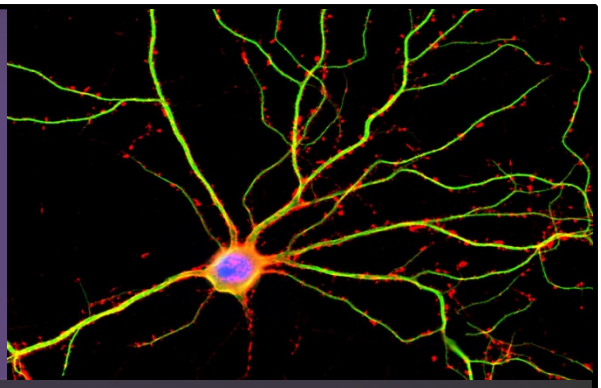
Eastern Carolina Chapter of the Society for Neuroscience Presents:



20th Annual Neuroscience Symposium
Catalyst for Collaboration

Featuring:

Mark Zylka, PhD

Distinguished Professor and Director of the Neuroscience Center at UNC-Chapel Hill

"Exploiting Genetics to Identify Environmental Risks for Autism"

Monday, October 22nd, 2018
East Carolina Heart Institute
www.ecu.edu/neurochapter

ECCSfN

Funding for this event was made possible by contributions from:

ECU

Anatomy & Cell Biology
Biology
Pharmacology & Toxicology
Physiology
Psychology

North Carolina Biotechnology Center

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 2018 Neuroscience Symposium:

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20th Neuroscience Symposium Schedule
October 22nd, 2018
East Carolina Heart Institute
115 Heart Drive, Greenville, NC 27834

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

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

10:15-11:30 Conference Room **Graduate Student and Postdoc Presentations**
10:15-10:40 PhD Candidate: Yi Zhu
"*Targeting RhoA-NF-kB Signaling in Chemotherapy-Induced Peripheral Neuropathy"*
10:40-11:05 Postdoc: Helen Rodgers, PhD
"*Dopamine D1 and D3 Receptor Modulators Restore Opiate Analgesia in a Model of Spinal Cord Injury Neuropathic Pain*"
11:05-11:30 PhD Candidate: Jessica McDonnell
"Neurobehavioral Indicators of Skill Acquisition in Left and Right Hand Dominant Individuals"

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

11:45-12:00 **Opening Remarks**: Dr. Peter Schmidt, Vice Dean of the Brody School of Medicine, ECU

12:00-1:00 **Keynote Address:** Mark Zylka, PhD, Distinguished Professor, UNC-Chapel Hill
"*Exploiting Genetics to Identify Environmental Risks for Autism*"

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

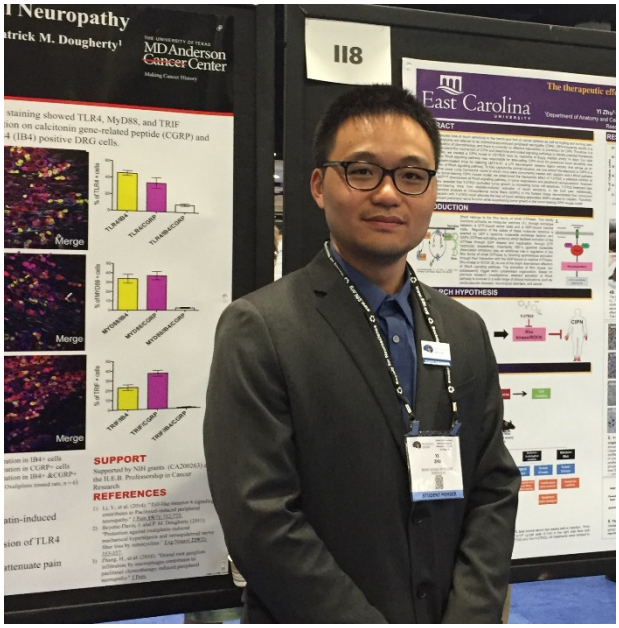
2:45-4:00 Conference Room **Faculty Presentations**
2:45 – 3:10 Yiyang Gong, PhD, Duke University
"*Optical Technologies for Neural Recordings*"
3:10 – 3:35 Erzsebet M Szatmari, PhD, Physical Therapy, ECU
"*Identifying New Signaling Pathways Involved in Alzheimer's Disease Progression*"
3:35 – 4:00 Sunghan Kim, PhD, Biomedical Engineering, ECU
"*EEG-Based Mild Cognitive Impairment Screening in an Outpatient Setting: A Pilot Study*"

4:00-4:15 **Closing Remarks and Awards from ECCSfN President:**
Alex Murashov, PhD

5:00-7:00 **After-Event Social at Pitt Street Brewing** with food (except alcohol) courtesy of the chapter and its sponsors. Please RSVP through the symposium registration link.

Podium Presentations

(in order of appearance)



Targeting RhoA-NF-kB Signaling in Chemotherapy-Induced Peripheral Neuropathy

Yi Zhu, PhD Candidate

Anatomy & Cell Biology
East Carolina University
Brody School of Medicine

During cancer therapy, many acute toxicities can be managed (e.g., febrile neutropenia, acute nausea and vomiting) and will resolve once therapy has been completed (e.g., mucositis). However, there are other severe adverse sequelae that persist after completion of therapy. There are no effective management strategies for the problem which impacts patient quality of life. For example, there are significant gaps in our understanding of chemotherapy-induced peripheral neuropathy (CIPN). CIPN frequently requires a reduction or cessation of chemotherapy, as there is currently no effective means to prevent or treat CIPN.

Until now, most studies use non-tumor-bearing animal models to investigate the mechanism of CIPN and its associated signaling pathways for identification of potential therapeutic targets. To conduct more clinically relevant studies, we induced CIPN in a syngeneic murine Lewis Lung Carcinoma model. In this tumor-bearing CIPN mouse model, we determined that Y-27632, a selective inhibitor of Rho kinase/p160^{ROCK}, is an effective adjuvant in tumor suppression and peripheral neuroprotection. Y-27632 not only preserved cisplatin's efficacy towards tumor suppression but also independently inhibited tumor growth by promoting tumor cell apoptosis. Furthermore, by alleviating the cisplatin induced-loss of epidermal nerve fibers (ENFs) and Meissner corpuscles (MCs), Y-27632 protected tumor-bearing mice from cisplatin-induced reduction of touch sensation. The quantitative proteomic analysis of mouse foot pad tissues revealed the striking cisplatin-induced dysregulation in inflammation, DNA repairing, and cellular stress associated proteins. Y-27632 not only reversed the changes of these proteins that are associated with Rho GTPase and nuclear factor-κB (NF-κB) signaling networks, but also suppressed cisplatin-induced NF-κB hyperactivation in foot pad tissues. These studies highlight the potential of targeting the RhoA-NF-κB axis with Y-27632, a promising therapeutic adjuvant to chemotherapy that protects peripheral nerve function while suppressing tumor growth.
Supported by NIH CA165202

Dopamine D1 and D3 Receptor Modulators Restore Opiate Analgesia in a Model of Spinal Cord Injury Neuropathic Pain

Helen Rodgers, PhD
Postdoctoral Scholar

Emergency Medicine
East Carolina University
Brody School of Medicine

A common consequence of spinal cord injury (SCI) is debilitating chronic neuropathic pain. Current treatment options (such as opioids) have inherent risks and often inadequately manage the pain. Consequently, new pain management therapies are desperately needed. We previously reported that dopamine D3 receptor (D3R) system dysfunction was associated with opioid resistance and that block of D1 receptor (D1R) function could restore morphine analgesia in these D3KO animals. Here, we demonstrate that in a model of SCI neuropathic pain, adjuvant therapy with a D3R agonist (pramipexole) or D1R antagonist (SCH39166) can restore the analgesic effects of morphine while reducing reward potential. Thermal and mechanical thresholds were tested in rats prior to and after contusion SCI under the following paradigms: after 1) saline, 2) morphine, 3) morphine + pramipexole, 4) pramipexole, 5) morphine + SCH39166, and 6) SCH39166. Reward potential of each drug combination was assessed using conditioned place preference. Following SCI, morphine + pramipexole and morphine + SCH39166 significantly increased both thermal and mechanical pain thresholds. Conditioned place preference was not induced by either drug combination. These data suggest that adjunct therapy with receptor-specific dopamine modulators represents a new target for pain management therapy after SCI.

Neurobehavioral Indicators of Skill Acquisition in Left and Right Hand Dominant Individuals

Jessica McDonnell, PhD Candidate

Department of Kinesiology
East Carolina University

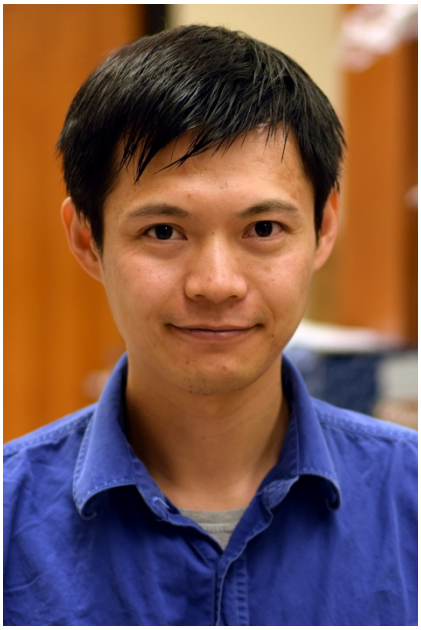
Background: Hand dominance is among the most obvious asymmetrical human attributes, meaning there is an innate preference for the use of one hand over the other. This asymmetrical behavior is biologically predetermined at some level; however, none of the contemporary explanations for hand dominance adequately explain the distribution of left hand dominance (12 – 16% of the Western population). Hand dominance is represented by more than an unprompted predisposition for one hand over another when executing fine motor skills. For example, the volume of the hand motor area contralateral to the dominant hand is markedly enlarged compared to the volume of the ipsilateral hand motor areas. This is true for both left- and right hand dominant individuals, so in the context of motor control it has historically been assumed that the brain of a left handed individual would mirror that of a right hand dominant individual when observing or executing an upper extremity motor tasks. We now know this to be false. Previous studies have found both left/right and anterior/posterior differences in neural activation patterns between the left and right hand dominant populations. The central objective of the proposed studies is to understand how sensorimotor information is integrated in the left and right hand dominant populations. **Methods:** We used a simple experimental paradigm that required subjects to modulate hand grip force in order to manipulate a cursor, displayed real time, to track a target moving in a repetitive pattern. Subjects initially performed the task unimanually; using dominant and nondominant hands to manipulate the curser in either lateral or vertical direction. The task was then completed bimanually; a single curser simultaneously receiving input from both hands. One hand controlled lateral movement while the other hand controlled vertical movement of the cursor. The target repeated the same pattern used in the unimanual conditions with equal input from each hand. Neurological and behavioral changes were measured as the visual tracking and force matching task was repeated and the skill was acquired. **Results:** EEG signals were decomposed into distinct frequency bands revealing that the two populations differed in brain states throughout the motor tasks. Right hand dominant individuals displayed increased beta activity in distinctly lateral regions of the brain, consistent with a motor strategy. Alternately, left hand dominant individuals expressed comparatively more theta activation, particularly over the frontal lobe thereby suggesting the population adopted a strategy more cognitive in nature. Further, right hand dominant individuals displayed a more disperse alpha band activation pattern compared to the left hand dominant population's more discretely lateralized pattern. The right hand dominant population expressed greater cross hemisphere connectivity while executing the bilateral motor task as compared to the left hand dominant population, consistent with the idea that left handers are more bilateral in behavior. This finding is mirrored by the behavioral measures showing increased performance (decreased error) in the left hand dominant population as the bilateral task progressed. **Significance:** It is clear from the data the two populations differ in the strategies adopted to complete the task. Implications of these findings may be relevant in rehabilitation settings.

Exploiting Genetics to Identify Environmental Risks for Autism

Mark Zylka, PhD

Distinguished Professor and Director of the Neuroscience Center at UNC-Chapel Hill

Our inability to identify environmental threats to the brain early—before they cause disease—represents one of the major challenges of our time. This challenge is particularly relevant to autism, which now affects 1 in 68 individuals, and where heritability studies indicate that environmental factors contribute to autism risk. In this seminar, Dr. Zylka will describe how candidate environmental risks for autism can be identified rationally, by identifying chemicals that interfere with the same molecular pathways that are affected in autistic individuals. His lab developed and optimized a transcriptomics-based approach to identify environmental-use chemicals that target several autism-linked molecular pathways. This approach makes use of a liquid handling robot and partial automation to test thousands of environmental-use chemicals and chemical mixtures on cultured neurons and neural progenitors. In addition, Dr. Zylka will discuss how his group is assessing the exposure threat to humans with environmental sampling data and is validating risk potential using high-confidence animal models of autism.



