

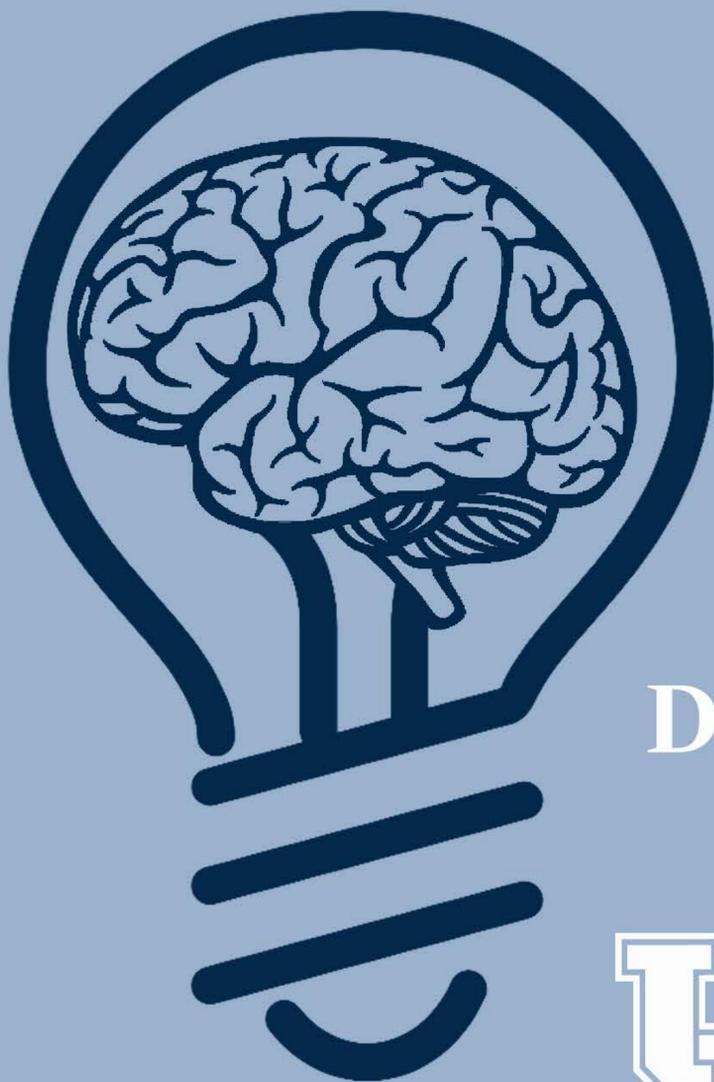
2020

University of Kentucky - Neuroscience Research Priority Area

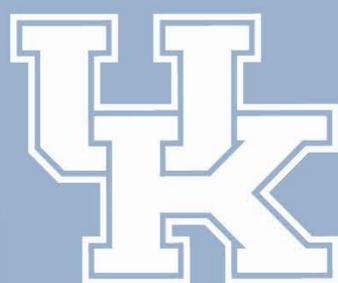
# NEUROSCIENCE CLINICAL TRANSLATIONAL RESEARCH SYMPOSIUM

Organized by the Kentucky Neuroscience Institute and  
UK Department of Neurology

**October  
23rd**



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NEUROSCIENCE  
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# Behavioral & Cognitive

Amanda Glueck, PhD<sup>1</sup> • Dong Han, Other<sup>1</sup>  
Neurology University of Kentucky<sup>1</sup>

### **Resilience and Changing Jobs Reduces the Likelihood of Depression during the COVID-19 Pandemic**

#### **Faculty**

**Introduction:** The coronavirus (SARS-CoV-2; COVID-19) pandemic mirrors the global disruption of the Spanish influenza (H1N1) pandemic of 1918. While data on viral mechanism, physical spread, and preventative measures have been documented, the pandemic's effect on related neuropsychiatric sequelae remain comparatively limited. The present study investigated the effects of social distancing on Personality Mood Scales (POMs) depression scores during the COVID-19 pandemic.

