigating a soft tissue lesion of the hand. Swift diagnosis can lead to earlier intervention and rehabilitation.

A Thoracic Spine Fracture in an Elderly Patient with Osteopenic and Rigid Spine

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Introduction

Thoracic spine fractures are thought to be less prevalent in comparison to cervical and lumbar spine fractures due to rib cage stabilization. Thoracic spine fractures are often associated with diffused idiopathic skeletal hyperosteosis (DISH) and ankylosing spondylosis.

Case Report

The patient is an 80-year-old male with history of chronic back pain who presented after sustaining a mechanical fall from standing, resulting in a tentorial subdural hematoma. The hematoma did not require neurosurgical intervention. The patient was admitted to our rehab facility and was unable to tolerate out of bed activity due to continued severe back pain. A lumbar computer tomography (CT) demonstrated a rigid and osteopenic spine with moderate anterior osteophytes at multiple levels. An oblique T11 vertebral body fracture was also noted. Neurosurgery was consulted immediately. No neurological compromise was noted on exam. Neurosurgery recommended conservative management with thoracic lumbar sacral orthosis (TLSO) given the patient's non focal exam and current comorbidities. The patient's pain regimen was also adjusted. Although he needed further assistance with donning on TSLO, his transfer and ambulation markedly improved throughout his rehabilitation course.

Discussion

Although this patient does not meet the criteria for DISH, a rigid and osteopenic spine is often noted as a late finding of DISH. This results a mechanically inferior brittle spine, susceptible to fracture with a relatively low impact fall. If the patient did not undergo further imaging, his fracture may have become unstable, leading to secondary spinal cord injury.

Conclusion

Although thoracic spine fractures are generally regarded as less common compare to cervical or lumbosacral fractures due to stabilizing effect provided by thoracic cage, patients with rigid and osteopenic spines are at risk to sustain thoracic spine fractures, and these fractures may ultimately cause neurological deterioration. Appropriate imaging is warranted to look for occult fractures even with low impact injury.

Subacute Combined Degeneration Secondary to Nitrous Oxide Abuse: Quantification of Use with Patient Follow-up

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Background: Subacute Combined Degeneration (SCD) is caused by demyelination of spinal cord white matter secondary to Vitamin B12 (Cobalamin) deficiency leading to core symptoms of spastic paresis, vibratory and proprioceptive deficits. Most common causes of B12 deficiency revolve around malabsorption and pernicious anemia. Nitrous Oxide (N2O) can also indirectly cause B12 deficiency by inactivating its biologically active form.

Methods and Materials: Clinical, imaging, and laboratory findings from the patient were summarized. A literature search using keywords including "subacute combined degeneration", "b12", nitrous oxide", "abuse", and "recreational" was undertaken.

Results:
Case Report: A 53-year-old man presented with bilateral leg weakness, repeated falls, and general paresthesia with bilateral numbness in upper and lower extremities. Prior to symptom onset, the patient reported ~3,000g of N2O inhalation within five days prior to symptom onset in addition to daily use. Work up revealed neurologic abnormalities consistent with SCD, normal B12 levels, increased homocysteine and methylmalonic acid, positive intrinsic-factor-blocking (IFB) antibodies, and sensorimotor polyneuropathy without major demyelination features. T2-weighted imaging revealed increased dorsal column signal attenuation. Treatment consisted of a seven-day course of intramuscular Vitamin B12, then once weekly injection. At 2-month follow-up, the patient reported improvement in mobility and sensation functionality between 30-70% of baseline.

Literature Search: Similarities of this patient to literature include the classic presenting symptoms of SCD and the gradual symptomatic improvement with B12 injections and N2O abstinence.

Conclusions: This case is remarkable due to SCD occurrence after recreational N2O abuse, objective quantification of N2O intake over a specified time period to induce SCD, occurrence secondary to N2O inhalation, positive IFB antibodies, and symptomatic presentation with B12 values within normal limits. This report highlights the dangers associated with N2O abuse and moving forward can be referenced in educating at-risk young adults.