Optical Technologies for Neural Recordings

Yiyang Gong, PhD

Assistant Professor
Department of Biomedical Engineering
Duke University

Studying individual neural microcircuits composed of numerous neuron types necessitates the creation of new technologies that record neural dynamics in a genetically targeted manner. Genetically encoded fluorescent voltage indicators have the potential to simultaneously report voltage waveforms from many neurons in a genetically identified population of cells. Although past probes of this class lacked the requisite kinetics, brightness, or optical response to resolve single action potentials, recently developed voltage sensors based on rhodopsin proteins exhibit high brightness and large optical responses, and provide multi-fold increases in signal-to-noise ratio. The rapid development of these indicators has enabled visualization of voltage activity in a variety of live organisms, and the imaging of single action potentials in awake behaving mammals is on the cusp experimental possibility. By applying formalisms that incorporate photon statistical noise and voltage waveforms to benchmark these various sensors, I will review the past progress of the field and describe future advancements needed to attain parallel optical recordings of voltage from many neurons. Finally, I will discuss complementary optical and signal processing methods in addition to genetically encoded sensors that will contribute to implementing voltage imaging in live-animal experiments.

Identifying New Signaling Pathways Involved in Alzheimer's Disease Progression

Erzsebet M Szatmari, PhD

Assistant Professor
Department of Physical Therapy
East Carolina University

Centaurin-α1/ADAP-1 (CentA1) is a Ras-anchoring protein highly enriched in the brain. Previous studies from our lab and others, demonstrated involvement of this protein in pathogenesis of Alzheimer's disease. However, the neurobiological function of CentA1 and its signaling in the brain is largely unknown. Here we report that mice knockout (KO) for CentA1 exhibit age-dependent increase in dendritic spine density and enhanced spine structural plasticity in the CA1 region of the hippocampus. Moreover, deletion of Centa1 leads to improved performance in a hippocampus-dependent memory task. Thus, we provide evidence for the first time that in normal brain, CentA1 functions as a negative regulator of dendritic spine density and plasticity and of hippocampus-dependent memory formation. Our findings suggest that CentA1 and its downstream signaling is a potential therapeutic target to prevent memory decline associated with aging and age-dependent brain disorders.

EEG-Based Mild Cognitive Impairment Screening in an Outpatient Setting: A Pilot Study

Sunghan Kim, PhD

Associate Professor
Department of Biomedical Engineering
East Carolina University

Mild cognitive impairment (MCI) and Alzheimer's Disease (AD) affect millions worldwide, yet no curative treatments for these near-degenerative disorders have been developed to date. The current study aims to propose a non-invasive, cost-effective, early diagnostic protocol for individuals suffering with MCI in an outpatient setting. Elderly participants (n=11) were screened for MCI utilizing the Montreal Cognitive Assessment (MoCA) questionnaire preceding a visual stimuli task. Participants were presented with facial stimuli to elicit event-related potentials (ERP) while their cortical activity was recorded utilizing electroencephalogram (EEG). Combining regional neurophysiological biomarkers into a multi-dimensional feature space allowed for differentiation between healthy and MCI participants based on their respective MoCA scores. This study illustrates the feasibility of recording reliable EEG in an outpatient setting while presenting a novel method for diagnosing MCI in elderly (age>60) populations.

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 and starting this year, we are delighted to name these awards after them. Prior to the announcement of the Larry Means Award for Best Undergraduate Poster and Edward Lieberman Award for Best Graduate Poster, Dr. Tuan Tran and Dr. Kori Brewer will provide brief introductions about each gentleman. Furthermore, we hope that you are able to enjoy the accompanying biographies written about them.

Larry Means, PhD
Author: Dr. Tuan Tran

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: Dr. Kori Brewer

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

Basic and Clinical Research

(**presenters** are in alphabetical order)

Altering Task-Practice Difficulty by Proportion of Errors in Stroke Rehabilitation: A Pilot Study

Kelly Anderson[1], DeAnna Adkins[2], Jill Stewart[3], Annie Simpson[4], Michelle Woodbury[5]

[1]Department of Occupational Therapy, East Carolina University, [2]NIH/NIND Scientific Review Officer, [3] Dept of Physical Therapy, University of South Carolina, [4]Department of Healthcare Leadership and Management, Medical University of South Carolina, [5]Department of Health Science and Research

Introduction: Challenge is a critical component of effective neurorehabilitation. Neuroscience and motor learning literature provide evidence that both errors and success have unique contributions to the neurophysiology of learning. Challenge impacts skill learning but also influences motivation and confidence. Therefore, we need a better understanding of how errors and success influence the neurorehabilitation process and affect recovery.

Objective: Determine the impact of success/error rate on motor learning, confidence and frustration, and perceived demand of a post-stroke rehabilitation intervention.

Methods *Setting:* Research lab *Design:* Cross-over study. *Participants:* 13 participants (5 female), ages (22-80, μ=60.3 years), (8-76mos) post stroke *Intervention:* Subjects completed 3 days of reaching training in a virtual reality environment, each day randomized to a different training condition. The difficulty of the reaching task was adjusted throughout practice to keep success rate constant (hard: 30-40% success, moderate: 60-70%success, easy:90-100% success). *Measurement:* Confidence- Questionnaire regarding ability to reach quickly and accurately to the target shown; Perceived demand- NASA-TLX assessment of training demand.

Results There were no significant differences noted in retention rates between the three conditions. Participants reported statistically significantly increased confidence after the easy training condition when compared to the hard condition (p=0.004; mean difference -5.39± 1.31; CI-9.04, 1.74) and the medium condition (p=0.004; mean difference -5.39± 1.31; CI-9.04, 1.74). Differences were not noted between the hard and moderate conditions. Significant differences were noted in the overall perceived demand of training only between the hard and easy conditions (p=0.004; mean difference -5.39± 1.31; CI-9.04, 1.74). Sub-analyses of individual items on the NASA-TLX revealed statistically significant differences between conditions for perceived performance, effort required, and frustration levels but there were no significant differences noted in the physical, mental, or temporal demands of the tasks.

Conclusions: None of the assessments or individual question items revealed significant differences between all conditions suggesting that perhaps the contrast between conditions could have been made even greater. Differences were most noted between the easy (90-100% success) and hard (30-40% success) conditions suggesting that individuals may be able to accept greater levels of failure before it affects the confidence and level of frustration.

A New Approach To Remotely And Non-Invasively Assess REM And Non-REM Sleep In A Mouse Model Of Restless Legs Syndrome

Joseph Basco[1], Heidi Kloefkorn-Adams[2], Bill Goolsby[2], Shawn Hochman[2], Stefan Clemens[1]

[1] Department of Physiology and Brody School of Medicine, East Carolina University, Greenville, NC
[2]Department of Physiology, Emory University School of Medicine, Atlanta, GA

Restless Legs Syndrome (RLS) is a neurological condition in which the patient experiences the urge to move their legs and is often reported as a painful or a burning sensation. RLS symptoms follow a circadian cycle and are most prevalent and severe late in the day and at night, thus compromising sleep. RLS responds well to dopamine receptor agonists that target the D3 receptor (D3R) subtype, and while the D3R knockout mouse (D3KO) mimics several of the behavioral deficits observed in the clinic, its sleep behavior has not yet been explored. We here present a novel approach to record sleep activity, REM and non-REM sleep stages, and breathing activity in non-contact mode.

A standard mouse cage was divided into compartments that contained food, water, and that each were shielded from each other and fitted with a pair of commercially-available Plessey electric field sensors. A low-light capable HD camera was placed in front of the cage to monitor the animal behavior and correlate it with the Plessey recordings. One animal (male wild type, WT, or D3KO) was placed in each compartment and the activities were recorded for 2 subsequent days, for up to 5 hours each. Plessey data were captured with pClamp and exported and analyzed as sonograms with Spike 2. Frequency analyses detected a 4-5 Hz band in resting animals that has been associated with breathing.

Our pilot data show that WT and D3KO animals show different sleep patterns. We regularly observed that WT animals coil up in their nest and display the 4-5 Hz frequency band as early as in the 2nd hour of the 5 hour-observation period. In contrast, D3KO continued to pace and climb around the cage and we did not detect any sleep activity in D3KO before the 3rd hour of observation.

In future studies we will utilize this approach to observe sleep behaviors for several different comorbidities, drug treatments, and to better understand RLS in its entirety.

The Effects Of Acute Exercise On NPY/AgRP and POMC Neuron Activity In The Arcuate Nucleus Of The Mouse Hypothalamus

Wyatt Bunner[1, 2], Brenton Thomas Laing[1,2,3], Taylor Landry[1,2,3], Hu Huang[1,2,3,4]

[1]Department of Kinesiology, East Carolina University, Greenville, North Carolina, USA
[2]East Carolina Diabetes and Obesity Institute, East Carolina University, Greenville, North Carolina, USA
[3] Human Performance Laboratory, Collage of Human Performance and Health, East Carolina University, Greenville, North Carolina, USA
[4]Department of Physiology of East Carolina University, Greenville, North Carolina, USA

Background: While much is known about the role of NPY/AgRP and POMC neurons to regulate energy homeostasis, less is known about how forced energy expenditure modulates these neurons and how this relates to energy intake. Therefore, we investigated the effects of acute exercise on neuronal activity in the arcuate nucleus of the hypothalamus.

Methods: NPY-GFP reporter mice were utilized to measure neuron activity. Exercise was performed by introducing the mice to a treadmill and running at a speed of 13.0 m/min for an hour and compared to a sedentary control group. Mice were sacrificed immediately after the bout of exercise by intracardial perfusion and brains were harvested and sectioned. For POMC identification, immunofluorescence staining was conducted. Co-localization of cFOS with the neurons of interest was used as a proxy of neural activation. To measure changes in firing rate, the same exercise protocol was employed, and cell attached patch-clamp electrophysiology recordings were performed on arcuate NPY-GFP expressing neurons. AAV carrying inhibitory DREADD was injected into arcuate of AgRP-ires-CRE mice along with CNO injection before acute exercise to inhibit AgRP neuronal activation. Food intake was measured at different time points after acute exercise.

Results: While we observed no difference in c-FOS in POMC neurons, immediately after exercise, c-FOS in arcuate NPY/AgRP neurons are significantly increased compared to the control group. This result was further confirmed by a significant increase in firing rate in NPY/AgRP neurons by electrophysiology recording. Food intake was significantly increased immediately after an acute bout of exercise. This exercise induced food intake was abolished while the AgRP neurons are inhibited.

Conclusion: We demonstrated significantly greater arcuate NPY/AgRP activation immediately after exercise compared to sedentary control, while POMC neurons remained unaffected. Notably, this exercise induced energy deficit also causes a significant increase in food intake post-exercise. Inhibition of AgRP neuron significantly negates this increase in food intake, suggesting that NPY/AgRP activation is critical for acute exercise induced food intake in un-trained mice.

Social Regulation of Dopaminergic Modulation of Spinal Motor Circuit Activation in Zebrafish (*Danio Rerio*)

Katie N. Clements, Faith K. Heagy, Fadi A. Issa

Department of Biology, East Carolina University, Greenville NC 27858

Social relationships are an important component of social communities; allowing resources to be properly allocated based on social role. One of the main focuses of our lab is to understand how social relationships influence motor behaviors and what underlying neural changes are modulating these behaviors in a status-dependent manner. Using zebrafish as a model, we were able to determine that stable social relationships influence motor output. When paired, male zebrafish form a social relationship consisting of a dominant and subordinate fish. Using a non-invasive electrophysiological approach, we were able to record field potentials generated by two distinct motor behaviors – escape and swim. We found that fish who assume a subordinate role modify their motor behavior in that they swim less and escape more than their dominant counterpart. Because of its known role in social regulation, motivation, and movement, we initially focused on whether dopaminergic signaling changed in a status-dependent manner. Using a pharmacological approach, we systemically injected L-DOPA along with D1R, D2R, and D3R agonists and antagonists. Most interestingly, blocking the D1R caused dominant fish to produce motor behaviors mimicking a subordinate phenotype; elevated escape sensitivity and suppressed swimming activity. Subordinate fish were not affected by D1R agonist or antagonist. These results correlate with our gene expression analysis performed on zebrafish whole brains, where subordinates shows a significant decrease in expression levels of D1R. To corroborate these findings we tested the escape and swim behaviors of a transgenic zebrafish line that lacked the D1R. We found that homozygous D1R fish escape and swim with a subordinate phenotype. Taken together, it appears that dopaminergic signaling is modified by social relationships; however, it is important to note that the dopamine results are contradictory to what was expected. D1R is known for its excitatory post-synaptic potential and yet blocking the D1R elicited a more sensitive escape response in dominants. This led us to hypothesize that dopamine signaling may be influencing these behaviors indirectly through an inhibitory interneuron. Two of the primary inhibitory inputs on the Mauthner neuron are GABA and Glycine. To determine which inhibitory input the D1 is acting through, we administered GABA and Glycine antagonists. With the GABA antagonist, subordinates showed a decrease in escape response while dominants showed no change. If D1 was acting through GABA, we would expect dominants to show an increase in sensitivity, mimicking the D1 antagonist results; but with these results, it does not appear that D1 is acts through GABA to influence the Mauthner. After Glycine antagonist administration, dominants showed an increase in sensitivity while subordinates showed no significant changes. This results supports that the D1 may be acting through a glycinergic neuron to mediate the escape behavior in a status-dependent manner. Taken together, our results suggest that social relationships influence communication between neural networks responsible for motor behavior.