**Method:** One hundred and forty-two adult participants (78.2% female), age 18-70 years old ( $M = 35.97$ ,  $SD = 13.98$ ) consented to participate in the study and completed an online questionnaire. A binary logistic regression analysis was conducted to investigate if age, race, gender, currently engaging in social distancing, having recently changed jobs due to COVID-19, being currently employed or going to school, predicts whether participants would have clinically significant (great than 1.5 standard deviation of standardized mean) depression scores on the Personality Mood Scales (POMs).

**Results:** Hosmer and Lemeshow goodness-of-fit analysis was not significant, indicating that the model was a good fit for the data. Controlling for all other variables in the model, participants who switched jobs due to COVID were 83% less likely to report clinically significant POM's depression scores ( $CI = 0.04, 0.78$ ). Additionally, controlling for all other variables in the model, every one unit increase in resilience score reduced the likelihood by 1 of participants reporting clinically significant POM's depression scores ( $CI = 0.90, 0.96$ ).

**Conclusion:** Results from this analysis suggest that individuals who have recently switched jobs due to COVID-19 reduces the likelihood of reporting clinically significant depression symptoms during the current pandemic, possibly suggesting that a form of novel engagement in work activities may be protective against affective distress from uncontrollable exogenous variables. Likewise, participants with higher resilience are less likely to experience depression during the COVID-19 pandemic.

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**Combining Transcranial Magnetic Stimulation (TMS) with Pharmacotherapy for Psychiatric Disorders: A Systematic Review**  
**Student**

**Background** - Despite development of pharmacotherapy treatments for psychiatric illnesses, response and remission rates remain suboptimal at best. Non-invasive brain stimulation treatments have shown varying efficacy in treatment of psychiatric illnesses. Transcranial magnetic stimulation (TMS) is one method of non-invasive stimulation which has demonstrated a modest effect size in randomized controlled trials. There is some degree of mechanistic overlap between brain stimulation methods, like TMS, which cause neuroplastic changes in the brain and certain medication classes like antidepressants and antipsychotics. Therefore, the purpose of this systematic review was to determine if there is a beneficial therapeutic interaction from combining TMS and pharmacotherapy compared to using either treatment alone for the treatment of psychiatric disorders.

**Methods** - A systematic review of published literature was conducted within MEDLINE. Systematic searches were conducted using predefined terms and a PRISMA diagram was created. Studies included in the review needed to have investigated an initial concurrent use of TMS and pharmacotherapy for a psychiatric disorder and not the use of adjuvant TMS in response to a drug-resistant psychiatric disorder. Following PRISMA guidelines, the review included randomized controlled trials, open label trials, retrospective chart reviews, case reports, and case series.

**Results** - Of the studies that were included, 14 studies examined antidepressants with TMS, 4 studies examined benzodiazepines with TMS, 1 study examined antipsychotics with TMS, and 1 study examined atomoxetine with TMS.

**Discussion** - The existing literature overall suggested a positive effect of combining TMS and antidepressants for the treatment of major depressive disorder compared to antidepressants alone or in combination with sham TMS. There are mixed results on whether benzodiazepines lessen the beneficial effects of TMS for depression. There is limited support for the use of combined TMS and pharmacotherapy for other psychiatric disorders including ADHD and positive symptoms of schizophrenia. More high-quality randomized controlled trials are needed to validate current findings as well as to explore additional uses of combined TMS and pharmacotherapy.

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Psychiatry Yale School of Medicine<sup>1</sup> • Psychiatry University of Wisconsin<sup>2</sup>

### **Neuromodulation of the frontal pole influenced functional connectivity and therapeutic learning in OCD**

#### **Faculty**

**Background:** Psychotherapy based on fear extinction is a mainstay of treatment for obsessive-compulsive disorder (OCD). The default mode network (DMN) is important to safety signal processing, fear extinction, and exposure-based therapies. The medial prefrontal cortex (mPFC) is important to safety learning and is an anchor of the DMN. Neuromodulation targeting the mPFC might augment safety learning and thereby enhance response to exposure-based therapies.