Histologic, Immunohistochemical, and Molecular Overlaps Among Primary and Metastatic Neoplasms in the Central Nervous System of Adults: Potential Pitfalls

Deepak Donthi[1], Richard D. Jordan[2], Everette E. Hite[2], K. Stuart Lee[3], Richard T. Dalyai[3], Keith A. Tucci[3], Philip J. Boyer[1]

[1]Department of Pathology and Laboratory Medicine, [2]Brody School of Medicine, and [3]Vidant Neurosurgery, Vidant Health, Greenville, North Carolina.

Background: The histologic distinction between primary and metastatic neoplasms in the central nervous system of adults is usually straightforward. Most primary adult neoplasms are astrocytomas or oligodendrogliomas and have an infiltrative growth pattern in neuroparenchyma while most metastatic neoplasms manifest a pushing margin with gliotic neuroparenchyma. This distinction can be challenging when primary or metastatic neoplasms contain a small round blue cell component.

Methods and Materials: Clinical, imaging, and pathology findings from five cases of central nervous system neoplasm with histologic, immunohistochemical, and/or molecular overlap were compiled over a one-year period. Lymphomas were excluded from consideration.

Results: Cases included a small cell glioblastoma, a glioblastoma with primitive neuronal component, a medulloblastoma, a metastatic small cell carcinoma, and a metastatic non-pigmented melanoma. Histologic overlap included cells with small cell features with scant cytoplasm and a high nuclear to cytoplasmic ratio, nuclear juxtaposition and moulding, and striking perivascular orientation and spread with invasion of neuropil. Immunohistochemical evaluation revealed variable expression of CD56 in all five neoplasms, TTF1 expression in the metastatic small cell carcinoma and both glioblastoma cases (Leica anti-TTF-1 antibody SPT24), and GFAP expression in the glioblastoma and the medulloblastoma cases. Molecular overlap included TERT promoter mutation in the two glioblastoma cases and the medulloblastoma case.

Conclusion: Primary and metastatic neoplasms in the brain can have remarkable histologic overlap creating challenges at the time of frozen section evaluation and during sign-out. Adult medulloblastomas and metastatic small round blue cell neoplasms including lung small cell carcinoma and melanoma can show striking perivascular and to some extent parenchymal invasion that mimics glial neoplasm. Likewise, consideration of a metastatic disease process is evoked by the presence of small cell or primitive neuronal components in astrocytomas and by the rare adult medulloblastoma. Distinguishing primary from metastatic disease can be complicated by (1) variable expression of specific antigens including CD56 and TTF1 and (2) expression of TERT promoter mutation in both glial neoplasms (oligodendrogliomas, primary glioblastomas) as well as adult medulloblastomas. The pathologist must be aware of these overlaps to avoid errant diagnostic conclusions.

Astrogliosis and Microglia Activation in Acyl-CoA Synthetase 6 Deficient Mouse Brain

Regina F. Fernandez[1], Sora Kim[2], Yingwei Zhao[2], Jessica L. Counihan[3], Daniel K. Nomura[3], Julia Chester[4], Jason Cannon[5], Jessica M. Ellis[1]

[1]Department of Physiology and East Carolina Diabetes and Obesity Institute, East Carolina University, Greenville, NC, [2]Departments of Nutrition Science, [4]Psychological Science, and [5]Toxicology, Purdue University, West Lafayette, IN, and [3]Departments of Nutritional Sciences and Toxicology, University of California, Berkeley CA

The omega-3 fatty acid, docosahexaenoic acid (DHA), is enriched in the central nervous system and thought to protect against neurological dysfunction. Yet, the mechanisms regulating DHA enrichment in the CNS remain unclear. To elucidate these mechanisms, we have genetically targeted long-chain acyl-CoA synthetase isoform 6 to create an Acsl6-deficient mouse ($Acsl6^{-/-}$). Acsl6 is an enzyme particularly enriched in the CNS, required for activating fatty acids by ligating them to a Coenzyme A, and predicted to have preference for DHA. We found that Acsl6 deficiency reduces DHA-containing phospholipids in brain and spine by ~50% but did not alter peripheral lipid content in liver or muscle. $Acsl6^{-/-}$ mice perform poorly during a wire hang test and are hyporesponsive to acoustic stimuli. While DHA is predicted to act as an anti-inflammatory and antioxidant agent, $Acsl6^{-/-}$ mice are surprisingly resistant to an exacerbated inflammatory response to lipopolysaccharide and oxidative stress response to paraquat. However, aging in $Acsl6^{-/-}$ mice results in a profound increase in astrogliosis and microglia activation in the brain. Our findings suggest that Acsl6 is a critical enzyme for brain DHA enrichment and protection against astrogliosis and microglia activation.

Histologic Assessment of Specimens Removed by Endovascular Thrombectomy Provides Clinicopathologic Correlation for a New Procedure for Treatment of Acute Ischemic Stroke: Preliminary Evaluation in 11 Cases

Robin L. Harrison[1], Aisha Kousar[2], Richard T. Dalyai[3], Hilal A. Kaanan[3], Wen Zhong[2], Philip J. Boyer[2]

[1]Brody School of Medicine, [2]Department of Pathology, and [3]Vidant Neurosurgery, East Carolina University and Vidant Health, Greenville, NC

Background: Treatment of ischemic stroke has most commonly relied on therapeutic administration of tissue plasminogen activator with the goal of preserving salvageable, ischemically compromised brain parenchyma. Endovascular thrombectomy provides a new tool in the therapeutic armamentarium for treatment of ischemic stroke. Limited studies have evaluated histologic findings of thrombectomy specimens.

Methods and Materials: Following their removal, thrombectomy specimens were evaluated histologically on three levels using hematoxylin and eosin stain and by trichrome stain. Histologic features relevant to the age of the clot were assessed including evidence of white blood cell changes (neutrophil nuclear loss, macrophage activation and hemophagocytosis) and evidence of organization (endothelial cell or fibroblast invasion of the clotted blood) with reviewers blinded to clinical history. A review of the patients' electronic medical record was undertaken and correlation of histologic findings with clinical history was undertaken. A literature search was undertaken using pubmed.gov using key words including thrombectomy and endovascular.

Results: Thrombectomy specimens from 5 men and 6 women, 43- to 91-years-old, who presented with clinical and radiologic features of ischemic stroke were evaluated. Early organization including endothelial cell invasion was noted in 5 specimens consistent with days-old thrombus while minimal organization was noted in the other specimens, consistent with hours to days old thrombus. The two specimens with the most advanced organization were seen in a patient with a previous myocardial infarct and an apical left ventricle lesion being treated with warfarin and the left atrium in a patient with atrial fibrillation subtherapeutic on warfarin, respectively. The exact origin of the other emboli was not known clinically. While at least some of the thrombi originated from complex atherosclerotic plaques but no evidence of atherosclerotic debris was identified. In limited literature reports of histologic evaluation of thrombectomy specimens, a rigorous methodology was not employed and dating was not undertaken.

Conclusions: Histologic evaluation of thrombectomy specimens can provide important data to the clinical team with the goal of, when possible, identifying the source of the embolus and providing data to prevent recurrent emboli. In addition, this evaluation provides an important documentation in the clinical chart regarding the thrombectomy procedure.

Cytoskeletal Regulation of Neurodevelopment in an hIPSC-derived Autism Model

Taylor Rudisill, **Brenna Kirk**, Amanda Petritsch, Emily Wilson, Oleksandr Kirsanov, Adrienne Orbita, Colin Johnson, Pranaya Pakala, Haroon Dar, Storm Davis, Karen Litwa

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

Autism affects 1 in 59 children, and is rapidly increasing in prevalence. Emerging evidence suggests that altered neural connectivity, particularly at the synaptic level, contributes to disease pathology. The actomyosin cytoskeleton drives neural circuit formation, including the development of actin-enriched spines at excitatory synapses. Actomyosin regulatory pathways are commonly disrupted by Autism-associated genetic mutations. Yet, it is still unknown how actomyosin regulation shapes developing neural circuits and how specific regulators contribute to Autism pathology. To address the role of actomyosin regulation in human brain development, we create cortical brain organoids from human induced pluripotent stem cells of individuals with Autism or those who are typically developing. Autism-derived cortical organoids exhibit increased excitatory synapse formation, similar to post-mortem patient samples. Similarly, the RhoA kinase inhibitor, Y-27632, reduces myosin-II activation and increases excitatory synapse area in control organoids. We hypothesize that Y-27632 elevates synaptic Rac1 activity leading to excitatory synapse expansion. In support of this hypothesis, Y-27632 increases phosphorylation of Rac1 targets, LIMK1 and cofilin, in cortical organoids. These results suggest that Rac1 activity dysregulation can promote Autism synaptic pathology, leading us to investigate which Rac1 activity regulators are present during early brain development. Our previous research identified a novel synaptic Rac1 regulator, ArhGAP23, in rat hippocampal neurons. ArhGAP23 also localizes to excitatory synapses of developing cortical organoids. Our current research focuses on how actomyosin regulators, such as ArhGAP23, contribute to critical periods of brain development coinciding with the emergence of Autism pathology. Through the use of human cortical organoids, we demonstrate that coordinated myosin-II and Rac1 activities underlie excitatory synapse development, and alterations in the balance between these actomyosin pathways may promote Autism pathology.

Radical Prostatectomy and Androgen Deprivation Cause a Negative Cumulative Impact on Neuron Survival in the Major Pelvic Ganglia

Jennifer C. McMains[1], Shelby A. Powers[2], Michael R. Odom[2], Elena S. Pak[2], Johanna L. Hannan[2]

[1]Department of Health Education and Promotion, East Carolina University, [2]Department of Physiology, Brody School of Medicine

Prostate cancer is often treated with androgen deprivation therapy (ADT) to shrink the prostate before removal via radical prostatectomy (RP). Following RP, erectile dysfunction (ED) affects 25-100% of men, negatively impacting their quality of life. RP induced ED is primarily attributed to injury of the cavernous nerves which branch from the major pelvic ganglia (MPG). We hypothesize combined ADT+RP will further decrease neurite branching and axon length, increase apoptosis and lead to less nitrergic neurons compared to ADT or RT alone.

Male Sprague-Dawley rats (12 weeks) were separated into control (no surgery; CON), castration (CAST), bilateral cavernous nerve injury (BCNI), or CAST+BCNI (C+B) groups (n=4/grp). At 16 weeks rats underwent BCNI to mimic RP and 2 weeks post-BCNI neurons from the MPG were dissociated and cultured for 72 hours. Neurons were fixed and stained with immunofluorescence for neuron-specific class III beta-tubulin to measure axonal branching and length and then co-stained with TUNEL assay to identify apoptotic neurons. Additional neurons were stained with sympathetic (tyrosine hydroxylase) or nitrergic (neuronal nitric oxide synthase) markers.

Overall, CAST, BCNI and C+B decreased neurite growth and branching, increased apoptosis, and decreased nitrergic neurons compared to CON ($p<0.05$). CAST and BCNI alone both caused 25% decrease in neurite length, 20% decrease in branching and 2-fold increase in apoptosis ($p<0.05$ vs CON). C+B further decreased neurite length by 33%, decreased branching by 50% and doubled apoptosis ($p<0.05$ vs CON). The population of nitrergic neurons were reduced by 60% in the CAST or BCNI alone and by 70% in the C+B ($p<0.05$ vs CON). Sympathetic stained neuron populations are currently being analyzed.

Our preclinical model of ADT+RP demonstrated markedly impaired neuritogenesis and decreased nitrergic, pro-erectile neurons compared to controls, ADT or RP alone. These data indicate that recovery of erectile function following nerve damage due to RP in a state of ADT is unlikely and these patients will presumably have higher incidence and severity of ED. Future studies will examine if testosterone supplementation following RP can restore neurite health and increase the population of nitrergic, pro-erectile neurons in order to recover erectile function.

Timecourse of Synapse Development in Human IPSC-derived Neurons

Adrienne Orbita, Brenna Kirk, Karen Litwa

Autism is one of the fastest growing developmental disabilities, currently affecting 1 in 58 children in North Carolina. Thus, there is a pressing need to understand the molecular mechanisms leading to the development of Autism. Autism is a cognitive disorder characterized by social deficits and the presence of restricted and repetitive patterns of behaviors or interests. At a cellular level, post-mortem patient brains exhibit increased excitatory synapses.

The underlying mechanisms for this increase are still unclear. However, decreased inhibitory signaling may affect synaptic refinement. In order to capture synapse formation during early brain development, we culture human cortical neurons and brain organoids. Our Autism-derived cortical organoids exhibit increased excitatory synapse formation. I have developed a 2-dimensional timecourse assay to capture the development of excitatory and inhibitory synapses to determine when imbalances emerge in Autism. Thus, human IPSC-derived neurons and cortical organoids provide a unique opportunity to observe the development of Autism pathology and to test our hypothesis that decreased inhibitory synapses result in increased excitatory synapse formation.

Designing an Optically Triggered Biosensor Inspired by Cofilin-Actin Rods

Fatema Salem, Abu-Bakarr Kuyateh, Dr. Robert Hughes

ECU Department of Chemistry

Actin, one of the most abundant eukaryotic proteins, is a part of many different cellular processes, including muscle contraction, cell division, cell motility, cell signaling, and the establishment and maintenance of cell junctions and cell shape. Actin is also highly affected by the oxidative environment of the cell and is therefore a sensor of changes in the levels of reactive oxygen species (ROS). As a result, actin holds promise as the basis for an engineered biosensor for the detection of the oxidative changes in the cell. High levels of oxidative stress play a significant role in the progression of major number of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (AD). In AD, neuronal oxidative stress is associated with the formation of cofilin-actin rods, a morphologically distinct protein-protein complex that has been implicated in synaptic loss in neural cells. A biosensor based on the phenomenon of cofilin-actin rod formation may have utility in characterizing neurodegenerative disease progression in model systems, or in the screening of compounds targeted towards slowing the effects of AD. In this work, we have created an optogenetic switch that enables light-activated cofilin-actin complex formation in cells undergoing oxidative stress. We demonstrate its sensitivity to various levels of oxidative stress by characterizing light dependent cofilin-actin clustering, spatial localization, and morphology in cell culture. Furthermore, we have undertaken a mutagenesis approach to further investigate the roles of key residues in the ATP-binding pocket of actin in cofilin-actin rod formation.

The Function of *Lipoma HMGIC Fusion Partner-Like 5* (*Lhfpl5*) Paralogs in Zebrafish Hair Cells

Alexandra Venuto[1], Itallia Pacentine[2], Teresa Nicolson[2], Timothy Erickson[1,2]

[1]Department of Biology, East Carolina University, Greenville NC
[2]Vollum Institute and Oregon Hearing Research Center, OHSU, Portland OR

Hair cells sense and transmit auditory and vestibular 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 / TMHS) acts is 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 contributions to MET channel assembly and function have not been analyzed.

Here, we show that teleost fishes possess paralogous *lhfpl5a* and *lhfpl5b* genes, likely originating with the teleost-specific whole-genome duplication event. In zebrafish, these paralogs are expressed in discrete populations of hair cells: *lhfpl5a* expression is restricted to auditory and vestibular hair cells, while *lhfpl5b* expression is specific to hair cells of the lateral line neuromasts. Consequently, *lhfpl5a* mutants exhibit defects in auditory and vestibular function, while disruption of *lhfpl5b* affects hair cells of the lateral line only. Using a stably integrated GFP-Lhfpl5a transgene, we show that Lhfpl5a localization at the tips of stereocilia is stabilized by MET complex proteins Pcdh15a and Cdh23. In agreement with results from mouse hair cells, we find that Pcdh15a localization in at the tips of stereocilia requires Lhfpl5a. However, in contrast to previous reports, we do not see evidence that Tmc protein localization depends upon Lhfpl5 function. In zebrafish *lhfpl5a* mutants, Tmc1-GFP and Tmc2b-GFP transgenes still localize to the hair bundle of vestibular hair cells. In summary, this work demonstrates the subfunctionalization of zebrafish *lhfpl5a* and *lhfpl5b* paralogs through cis-regulatory divergence, and provides a new perspective on the function of Lhfpl5 in regulating MET channel assembly.

Extramedullary Tanycytic Ependymoma of the Spinal Cord: Literature Review and Context of Variants and Topography Seen at a Single Institution

Niki T. Winters, B.S.[1], Jonathan D. Dvorak, M.D.[1], K. Stuart Lee, M.D.[2], Shawn M. Collins, B.S.[3], Regis Hoppenot, M.D.[2], Hilal Kanaan, M.D.[2], Wen Zhong, M.D.[3], Philip J. Boyer, M.D., Ph.D.[3]

[1]Brody School of Medicine, [2]Eastern Neurosurgical and Spine Associates, and [3]Department of Pathology and Laboratory Medicine, and East Carolina University Brody School of Medicine and Vidant Health, Greenville, North Carolina.

Background: Ependymomas are glial neoplasms which arise along ventricular surfaces in the cerebral hemispheres and posterior fossa and along the spinal canal of the spinal cord. Histopathologically, classic, papillary, clear cell, tanycytic, and myxopapillary variants of ependymoma are recognized and can be classified as grade I, II, or III (anaplastic) using World Health Organization (WHO) criteria. Myxopapillary ependymomas typically arise in an extraaxial location in association with the conus medullaris, cauda equina, or filum terminale and are WHO grade I lesions; other variants typically arise as intramedullary lesions and are grade II or III lesions.

Hypothesis: Tanycytic ependymomas of the spinal cord are rare at our facility and in the literature.

Methods and Materials: After encountering an extramedullary tanycytic of the spinal cord, a search of the East Carolina University Department of Pathology and Laboratory Medicine laboratory information system was conducted to identify all cases of ependymomas accessioned during the previous 30 years. Cases for which glass slides were available were reviewed and classified using 2016 WHO criteria and the site of occurrence was documented. A PubMed search was carried out using key words including ependymoma, tanycytic, myxopapillary, and spinal cord. Medical records for five representative spinal cord cases were reviewed and summarized.

Results: A total of 88 cases diagnosed as ependymomas have been treated neurosurgically at our center, 29 (33%) of which were identified in association with the spinal cord as intramedullary or extramedullary lesions. Spinal cord lesions have included classic (15), myxopapillary (14), and tanycytic (1) variants. Our tanycytic ependymoma occurred in the conus medullaris and, to date, only six other extramedullary cases have been reported. Literature search revealed that approximately 50% of adult ependymomas occur in the spinal cord and the uncommon tanycytic ependymoma most often occurs as an intramedullary lesion.

Conclusions: In our series, approximately a third of ependymomas occurred within the spinal cord, a lower proportion than reported in the literature. Tanycytic ependymomas are uncommon and usually occur as intramedullary lesions within the spinal cord. Given their spindle cell phenotype and general absence of rosettes, they can be confused with both astrocytomas and Schwannomas.

Novel Psychotropics, Perinatal Substance Abuse and Child Maltreatment: Qualitative Study

Mary Ellen Wright, PhD, ARNP, CPNP[1], **John E. Wright**, MD, FAAP[2]

[1]Clemson
[2]East Carolina University Brody School of Medicine

Alpha pyrrolidinopentiophenone (α-PVP) acts as a norepinephrine-dopamine reuptake inhibitor. Behavior post-use of this psychostimulant can cause hyperstimulation, paranoia, hallucinations, excited delirium overdose, and suicide. Reports of adult behavior characteristics after use of this synthetic neurostimulant contain descriptions of disturbing and severe child abuse and endangerment. Purpose: The purpose of the study is to describe the behaviors of parents and caregivers of children under the influence of α-PVP, from reported cases of child maltreatment. Design: The study was a qualitative case study phenomenological analysis using de-identified reports of child maltreatment with associated caregiver use of the synthetic neurostimulant drug α-PVP. Method: Collection of child maltreatment cases (n = 151) that were reported contained information that the child's caregiver/parent was using α-PVP during the time of the potential incidence of child maltreatment. Results: Phenomenological thematic analysis was performed (n=151) and categories of child maltreatment were identified that included: abandonment, child reports of unusual parent behaviors, physical abuse of the child, violent behaviors of the adult, and paranoid ideations of the adult.

Analysis of Functional Domains in Tomt, a Protein Required For Mechanotransduction in Sensory Hair Cells

Denise Zangwill[1], Teresa Nicolson[2], and Timothy Erickson[1,2]

[1]Department of Biology, East Carolina University, Greenville NC
[2]Vollum Institute and Oregon Hearing Research Center, OHSU, Portland OR

Mutations in the gene *transmembrane O-methyltransferase* (*TOMT* / *LRTOMT2*) causes non-syndromic deafness in humans, mice, and zebrafish. TOMT is enriched in the secretory pathway of sensory hair cells where it facilitates the apical trafficking of Transmembrane channel-like proteins TMC1 and TMC2, the putative pore-forming subunits of the hair cell mechano-electrical transduction (MET) channel. Co-immunoprecipitation experiments show that mouse TOMT and TMC1 can directly interact in cultured cells, but it remains unclear which regions of the proteins are involved in this interaction. Here, we describe our work to define the functional domains in TOMT with the goal of understanding how the TOMT-TMC interaction facilitates the formation of the MET channel in sensory hair cells.

TOMT is predicted to have an N-terminal transmembrane domain (TMD) followed by a "linker" region and a putative O-methyltransferase (O-MT) domain. Our preliminary results using a split GFP strategy suggest that TOMT's linker and O-MT domains are oriented towards the cytoplasm. Interestingly, genetic evidence suggests that the O-MT domain is playing a non-enzymatic role, since mutating critical residues of the predicted enzymatic active site have no effect on TOMT function in hair cells. Nonetheless, results of our structure-function experiments suggest that both the N-terminal and the O-MT domains are required together for TOMT's function in hair cells. Based on the available evidence, we hypothesize that TOMT's cytoplasmic linker and O-MT domains function non-enzymatically to interact with the TMCs and promote MET complex assembly. Future studies will define the protein-protein interaction interface between TOMT and the TMCs and determine their functional consequences.

Poster Session 1
Clinical Case Studies

(**presenters** are in alphabetical order)

Giant Cell-Rich Solitary Myofibroma of the Vertebral Column Causing Spinal Cord Compression in a Child: An Uncommon Presentation of an Uncommon Lesion

J. Christopher Carter[1], Jonathan D. Dvorak[1], Hilal Kanaan[2], Hyder Arastu[3], Philip J. Boyer[4], K. Stuart Lee[2]

[1]Brody School of Medicine, [2]Vidant Neurosurgery, and Departments of [3]Radiation Oncology and [4]Pathology and Laboratory Medicine, East Carolina University and Vidant Health, Greenville, NC

Background: Myofibromas are rare, benign neoplasms of mesenchymal origin that primarily occur in soft-tissues, occasionally involve osseous structures, and can be either solitary or multicentric. We report a case of a solitary myofibroma presenting with symptoms due to a T7 vertebral body lesion and spinal cord and nerve root compression.

Methods and Materials: Medical records were evaluated and summarized. A literature search was conducted using key words including myofibroma, giant cel myofibroma, and spinal column.

Results:
- Case Report: An 11-year-old boy presented with progressive back pain and lower extremity weakness leading to ambulation deficits. Imaging revealed a left extradural mass involving and apparently arising from the T7 vertebral bone with significant spinal cord compression. Biopsy and then surgical resection were undertaken. Histologic evaluation revealed a giant cell-rich myofibroma consisting of spindle-shaped cells in a distinct fascicular pattern with mild cytologic atypia, minimal mitotic activity, and absence of identifiable necrosis. Abundant multinucleated giant cells were noted compatible with osteoclasts. A radiation oncology consultant felt that there was no immediate role for radiation therapy in the absence of a lesion. During the subsequent two years of followup, MRI scans identified an apparent complete resection with no evidence of residual or recurrent neoplasm and with decompression of the spinal cord. Clinically, although initial symptom improvement were noted, there was return of some weakness not explained by imaging studies.
- Literature Search: Myofibromas typically present in infancy or adolescence and undergo periods of rapid growth, stabilization, and in some circumstances spontaneous regression. A total of 11 cases of involvement of vertebral column bone by solitary myofibroma were identified. None of the reported cases contain the large number of giant cells seen in our case. Given the paucity of cases in the literature, the optional treatment protocol is not known. Treatment of myofibromas at other sites range from observation to surgical resection with or without adjuvant radiotherapy.

Conclusions: We report a case of a solitary myofibroblastoma treated only with surgery to date. The location of the lesion is uncommon and the reporting of treatment outcomes may help guide therapy of subsequent patients.

Atypical Presentation of Neurotoxoplasmosis as a Solitary, Ring-Enhancing, Intraaxial Lesion: Initial Manifestation of Previously Undiagnosed Acquired Immunodeficiency Syndrome

Matthew W. Coco[1], Robin L. Harrison[1], K. Stuart Lee[2], Shawn M. Collins[3], Philip J. Boyer[3]

[1]Brody School of Medicine, [2]Vidant Neurosurgery, and [3]Department of Pathology, East Carolina University and Vidant Health, Greenville, NC

Background: The human immunodeficiency virus (HIV) is a retrovirus that replicates within and destroys CD4 lymphocytes, ultimately leading to acquired immunodeficiency syndrome (AIDS). AIDS is diagnosed if a patient has a CD4 count <200/uL or manifests an AIDS-defining illness. The most common of the AIDS-defining infections of the central nervous system is toxoplasmosis, a parasitic infection by Toxoplasma gondii. Neurotoxoplasmosis classically presents with neurological/motor deficits, intractable headaches, and multifocal, ring-enhancing lesions on MRI. We present a case with an atypical manifestation of neurotoxoplasmosis which lead to a new diagnosis of AIDS.

Methods and Materials: Clinical features of the case were summarized. A PubMed.gov literature search was undertaken. A search of our institution's pathology laboratory information system was undertaken and surgical and autopsy pathology toxoplasmosis cases were identified reviewed.

Results:
Case Report: A 45-year-old man with a history of hypertension presented with progressively severe left temporal headaches that did not respond to antibiotics for suspected sinus infection or pain medications along with progressive photophobia and blurry vision. Computed tomography and magnetic resonance imaging brain scans identified a solitary, 6.4 x 3.9 x 4.7cm, ring-enhancing lesion in the left temporal lobe which was surgically removed. Immunohistochemical evaluation of the specimen and serology were diagnostic of neurotoxoplasmosis. Additional laboratory studies revealed HIV infection, a CD4 count of 90/uL (normal 330-2540/uL), and a CD4:CD8 ratio of 0.2 (normal 1.2-2.6).
Literature Search and Laboratory Information System Review: Toxoplasmosis most commonly and characteristically presents in the central nervous system with multiple lesions, most commonly in the setting of AIDS and other immunodeficiency states. A solitary toxoplasmosis lesion is uncommon, represented in the literature as case reports. This is the only surgical or autopsy pathology case reviewed at our institution to date.

Discussion: This atypical presentation of neurotoxoplasmosis in a patient with a previously undiagnosed HIV infection shows the importance of entertaining a broad differential and being aware of confirmation bias. This case also emphasizes the importance of clinician awareness of Center for Disease Control guidelines and clinical triggers that may suggest a patient's risk for HIV, as well as the recommended screening guidelines for HIV.

Extradural Spinal Column Solitary Fibrous Tumor (Hemangiopericytoma Variant) Extending Through Vertebral Foramen into Pleural Space: Novel Presentation and New World Health Organization Classification

Sean C. Johnson[1], Kotaro Takeda[2], Hilal A. Kanaan[3], Keith A. Tucci[3], Jasmin Jo[4], Carlos J. Anciano Granadillo[5], Hyder Arastu[6], F. Zahra Aly[2], Philip J. Boyer[2]

[1]Brody School of Medicine, [2]Department of Pathology and Laboratory Medicine, [3]Vidant Neurosurgery, and Departments of [4]Internal Medicine (Hematology-Oncology), [5]Cardiothroacic Surgery, and [6]Radiation Oncology

Background: Primary dura-based neoplasms include, using the 2016 World Health Association classification of tumors of the central nervous system, solitary fibrous tumor (SFT) which now includes both the classic, low-grade SFT (grade I) and the higher grade and more biologically aggressive hemangiopericytoma variant (HPC, grade II or III). We describe a case of a SFT with both (1) intradural and extramedullary features and (2) pleural involvement.

Methods and Materials: Clinical features of the cases are summarized. A PubMed.gov literature search was undertaken using search terms including SFT, HPC, vertebral, spinal, and pleural.

Results:
Case Report: A 48-year-old woman presented with initially vague musculoskeletal complaints which progressed to right shoulder and arm pain followed by then sensory changes and progressively severe weakness in her bilateral lower extremities rendering her unable to walk. Imaging studies identified (1) a well-circumscribed intradural and extramedullary soft tissue mass on the right at T1-T2, (2) with extension into and widening of the right T1-T2 bony foramen, and (3) extension into the pleural region. The spinal and then pleural components were removed by separate procedures. Histological examination of both specimens revealed a grade II SFT, HPC variant, WHO grade II based on immunohistochemical analysis (expression of CD34 and STAT6 with no expression of epithelial membrane antigen). Posteroperative imaging revealed no residual neoplasm. The patient experienced initial resolution of most of her symptoms but some return of numbness subsequently.
Literature Review: SFTs are rare central nervous system neoplasms which can arise intracranially and along the spinal cord. No SFT or HPC case similar to this case was identified in the literature.

Conclusion: SFTs are neoplasms of presumed mesenchymal origin, possibly from pericytes, first described as rare tumors of the pleura. In this case, origin of the neoplasm in the extramedullary vertebral column with extension through the vertebral foramen into the plural space was favored by radiologists and surgeons rather than a primary pleural lesion extending into the vertebral column. The reclassification of HPC as a variant of SFT is concordant with WHO soft tissue tumor nomenclature but will lead to nomenclature challenges in the literature.

Fourth Ventricle Hemangioblastomas Arising from Medulla: Report of Two Cases and Review of Literature Regarding Unusual Location

Ghalib Khan[1], Richard T. Dalia[2], Jasmin Jo[3], Shawn M. Collins[4], Wen Zhong[4], K. Stuart Lee[2], Philip J. Boyer[4]

[1]Brody School of Medicine, [2]Vidant Neurosurgery, and Departments of [3]Medicine (Hematology-Oncology), and [4]Pathology and Laboratory Medicine, East Carolina University and Vidant Medical Center, Greenville, North Carolina

Background: Hemangioblastomas are a primary neoplasm of the central nervous system of uncertain histogenesis. They most commonly occur in the infratentorial region, within the cerebellum. Presentation in the fourth ventricle is uncommon. We present two cases of fourth ventricle hemangioblastoma arising in association with the medulla.

Methods and Materials: The patients' clinical records were reviewed and summarized. A search of the East Carolina University Department of Pathology and Laboratory Medicine laboratory information system was conducted to identify all cases of hemangioblastoma accessioned during the previous 30 years. Cases for which glass slides were available were reviewed and classified using 2016 WHO criteria and the site of occurrence was documented. A literature search was conducted including key words hemangioblastoma, fourth ventricle, and medulla.

Results: In case 1, a lesion arising from the right dorsolateral region of the medulla was identified on brain imaging of a 46-year-old man; it was interpreted to be incidental with no evidence of hydrocephalus and was examined at autopsy. In case 2, a 33-year-old man presented with vision deficits due to papilledema in the setting of obstructive hydrocephalus due a large fourth ventricle mass associated with the medulla which was resected. Hemangioblastoma was not in the radiologic or clinical differential diagnosis in either case. In the literature and in our institution, most hemangioblastomas arise within the cerebellum or, less commonly, the brainstem without involvement of the fourth ventricle. Only 5 other cases of hemangioblastoma arising in association with the medulla and involving the fourth ventricle have been documented in the literature while we have seen 1 case and 32 cases documented in the literature identified a fourth ventricle lesion arising from the ventricle surface or extending from sites other than the medulla, e.g. cerebellum in our case.

Conclusions: Hemangioblastomas are among the most common infratentorial neoplasms seen in adults and most occur within the cerebellum or, less commonly, the brainstem. A fourth ventricle location has been documented only occasionally, most commonly presenting with symptoms due to obstructive hydrocephalus. While uncommon, hemangioblastoma should be included in the differential diagnosis of an infratentorial neoplasm involving the fourth ventricle.

Atrial Myxoma Presenting with Hemorrhage and Multifocal Infarcts in Brain of a 59-Year-Old Man: Uncommon Outcome for the Most Common Primary Heart Neoplasm

Arthur M. Samia[1], Deepak Donthi[2], Wen Zhong[2], Eric M. Martin[3], Shawn M. Collins[2], Philip J. Boyer[2], K. Stuart Lee[4]

[1]Brody School of Medicine, [2]Department of Pathology and Laboratory Medicine, [3]Eastern Radiologists, and [4]Vidant Neurosurgery, Vidant Health, Greenville, North Carolina.

Background: Atrial myxomas are the most common primary neoplasm of the heart. In addition to effects on the heart and mitral valve, adverse effects on the brain include embolization of either (1) fragments of the neoplasm or (2) thrombus formed in the atrium in the setting of stasis due to obstruction by the lesion. We report the case of a man with symptoms from multifocal brain lesions and subsequent identification of a large atrial myxoma.

Methods and Materials: Clinical, imaging, and pathology findings from the patient were summarized. A literature search using key words including atrial myxoma, embolus, and brain was undertaken.

Results:
Case Report: The patient is a 59-year-old man who presented with headaches, visual changes, and difficulty with word finding. Computerized tomography (CT) and magnetic resonance imaging scans revealed (1) a large left occipital lobe enhancing mass, (2) multiple other hemorrhagic lesions in the cerebral hemispheres and cerebellum, and (3) multiple old lacunar infarcts. A CT scan of his chest, abdomen, and pelvis was initially read as negative. Review requested by the neurosurgical team revealed a lobular left atrial and left ventricular lesion spanning the mitral valve, 2.3 X 3.5 cm. Histologic evaluation of the resected heart lesion revealed an atrial myxoma with a very complex surface including multiple papillary excrescences. Resection of the left occipital lobe lesion revealed embolic myxoma in vessels and free in the brain along with acute, subacute, and chronic hemorrhage.
Literature Search: Symptomatic atrial myxomas have been identified in patients ranging from childhood to the eighth decade variably coming to clinical attention due to heart symptoms or symptoms related to embolization. When brain emboli are identified, ischemic infarcts are much more common than hemorrhages.

Conclusion: This case is remarkable due to late presentation, large size of the atrial myxoma, presence of abundant cerebral hemisphere and cerebellar lesions, and histologic comparison of the heart and brain lesions including the identification of myxoma within and damage to brain blood vessels. The exact nature of the multiple, smaller lesions in his brain is not known: they could be the result of either thromboemboli or myxoma emboli.

Spectrum of Proliferative Hematopoietic Lesions Seen in the Central Nervous System at a Single Medical Center: Diffuse Large B Cell Lymphomas (of Course) and Lots More

Nupur Sharma[1], Raven Delgado[2], Darla Liles[3], Brian Dangott[1], Jasmin Jo[3], Regis G. Hoppenot, K. Stewart Lee[4], Renuka Melanie[1], Lenwood P. Smith[4], Keith A. Tucci[4], Richard T. Dalyai[4], Shawn Collins[1], Philip J. Boyer[1], Bethany Vallangeon[1]

[1]Department of Pathology, [2]Brody School of Medicine, [3]Department of Internal Medicine (Hematology-Oncology), East Carolina University and [4]Vidant Neurosurgery, Vidant Medical Center, Greenville, North Carolina

Background: Improvements in the pathologic characterization of hematopoietic disease employing clinical, immunophenotype, and molecular data is reflected in the more complex 2017 World Health Organization classification of tumors of hemopoietic and lymphoid tissue. A relatively restricted group of hematopoietic lesions is recognized in the central nervous system (CNS). While diffuse large B cell lymphomas are the most common lesion seen in the CNS, both as primary and secondary processes, a limited range of hematopoietic lesions, mostly B-cell processes, can be identified.

Methods: This retrospective study gathered both typical and uncommon central nervous system lesions seen during the previous four years. Literature searches were conducted using the key words central nervous system, lymphoma, and hematopoietic neoplasm.

Results: A wide range of lesions, mostly B cell lymphoma variants, were identified during the four-year period. The most common neoplasm was diffuse large B-cell lymphoma, both primary (arising in the CNS) and secondary (arising outside of the CNS with secondary involvement) variants, seen in both immunocompetent and immunodeficient individuals. In addition, cases of marginal zone lymphoma, mucosal associated lymphoid tissue (MALT) type, follicular lymphoma; intravascular large B-cell lymphoma; and autoimmune lymphoproliferative syndrome type 5 (ALPS-5) were identified. Optimal diagnosis usually requires a biopsy. However, in some cases, the diagnosis of DLBD was identified on a cerebrospinal fluid tap avoiding the need for a biopsy. In several cases in which lymphoma was suspected clinically, including in a renal transplant patient, patients were treated with corticosteroids prior to biopsy and histopathologic findings were non-diagnostic. One of these patients was subsequently revealed to have a diffuse large B-cell lymphoma on re-biopsy after discontinuation of corticosteroid therapy.

Conclusions: For optimal diagnosis and treatment, it is critical for the clinician and the pathologist to be aware of the range of hematopoietic lesions seen in the brain and to gather sufficient specimen to perform all necessary testing.

Poster Session 2

Basic and Clinical Research

(**presenters** are in alphabetical order)

Recurrent Multifocal Parenchymal Hemorrhages in Multiple Sclerosis: A Case Report and Literature Review

N. Bellamy[1], GA. Koromia[2], D. Price[3]

[1]Brody School of Medicine at East Carolina University, Greenville, NC, [2]Joan C. Edwards School of Medicine at Marshall University, Huntington, WV, [3]Department of Neurology, Vidant Hospital, Brody School of Medicine at East Carolina University, Greenville, NC

Multiple sclerosis (MS) is a debilitating neurologic disease, which causes severe long-term morbidity and mortality. Our patient had an unusual presentation with recurrent multifocal parenchymal hemorrhages and a history of secondary progressive MS with previous cerebral microbleeds and absent vascular risk factors.

A 40-year-old male with past medical history of secondary-progressive MS with paraplegia, post-traumatic stress disorder (PTSD), and glucose-6-phosphate dehydrogenase deficiency presented to an outside hospital for seizures. At baseline, he could speak and feed himself independently; however, he could only perform simple motor functions. He presented encephalopathic and non-communicative after three seizures witnessed at home. In the emergency department an additional seizure was witnessed. A computed tomography (CT) scan of the head showed two intraparenchymal hemorrhages: a 3 x 2.6 cm frontal hemorrhage and a 1.2 cm midbrain hemorrhage causing a 2.4 mm left-to-right midline shift. Keppra was initiated and he was transferred to our facility's ICU for further management. Follow up magnetic resonance imaging (MRI) did not show an infarct but did show multiple areas of microbleeds in that region, in addition to a new subacute hemorrhage in the frontal lobe and left thalamus, and significant worsening of his MS lesions from four months prior.

Our patient was young, and lacked the traditional risk factors for intracerebral hemorrhage. Many of his bleeds occurred over his MS plaque sites and thus suggest MS as the precipitating factor of his recurrent multifocal parenchymal hemorrhages. Previous studies have suggested vascular fragility associated with neorevascularization around MS plaques as an underlying etiology. Unfortunately, the correlation between cerebral hemorrhages and MS has not been well elucidated. More studies are needed to ascertain the specific pathophysiology of how and why some patients with MS have an increased risk for cerebral hemorrhages. It is important for clinicians to consider this correlation when caring for patients with MS.

Targeting Mesocortical and Mesoaccumbens Dopamine Neurons with DREADDs Using the Combination of Adeno-Associated Viral Vectors and Retrograde Transported of Herpes Simplex Viral Vectors

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

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

The mesencephalic dopamine neurons have various physiological roles depending on the areas of the brain they innervate. Mesocortical dopamine innervating the prefrontal cortex has been found to be critical for executive control and working memory while mesoaccumbens dopamine in the nucleus accumbens signals salient or relevant environmental stimuli. Abnormalities in these pathways contribute to diseases including schizophrenia and addiction. Understanding how alterations in signaling to these neurons alters their function is critical to understanding how exposure to drugs of abuse or environmental risk factors for schizophrenia contribute to these diseases. This current work is focused on using viral vectors to specifically target these dopamine neuron populations based on area of the brain they innervate and activate or inhibit these neurons with DREADDs (Designer receptors exclusively activated by designer drugs). Preliminary studies tested independently the efficiency of retrograde transport of HSV from the nucleus accumbens and dopamine neuron infection with AAV. Both independently labeled dopamine neurons in the ventral tegmental area. We next tested a combination of a retrogradely transported herpes simplex viral vector injected into the nucleus accumbens or prefrontal cortex and adeno associated viral vector in the ventral tegmental area. The first trial used an HSV expressing Cre and YFP in combination with an AAV expressing hM3Dq and mCherry. Analysis found good retrograde transport of the HSV and expression of mCherry indicating co-infection of those neurons with AAV. Of interest was the observation that some mCherry labeled neurons did not show detectible YFP labeling, suggesting that only low levels of cre appear necessary for expression of mCherry. As expected a much larger dopamine neuron population was labeled with nucleus accumbens injection as compared to prefrontal cortex injection. Initial trials expressing hM3Dq and mCherry via the HSV has not been successful. Future studies will investigate molecular changes that result from altering activation of these dopamine neuron populations with DREADD agonist clozapine to help understand how environmental stimuli can alter dopamine function and contribute to disease.

α-Klotho Suppresses NPY/AgRP Neuron Activity and Regulates Glucose Metabolism in Mice

T. Landry[1,2], B. Laing[1,2], P. Li[1, 2], W. Bunner[1,2], Z. Rao[1,2], M. Naderi[1, 2], Y. Yuan[1, 2], H. Huang [1,2,3]

[1]Department of Kinesiology, East Carolina University, 115 Heart Drive Rm 4108, Greenville, NC, United States
[2]Diabetes and Obesity Institute, East Carolina Heart Institute, 115 Heart Drive Rm 4108, Greenville, NC, United States
[3]Department of Physiology, East Carolina University, 115 Heart Drive Rm 4108, Greenville, NC, United States

Background/Aims: α-Klotho is a circulating factor with well-documented anti-aging properties; however, the central role of this protein remains largely unexplored. We aimed to investigate the potential role of central αklotho as a novel regulator of energy balance and glucose homeostasis.

Methods: Central administration of α-klotho was performed by using intracerebroventricular (ICV) injection for seven days in diet-induced obesity (DIO) mice. Conversely, central inhibition of α-klotho was performed via ICV administration of anti-αklotho antibody in chow-fed mice. Whole-cell current-clamp electrophysiology and immunofluorescent staining were used on hrNPY-GFP reporter mice to investigate the effects of α-klotho on NPY/AgRP neurons. Furthermore, to determine downstream signaling pathways associated with hypothalamic α-klotho action, GT1-7 hypothalamic cells were treated and analyzed using western blot and qPCR.

Results: Central α-klotho administration decreased food intake and improved glucose tolerance in DIO mice. Post-mortem tissue analysis also revealed reduced liver lipid content and gluconeogenic gene expression. On the contrary, anti-α-klotho antibody administration impaired glucose tolerance. Both electrophysiology and immunofluorescent staining revealed AgRP/NPY neurons exhibit hyperpolarization and reduced firing rate in response to α-klotho treatment. These effects are, at least partially, explained by increased magnitude, but not frequency, of mIPSC's. *In vitro,* α-klotho blunted serum-starvation-induced AgRP gene expression and increased phosphorylation of $ERK^{44/42}$, AKT^{ser473}, and $Foxo1^{ser256}$. These downstream effects were abolished by pretreatment with inhibitors of either fibroblast growth factor 1 (FGFR1) or PI3kinase.

Conclusion: Overall these results suggest a hypothalamic function of α-klotho, via FGFR1 and Pi3kinase signaling, to suppress AgRP/NPY neuron activity. Coupled with *in vivo* results, these data indicate a prominent role of α-klotho in regulation of energy balance and glucose homeostasis, thus providing new insight into the pathophysiology of metabolic disease.

The Endocannabinoid System as a Regulator of Dendritic Spine Density

Alexis R. Papariello[1], Karen Litwa[2], Ken Soderstrom[1], David Taylor[1], Brenna Kirk[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 at East Carolina University, Greenville, NC

Autism Spectrum Disorder (ASD) is a heterogenous developmental disorder that affects 3.5 million individuals in the United States of America. Symptoms of autism in young children include poor social functioning, limited communication, and restricted interests that present between the ages of one and three years. The endocannabinoid system is a retrograde synaptic regulator that plays an important role in early, embryonic development; thus, this system represents a valuable target for pharmacological intervention of autism. Changes to cannabinoid receptor density, metabolizing enzyme levels (fatty acid amide hydrolase, monoacylglycerol lipase, and diacylglycerol lipase), and signaling molecules (2-arachidonoylglycerol and anandamide) may underlie phenotypic neuronal abnormalities such as increased dendritic arborization and increased dendritic spine density. With the recent development of human induced pluripotent stem cells (hIPSC), we can now culture 2D and 3D brain models of autism patients and typically developing individuals to better understand how altered endocannabinoid signaling affects human fetal brain development. Preliminary RNASeq and qRT-PCR studies indicate that monoacylglycerol, the enzyme responsible for metabolizing and terminating the effects of 2-arachidonoylglycerol (the principal endocannabinoid in CNS) is expressed at elevated levels in autism-derived reprogrammed neurons relative to typical controls. We hypothesize that the decreased functionality within the endocannabinoid system leads to increased dendritic branching and increased dendritic spine density. Our first goal is to determine if the protein expression and functionality of the endocannabinoid system varies across neurons derived from autistic and typically-developing individuals. This step is currently under progress. Our second goal is to determine if typical dendritic and synaptic morphology can be rescued in autism-derived neuronal cultures through an endocannabinoid agonist and if autism-associated morphology can be emulated via antagonism.

Establishment of Methods to Measure Endocannabinoid Levels in Cultured Neurons

Rohan Parekh[1], Kimberly Kew[2], Ken Soderstrom[1]

[1]Department of Pharmacology & Toxicology, Brody School of Medicine, East Carolina University
[2]Department of Biochemistry and Molecular Biology, Brody School of Medicine, Greenville, NC, USA.

Vertebrates have an endocannabinoid system driven by lipid transmitters that bind to CB1 and CB2 cannabinoid receptors. Endocannabinoids and their receptors can be found both peripherally and within the CNS. Endocannabinoid signaling plays a significant role in CNS development. Lower endocannabinoid levels may be associated with altered neuronal development. This hypothesis will be tested by comparing endocannabinoid levels in neuronal cultures that are typically-developing and others that have been that have been mutated to develop abnormally. Differences in endocannabinoid levels will suggest a potential link between altered neuronal development and endocannabinoid signaling.

To begin to explore this possibility, a mass spectrometry method was established to measure levels of endocannabinoids. Anandamide (AEA), 2-arachnidonyl glycerol (2-AG) and their d8-deuterated forms were analyzed on a triple quadrupole mass spectrometer (LCMS) and a calibration curve was established to determine limits of detection. Then, typically-developing and mutant neurons were cultured and endocannabinoids were isolated via a lipid-lipid extraction method. Endocannabinoid levels were then measured quantitatively via LCMS. These measures will be corrected for recovery and compared across normal- and abnormally-developing neuron types.

Establishment of methods to measure 2-AG and ANA levels in typically- and abnormally-developing neuronal cultures will allow us to determine the extent to which altered endocannabinoid signaling may be responsible for the neurophysiological differences observed.

Stereological Assessment of Dopamine Neuron Count in Paired Male and Female Mice

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

East Carolina University Brody School of Medicine

Parkinson's disease, the second most common neurodegenerative disorder, costs over $14 billion annually in the United States alone and is projected to double in prevalence by 2040. Parkinson's disease symptoms are caused by the loss of a dopaminergic phenotype and ultimately dopamine neuron cell death in the substantia nigra pars compacta (SNpc). Based on recent data, the number of SNpc dopamine neurons can fluctuate relatively rapidly under a variety of conditions. For example, 14d of exercise and environmental enrichment increases the number of SNpc dopamine neurons by ~15%. Additionally, 7 d in a male-female pair increases dopamine neurons in males but decreases them in females. This determination is made by comparing cell counts of dopamine neurons in the SNpc of different treatment groups. Stereological assessment is an essential technique to accurately determine cell numbers. The purpose of this study was to implement the stereological assessment of SNpc dopamine neuron numbers using Z-stacks from a standard fluorescent microscope and the readily available image processing software ImageJ. We used sections from male-female pairs to assess differences in dopamine neuron numbers, since prior work has shown sexual dimorphism in dopamine neuron cell populations and response to pathology. Two left midbrains from C57 black 6 mice were used for pilot data, one male and one female. Cell counts from the female mouse were estimated at 7238.4 SNpc dopamine neurons with a CE of 0.14. Cell counts from the male mouse were estimated at 7662.8 SNpc dopamine neurons with a CE of 0.08. These results are similar to previous work estimating dopamine neurons in the SNpc and indicate that we are able to accurately estimate dopamine neuron numbers in the SNpc. In future studies, using conditions that alter dopamine neuron numbers, we will be able to accurately estimate and compare dopamine neuron numbers. By understanding how dopamine neurons lose or retain their phenotype, it may be possible to develop treatments to help Parkinson's disease patients by maintaining dopamine.

Relationship Between Dopamine and Morphine Responsiveness After Spinal Cord Injury

Ryan Patton[1], Helen Rodgers[1], Jacob Yow[1], Stefan Clemens[2], Kori Brewer[1,2]

Brody School of Medicine, East Carolina University, [1]Department of Emergency Medicine and [2]Physiology

Opioids are commonly prescribed to relieve neuropathic pain after a spinal cord injury, but often fail to be effective due to an injury-induced state that mimics opioid tolerance. Previous studies have shown that the analgesic effects of morphine can be restored if morphine is administered in combination with a dopamine 3 receptor agonist or a dopamine 1 receptor antagonist, demonstrating that dopamine receptor activity modulates the response to opioids after spinal cord injury. This study investigated the effects of spinal cord injury on levels of dopamine and its metabolites in the CNS and determined if changes in dopamine levels are associated with injury-induced morphine tolerance. Baseline nociceptive thresholds were measured in 8 uninjured and 16 injured rats before and after injection of morphine (2mg/kg) or saline (control). Rats were then randomized to have thresholds re-assessed after injection of morphine + pramipexole (D3 agonist), morphine + SCH (D1 antagonist), pramipexole, or SCH. Lumbar spinal cord and striatum tissue were collected and processed for mass spectrometry to quantify and compare levels of dopamine and its metabolites in injured versus uninjured animals. Morphine alone increased sensory thresholds in all uninjured but only 33% of injured rats. Both morphine + pramipexole and morphine + SCH increased sensory thresholds in all injured animals, while pramipexole and SCH alone had no effect. Striatal dopamine levels in injured animals that were not responsive to morphine were significantly decreased compared to uninjured animals. Dopamine levels in injured animals that responded to morphine (n=5) compared to animals that did not respond to morphine (n=10) are currently being analyzed. These data suggest that reduced dopamine levels in the striatum may contribute to morphine tolerance after SCI.

Focal Adhesion Characterization of Developing Human Neurons

Amanda Petritsch, Brenna Kirk, Karen Litwa

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

Neurons function in highly organized circuits which rely on specific cell morphology and localization. Cell signaling, morphology, and motility are dependent on focal adhesions, which are points of contact between the cell and its environment. Focal adhesions undergo dynamic alterations as they form, mature, and dissociate. It is currently unknown how focal adhesions change during neuronal differentiation. Neurons extend processes to locate synaptic partners through the soft environment of the developing brain; we use human induced pluripotent stem cells (hIPSCs) as a tractable model to study changes in focal adhesions within the first 120 hours of neuronal differentiation. In order to produce a soft three-dimensional substrate that more accurately recapitulates the in vivo neuronal environment, we develop Collagen-Hyaluronic Acid-Laminin hydrogels. We use hydrogels to investigate the impacts of dimensionality and stiffness on focal adhesions during neuronal differentiation. In order to evaluate focal adhesion complexes, we stain neuronal progenitor cells and neurons for focal adhesion proteins including α-actinin found in nascent adhesions, and paxillin, vinculin, and zyxin which are recruited during focal adhesion maturation. Total Internal Reflection Fluorescence (TIRF) microscopy resolves the adhesion complexes into discrete structures throughout the timecourse of neuronal differentiation. We then use ImageJ and Sigma Plot software to analyze focal adhesion composition, size, area, and distribution. With this study, we hope to further our understanding of the impacts of dimensionality, substrate stiffness, and cell differentiation on focal adhesion complexes. Future studies will explore how neurodevelopmental disorders alter focal adhesion dynamics using 3-D cortical organoids to mimic both the dimensionality and stiffness of the brain.

Neural Dynamics of Sarcasm Perception: The Role of Empathy

Rothermich, K.[1], Ahn, S.[2], Dannhauer, M.[3], & Pell, M.D.[4]

[1]Department of Communication Sciences and Disorders, East Carolina University
[2]Department of Mathematics, East Carolina University
[3]Department of Psychiatry & Behavioral Sciences, Duke University
[4]School of Communication Sciences and Disorders, McGill University

Previous studies have shown that individual differences in cognitive processes (i.e., empathy) can mediate social-pragmatic language processing (van den Brink et al., 2012). However, most studies to date used written stimulus materials, leaving open how the brain reacts to naturalistic, everyday interactions. In an electro-encephalography (EEG) study, we investigated the influence of empathy measures on brain correlates of processing communicative intentions in the form of sincere compliments and sarcasm. Twenty-three healthy adults rated short audio-visual scenes between two actors on social appropriateness, while we recorded brain activity using a 64-channel EEG system. Participants also filled out questionnaire about their level of empathy. The analysis of event-related potentials (ERPs) showed that participants with *lower* empathy showed higher ERP amplitudes in response to sarcasm between 250 – 400 ms in contrast to the high empathy group. The time-frequency results reveal increased alpha power (8-12 Hz) in the same time window after the onset of a sarcastic remark compared to sincere compliments, which seems to be driven by a significant effect in the *high* empathy group. We will discuss our results with respect to the integration of semantic meaning with social-pragmatic information as well as the allocation of cognitive resources during nonliteral communication.

Development of Small Molecule Inhibitors of the Classical Pathway of Complement

Blake R. Rushing, Denise Rohlik, Ryan J. Garrigues, Brandon L. Garcia

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

The complement system is part of the innate immune system that is composed of a network of blood serine proteases. Complement is important for recognizing and clearing a variety of foreign substances and damaged host tissues. Despite its role as a 'first-line-of-defense' against invading pathogens, dysregulation of complement is implicated in a number of autoimmune diseases, inflammatory conditions, and neurological disorders including Alzheimer's disease (AD). In AD, the overactivation of one of the three canonical pathways of complement - the classical pathway - has been causally-linked to disease progression in animal models. Increasing evidence has shown that the pattern recognition molecule of the classical pathway, C1q, recognizes β-amyloid plaques, and inappropriately activates complement in early stages of AD. However, this complement activity fails to clear these plaques, contributing to a chronic inflammatory environment which mediates the loss of neural synapses, leading to the progression of this disease.

We used a fragment-based drug discovery approach to search for small molecule inhibitors that specifically target the initiating protease of the classical pathway, C1r. Initially a 2,000-compound library was screened for C1r binding using surface plasmon resonance (SPR). Of these, 60 compounds were selected and verified to bind to C1r in a dose dependent manner. Using a classical pathway specific complement ELISA assay, these 60 compounds were then screened for their ability to inhibit CP. From this assay, one compound – named 3D4 – was found to be the most potent inhibitor. 3D4 was tested in a secondary complement hemolysis assay, where it was shown to protect cells from complement-mediated lysis. Further analysis showed that 3D4 could inhibit C1r specifically in a synthetic peptide cleavage assay, and *in silico* molecular docking analysis determined the catalytic cleft of the serine protease domain of C1r as 3D4's predicted binding site. These data indicate that 3D4 is a promising molecular scaffold which can be built upon to synthesize a more optimal C1r inhibitor which could potentially be used in the study of and treatment of AD.

Relationship of N-glycans to Neuroblastoma

Austin Whitman, Kristen Hall, Dr. Douglas Weidner, Dr. Ruth Schwalbe

Brody School of Medicine

Cell development, maintenance, and signaling pathways are influenced by N-glycosylation. Changes in branching of N-glycans are linked to tumor development and progression. All N-glycans share a common core sugar sequence and are classified as three major types: oligomannose, complex, and hybrid. Previously, we showed that cell structure and function was modified by altering the type of N-glycan. We hypothesize that modifying the levels of complex N-glycans will alter aberrant cell behavior in neuroblastoma (NB). CRISPR/Cas9 technology was used to create a N-glycosylation mutant cell line HuNB(-*Mgat2*) by silencing *Mgat2* (encodes for GnT-II) in the human neuroblastoma BE(2)C cell line, called HuNB. The action of GnT-II is to convert hybrid type glycans to complex, producing a cell line with complex type N-glycans. To study the partial absence of complex type N-glycans, the HuNB(-*Mgat2*) cell line will be transiently transfected with Mgat2, known as HuNB(-/+*Mgat2*). Two independent lectin binding assays showed the presence of complex N-glycans in the HuNB and HuNB(-/+*Mgat2*) cell lines and an absence of complex N-glycans in the HuNB(-*Mgat2*) cell line. Further, western blotting of total cell membranes was used to show that an N-glycosylated voltage-gated K+ channel (Kv3.1b) protein has hybrid type N-glycans and complex type in the HuNB(-*Mgat2*) and HuNB cell lines, respectively. Soft agar assay showed that elimination of the complex N-glycans lessens the transformed phenotype. Wound healing assays reveal elimination of complex N-glycans increases migratory rates of NB cells. Thus, our results reveal that the malignant transformation phenotype in NB cells ameliorates by lowering the levels of complex type N-glycans.

Facial Processing Across Age Groups

Austin T. White, Patrick M. Williams, Sunghan Kim Ph.D.

Biomedical Instrumentation and Data Analysis Lab, East Carolina University, Greenville, NC

Mild cognitive impairment (MCI) and Alzheimer's Disease (AD) affect millions worldwide, yet no curative treatments for these neuro-degenerative disorders have been developed to date. The current study aims to evaluate electrophysiologic facial processing differences across age groups (18-25, 26-40, 41-69, 60+) in order to work towards early detection of AD and MCI. Participants (n=8) were screened for cognitive well-being with the Montreal Cognitive Assessment (MoCA) and for confounding variables with a questionnaire preceding a visual stimuli task. Participants were presented with facial stimuli to elicit event related potentials (ERP) while their cortical activity was recorded with electroencephalogram (EEG). Source localization, independent component analysis, and regional neurophysiological biomarkers will be combined into a multi-dimensional feature space to facilitate differentiation between age groups. This study aims to illustrate the feasibility of a standard model of facial processing across healthy aging.

Hyaluronic Acid Regulation of Synapse Development

Emily Wilson, Warren Knudson, and Karen Litwa

Dept of Anatomy and Cell Biology, East Carolina University

Parent-reported diagnoses of neurodevelopmental disorders totaled nearly 15% of children in the United States ages 3 to 17 years in 2006-2008. Changes in the ratio of excitatory to inhibitory synapses is a common underlying mechanism in 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 excitatory/ inhibitory ratio is regulated. It is thought that alterations in spacing between neural cells and synapses as well as changes in cytoskeleton-dependent signaling are involved in the regulation of the excitatory/inhibitory ratio. Changes in the extracellular matrix (ECM) during neurodevelopment may alter the formation of the brain and its function. Hyaluronan (HA) is the major component of the brain ECM, is a space-filling macromolecule that controls cellular spacing, but also acts as a ligand for matrix receptors such as CD44. We hypothesize that manipulation of hyaluronan (HA) levels in the extracellular matrix of neural cells will reveal underlying mechanisms of altered synaptic signaling leading to neurodevelopmental disorders. Because alterations in cytoskeleton remodeling can change synaptic morphology and overall excitatory synapse transmission in the brain, the ECM provides a new angle to analyze neurodevelopment, particularly synapse formation and signal transduction.

RhoGTPases control the morphology of the synapse through regulation of the actin cytoskeleton. We hypothesize that HA, through interaction with a receptor, CD44, regulates RhoGTPases, such that the removal of HA from the ECM will alter the excitatory to inhibitory synaptic ratio in favor of excitatory synapses and lead to synaptic disorders such as ASD and epilepsy.

Preliminary results indicate HA, its synthases and CD44 are present within or around neural cells. We expect that manipulation of these molecules will alter the actin cytoskeleton, through RhoGTPase signaling, ultimately affecting synaptic development. We hypothesize that ECM alterations lead to disorders with characteristic imbalances in the excitatory to inhibitory ratio. These results will provide valuable insight into an understudied aspect of neurodevelopment.

Using Fluorescently Tagged α-Synuclein to Monitor TG2 Macromolecular Assembly: Determining Biophysical Properties That Promote Lewy Body Amyloidosis

B. A. Zeches[1], J. Washington[2], A. H. DeSantis[2], A. Hondros[2], T. N. Zeczycki[1,2]

[1]Brody School of Medicine at East Carolina University, [2]East Carolina Diabetes and Obesity Institute

Parkinson's Disease (PD), the second most common neurodegenerative disorder, is characterized by dopaminergic-neuronal cell death; this neuronal cell-death contributes to the clinical presentation of bradykinesia, resting tremor, and rigidity. In addition to progressive neurodegeneration within mesencephalic nuclei, PD is marked by proteinaceous intraneuronal inclusions, termed Lewy bodies. These inclusions largely contain aggregated, insoluble α-synuclein amyloids. As recent studies have shown, the formation of these aggregates is exacerbated by the Ca^{2+}-dependent activity of transglutaminase 2 (TG2); TG2 directly catalyzes α-synuclein inter- and intramolecular crosslinking. These post-translationally modified α-synuclein exhibit increased oligomerization and aggregation proclivities; as such, they are major contributing factor to PD and α-synucleinopathy amyloidosis.

The TG2-mediated, post-translational modification of α-synuclein, and other intrinsically disordered, pathogenic proteins—i.e. tau, Huntington, and amyloid β A4 peptide—is initiated by the formation of a TG2:α-synuclein encounter complex; however the molecular mechanisms underlying the formation of this complex is poorly understood. Considering the successful use of therapeutics targeting protein-protein interactions, preventing the formation of the TG2: α-synuclein complex by small molecular inhibitors may offer a tractable avenue for therapeutic development. To this end, our focus is to understand the the molecular mechanisms governing the macromolecular assembly of TG2: α-synuclein complex. As is our objective here, using both equilibrium binding studies and fluorescence anisotropy with fluorescently tagged α-synuclein at the N-and C-termini (G7C and A91C mutants, respectively), we have monitored the binding interactions between TG2 and α-synuclein. Thermodynamic parameters associated with complex formation were determined both by SPR and fluorescent binding experiments. Assessment of the binding microenvironment – by both using different, environmentally sensitive, fluorescent probes (i.e. BADAN, pyrene, and acrylodan) and steady-state anisotropy measurements- has allowed us to qualitatively probe the topography of the TG2:α-synuclein complex; as our results suggest, the terminal regions of α-synuclein remain untethered when α-synuclein is bound to TG2. Taken together, these results represent the first steps towards understanding the kinetic, thermodynamic, and structural mechanisms driving the formation of the pathogenic TG2:α-synuclein complex; understanding these mechanisms is key to advancing TG2 containing macromolecular complexes as molecular targets for the development of tractable therapeutics for PD and other neurodegenerative diseases.

Poster Session 2
Clinical Case Studies

(**presenters** are in alphabetical order)

Subacute Cerebral Cortical Infarct in a 14-Year-Old Boy with New Diagnosis of Type 2 Diabetes Mellites: Etiologic Considerations

William M. Clark[1], Connor L. Pratson[1], Cathleen Cook[2], Lenwood P. Smith[3], John S. Stalls[2], Philip J. Boyer[4]

[1]Brody School of Medicine, [2]Department of Pediatrics, [3]Vidant Neurosurgery, and [4]Department of Pathology and Laboratory Medicine, East Carolina University and Vidant Health, Greenville, North Carolina

Background: Infarcts in the brains of children are very uncommon and their etiology can be challenging to determine. We report the case of a 14-year-old boy with a focal cerebral infarct.

Methods and Materials: Clinical data were reviewed and compiled. A literature search using key words including infarct, childhood, and diabetes was undertaken.

Results:
- Case Report: A 14-year-old boy with no history of previous seizures presented in status epilepticus of about 1.5 hours duration. He had by report been stuck on the head by a basketball several weeks prior to admission with no known sequala. During the time of admission, he was diagnosed with diabetes type 2 with a high blood glucose and an elevated hemoglobin A1c; he was not in diabetic ketoacidosis; beta-cell auto-antibody screen was negative. Imaging studies of the brain identified a single lesion in the right frontal lobe, superficial, 1.5 cm in greatest dimension with no abnormality of adjacent blood vessels with the differential diagnosis of neoplasm and subacute injury; the lesion was at the middle cerebral artery-anterior cerebral artery boundary zone. Imaging of the spinal column and cord was negative. The lesion was excised and histologic evaluation revealed findings consistent with hemorrhagic infarct, early subacute, days to weeks old, with no evidence of embolic material in vessels and no evidence of contusion. The infarct very likely served as focus for the seizure leading to status epilepticus; he has had no recurrent seizures postoperatively. Echocardiogram was negative.
- Literature Review: No case reports were identified which identified stroke in a type 2 diabetic child and in a large series of stroke in childhood only a very small proportion of cases (51/3156) had the coincident diagnosis of diabetes. However, some studies suggest that specific organ system damage may be seen in young individuals with DM and impairment in vascular health can be demonstrated.

Conclusions: The exact cause of this patient's cerebral infarct is uncertain. The location of the infarct at the boundary zone of the middle and anterior cerebral arteries is noted. The role if any of diabetes in the development of the infarct is uncertain.

"Typical" vs. "Atypical": Two Presentations of Atypical Teratoid/Rhabdoid Tumor Presenting Challenges in Diagnosis and Treatment

Carolyn A. Early[1], Abraham O. Kurunga[2], Lenwood P. Smith[5], Cathleen Cook[2], Hyder Arastu[3], Philip J. Boyer[4], Beng R. Fuh[2]

[1]Brody School of Medicine and Departments of [2]Pediatrics, [3]Radiation Oncology, and [4]Pathology and Laboratory Medicine, East Carolina University, Greenville, NC; [5]Vidant Neurosurgery, Vidant Health, Greenville, NC

Background: Atypical teratoid/rhabdoid tumors (AT/RT) are rare and aggressive embryonal neoplasms, representing 2-3% of all CNS tumors of childhood and 20% of all CNS tumors in children under the age of 3. Diagnosis is made by biopsy. Even with aggressive treatment, AT/RTs are associated with a poor prognosis with mean survival ranging from 6 months to 2 years. A "typical" and "atypical" case highlight the neuroradiologic, pathologic, and clinical challenges associated with their diagnosis and treatment.

Methods and Materials: Clinical features of the cases are summarized. A PubMed.gov literature search was undertaken using key words including atypical teratoid/rhabdoid tumor.

Results:
Case Reports: Case 1: A 2-year-old boy, recently diagnosed and treated for influenza, presented with decreasing physical activity, bilateral leg weakness, and loss of gait. Computer Tomography (CT) and magnetic resonance imaging (MRI) scans identified a posterior fossa mass with obstructive hydrocephalus along with spinal metastatic disease. Pathologic evaluation of the resected lesion identified characteristic histologic and immunohistochemical features diagnostic of AT/RT. He was treated with chemotherapy and radiation therapy but died within one year of diagnosis. Case 2: An 8-year-old girl with recurrent streptococcal pharyngitis and headaches developed blurry vision and difficulty with upward gaze. Head CT and MRI scans showed obstructive hydrocephalus with ventricular enlargement of the third and fourth ventricles and a pineal region tumor. Partial resection was undertaken, and initial pathologic workup revealed a poorly differentiated neoplasm lacking classic rhabdoid features and antigen expression; further testing revealed loss of expression of INI-1, diagnostic of ATRT. She has received chemotherapy and radiation therapy and is alive nearly three years after diagnosis.
Literature Review: AT/RT typically present during the first few years of life, usually by 2 years of age. They may arise in either supratentorial or infratentorial locations. Pineal ATRTs represent only 3% of cases. Case 2 represents an "atypical" AT/RT with respect to age at presentation, location, antigen expression, and favorable response to therapy.

Conclusions: AT/RT's are a rare primary, childhood central nervous system neoplasm that require aggressive treatment. These cases illustrate "typical" and "atypical" clinical and pathologic features that can be seen in AT/RTs.

Lethal HPA-5b-Associated Fetal and Neonatal Alloimmune Thrombocytopenia Due to Intrauterine Intracranial Hemorrhage During First Pregnancy: Case Report and Review of the Literature

Lauren E. Geisel[1], Ding Dai[2], Christy M. Isler[3], Diana M. Strickland[3], Philip J. Boyer[2], Qi Cai[2], Brian R. Curtis[4], Karen L. Kelly[2]

[1]Brody School of Medicine at East Carolina University, and Departments of [2]Pathology and [3]Obstetrics and Gynecology, Greenville, NC and [4]Blood Center of Wisconsin, Milwaukee, WI

Background: Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare disease process due to maternal alloantibodies generated against fetal human platelet antigens (HPAs) which cross the placenta and can lead to thrombocytopenia with intracranial hemorrhage (ICH). A high rate of placental abnormalities is also seen in FNAIT pregnancies. The majority of FNAIT cases, 80-85%, are caused by anti-HPA-1a. We report a case of anti-HPA-5b FNAIT leading to intracerebral hemorrhage, placental abnormalities, and intrauterine fetal death.

Study Design: Medical records were evaluated. Maternal serologic studies and evaluation of maternal and paternal HPA genotype were conducted by polymerase chain reaction employing fluorescent hydrolysis probes. Autopsy and placenta pathologic evaluation were undertaken. A literature search was conducted including key words FNAIT, HPA-5b, and intracranial hemorrhage.

Results: A 22-year-old woman, G1P0, at 25 1/7 weeks gestation, presented with decreased fetal movement. Her pregnancy was complicated by significant first-trimester vaginal bleeding without transfusion therapy. Ultrasound identified fetal demise and showed a large, echogenic brain mass consistent with intracranial hemorrhage. Vaginal delivery was induced. Autopsy identified acute and subacute intracerebral hemorrhage with subarachnoid extension and diffuse, acute hypoxic-ischemic injury. Placental examination identified acute and chronic intervillitis. Maternal serologic evaluation identified antibodies to HPA-5b, seen in up to 10% of FNAIT cases. Genotypes were determined: maternal HPA-5a/5a, paternal HPA-5a/5b, fetus HPA-5a/5b. Literature review identified 53 case reports of FNAIT in which the antibody was specified, with ICH in 24 and with anti-HPA-5b antibodies and ICH in 7. No reports to date provide photographic documentation of the ultrasound findings and pathologic findings in the fetus and only a few cases document histopathologic findings in the placenta.

Conclusions: Maternal HPA-5b anti-platelet alloantibodies can be formed and cross into the fetal circulation and lead to the destruction of fetal platelets in the first at-risk pregnancy. Intracranial hemorrhage was the cause of intrauterine fetal death in this patient with histologic findings suggesting hemorrhage at least 2-3 days prior to death. Clinical suspicion and appropriate laboratory testing is essential for determination of the correct diagnosis and is crucial for the optimal management of subsequent pregnancies.

Spectrum of Intracranial Neoplasms and Locations Manifesting with Visual Symptoms: A Low Threshold for Brain Imaging Is Necessary to Prevent or Minimize Irreversible Vision Loss

Brent B. Hoffman[1], Mitchell G. Nash[1], When Zhong[2], J. Stuart Lee[4], John W. Prenshaw[3], Regis G. Hoppenot[4], Richard T. Dalia[4], Keith A. Tucci[4], Philip J. Boyer[2]

[1]Brody School of Medicine and [2]Department of Pathology and Laboratory Medicine, East Carolina University, [3]White Eye Associates Ophthalmology, and [4]Vidant Neurosurgery, Vidant Health, Greenville, North Carolina

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 during the past five years 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: Lesions compressing the optic nerve(s) and/or optic chiasm included meningiomas (including one case in which a separate lesion, an unsuspected metastatic lung carcinoma, was also resected), pituitary adenomas, a Rathke cleft cyst, and craniopharyngiomas. Intraaxial or extraaxial neoplasms disrupting optic tract fibers or geniculocalcarine fibers included primary neoplasms (glioblastomas, pleomorphic xanthoastrocytoma), metastatic neoplasms (lung (adenocarcinoma and squamous cell carcinoma) and heart (atrial myxoma) primary sites), 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, a significant delay resulted prior to imaging studies of the head and then the correct diagnosis and treatment being undertaken. 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.

Sisters with Rheumatoid Arthritis and the Rare Extraarticular Manifestation Rheumatoid Meningitis: Case Reports

Kyle Luke[1], Julia A. Castle[3], Karen L Furie[4], Peter A. Merkel[5], Philip J. Boyer[2]

[1]Brody School of Medicine, [2]Department of Pathology and Laboratory Medicine, East Carolina University, Greenville, North Carolina; [3]Naval Health Clinic, Annapolis, MD; [4]Department of Neurology, Lifespan Miriam and Bradley Hospitals, Providence, RI; [5]Department of Medicine, University of Pennsylvania

Background: Rheumatoid arthritis (RA) is an autoimmune disease which has both effects on joints and extraarticular and systemic effects. Involvement of the brain as rheumatoid meningitis (RM) is rare, most often seen in long-standing RA with severe articular erosions and deformation. We present the cases of elderly sisters with RA and RM.

Methods and Materials: Clinical data were reviewed and compiled for both patients. A literature search using key words including rheumatoid arthritis, rheumatoid meningitis, brain, leptomeninges, and rheumatoid nodule was undertaken.

Results:
- Case Reports: Two sisters with the clinical diagnosis of RA developed neurologic symptoms, underwent imaging studies demonstrating leptomeningeal abnormalities, underwent biopsy, and ultimately underwent autopsy. The first sister presented at age 73 and biopsy revealed dense subarachnoid fibrosis with scant inflammatory cells present along with cortical gliosis, possibly resolved rheumatoid meningitis. The second sister presented with neurologic symptoms at age 78 and biopsy revealed nodular, centrally necrotic granulomatous inflammation with negative special stains and culture, diagnostic of rheumatoid meningitis and consistent with rheumatoid nodule formation. For both patients, increased immunosuppressive therapy resulted in some improvement in neurologic function and reduction in the volume of leptomeningeal lesions was seen on repeat imaging studies in both. At autopsy both sisters showed patchy persistence of meningitis. HLA typing revealed HLA DR4 in one case and not the other; DRB1*1301 and DRB3*02XX were present in both sisters, implicated in some cases of RA.
- Literature Review: To date, only approximately 50 cases of histopathologically confirmed rheumatoid meningitis have been presented in the literature; patients range in age from approximately 30 to 90 years with a slight female predominance and to our knowledge not reported previously in sisters. The most common findings included meningeal inflammation, rheumatoid nodules, and vasculitis or some combination of features.

Conclusions: The identification of rheumatoid meningitis in sisters with RA is highly unusual and raises the question of a familial and genetic underpinning with a possible DRB1*1301 or / and DRB3*02XX association.

Spurious Extraaxial Appearance of a Cystic Spinal Cord Lesion on Imaging Studies: Unusual Presentation of Hydromyelia

Zachary D. Pruitt[1], Wen Zhong[2], Philip J. Boyer[2], K. Stuart Lee[3]

[1]Brody School of Medicine, [2]Department of Pathology, and [3]Vidant Neurosurgery, East Carolina University and Vidant Medical Center, Greenville, North Carolina

Background: Cystic lesions involving the spinal cord include hydromelia, syringomyelia, arachnoid cyst, endodermal cysts, and cystic neoplasms in or adjacent to the spinal cord. These lesions present clinically due to compression of or damage to adjacent axons, neurons, and nerve roots. Imaging studies are usually able to discern the intramedullary vs. extramedullary location of a lesion allowing for generation of a differential diagnosis. We present a case report of a cystic spinal cord lesion which challenged radiologic localization and then generation of a differential diagnosis.

Methods: The patient's clinical record was reviewed and summarized. A literature search was conducted using Pubmed.gov and GoogleScholar.com with key words including hydromyelia and syringomyelia.

Results: A 64-year-old woman presented with a months- to years-long history of slowly progressive back pain and then weakness greater than sensory deficits in the bilateral lower extremities including left-sided greater than right-sided footdrop. She was status-post thyroidectomy 30 years previously for papillary thyroid carcinoma. Computer Tomography (CT) and magnetic resonance imaging (MRI) scans identified a large cystic structure, non-enhancing, extending from T11 to L1, with posterior displacement and moderate flattening of the conus. An extramedullary location was favored, but a myelogram suggested it was intramedullary, confirmed intraoperatively. Myelotomy released clear, straw-colored fluid under pressure. Endoscopic evaluation revealed a smooth walled, yellowish cyst cavity with no nodular excrescences and biopsy was not undertaken. Histologic evaluation of the cyst fluid identified acellular, proteinaceous material. Literature search identified no other cases where a hydromelia or syringomyelia was challenging to classify radiologically as either intramedullary or extramedullary. MRI at 4 months post-operative showed no residual cyst(s). At 7 months postoperative, while some degree of weakness and sensory deficit remained, the patient's footdrop had nearly resolved.

Conclusions: This patient's spinal cord lesion is best classified as a hydromyelia rather than syringomyelia or cystic neoplasm based on radiologic, intraoperative, and clinical improvement features. Its appearance on CT and MRI studies was misleading and raised concern for a neoplastic process. A conservative surgical approach appears to have been warranted. Imaging followup will continue to track the course of the lesion and any additional improvement.