**Methods:** To characterize the effects of mPFC neuromodulation, 17 community volunteers completed resting-state fMRI scans before and after receiving 20 minutes of anodal frontopolar multifocal transcranial direct current stimulation (tDCS). To examine the effects of tDCS on therapeutic learning, 24 patients with OCD were randomly assigned to receive active or sham tDCS before completing a two-day exposure and response prevention (ERP) challenge.

**Results:** After tDCS, functional connectivity decreased between the frontal pole and clusters in the anterior insula and basal ganglia, while connectivity with the middle and superior frontal gyri increased ( $p < .001$ , corrected). Functional connectivity between DMN and salience networks increased after tDCS ( $p < .001$ ). OCD patients who received active tDCS exhibited more rapid within- and between-trial therapeutic extinction learning ( $p < .05$ ) during the ERP challenge compared to those who received sham tDCS.

**Conclusion:** tDCS targeting the mPFC may modulate functional connectivity between the DMN and salience network and can accelerate therapeutic learning in OCD. Though limited by small samples, these promising findings motivate further exploration of the effects of tDCS on neural and behavioral targets associated with exposure-based treatments for OCD and for other anxiety and related disorders.

Thomas Adams Jr, PhD <sup>1</sup>

Psychology University of Kentucky <sup>1</sup>

### **Effect of Transcranial Direct Stimulation (tDCS) on Graph Theory Measures**

#### **Faculty**

**Background:** There is limited literature on effect of prefrontal transcranial direct current stimulation (tDCS) on resting brain functional connectivity, particularly graph theory derived functional connectivity measures. Two previous studies have shown an increase in global brain efficiency, decrease in global nodal clustering with tDCS as well as changes in centrality measures following administration of prefrontal tDCS. We sought to examine changes in graph theory measures of functional connectivity in 17 community volunteers following administration of anodal frontopolar tDCS.

**Methods:** Seventeen community volunteers completed resting-state functional magnetic resonance imaging (fMRI) scans (12 min) before and after receiving multifocal tDCS targeting the frontal pole. A single anode was placed over the frontal pole, which was surrounded by five return electrodes (cathodes) and 1.5 mA of current was administered for 20-min. Resting state fMRI data were processed through Conn Toolbox ([www.nitrc.org/projects/conn](http://www.nitrc.org/projects/conn)) and second-level analyses were completed with GraphVar 2.0 (<https://www.nitrc.org/projects/graphvar/>). Global and local efficiency, clustering, and assortativity were measured. Degree centrality was also measured in preselected regions of interest (ROIs). False discovery rate (FDR) correction was applied to all p-values.

**Results:** No significant differences in global clustering, efficiency and assortativity were found between pre-tDCS and post-tDCS sessions. Local clustering was significantly decreased in right frontal pole ( $p=0.001$ ), left frontal pole ( $p=0.002$ ) and right putamen ( $p=0.002$ ) following tDCS. Local efficiency was increased in bilateral insula and the dorsal anterior cingulate cortex (dACC,  $p<0.05$ ) following tDCS. Local efficiency decreased significantly in right putamen and right pallidum ( $p<0.05$ ) following tDCS. There was also a significant increase in degree centrality measures in the right and left insula ( $p<0.05$ ) following tDCS.

**Conclusion:** We did not find significant differences in global connectivity measures, we did find significant local differences. Increased local efficiency observed in the insula and dACC suggest that tDCS may have modulated functional connectivity with core hubs of the salience network. Graph theory measures of degree and betweenness centrality show covariance of functional connectivity, implying significant increase or decrease in connectivity measures at nodes with changes in these measures when comparing sessions before and after tDCS. Nodes with higher centrality measures are usually important drivers of the network and in this regard, our finding highlights downstream effects of tDCS on brain networks in healthy volunteers. Graph theory measures are complementary to traditional methods of fMRI analyses like functional connectivity and can highlight changes within a particular network.

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Psychology University of Kentucky<sup>1</sup> • Neurology University of Kentucky<sup>2</sup>

### **Exploring the Effects of Social Distancing, Employment Status and Resilience on Anxiety during the COVID-19 Pandemic Student**

**Introduction:** The coronavirus (SARS-CoV-2; COVID-19) pandemic has created an unprecedented disruption worldwide. Both the Center for Disease Control and the World Wide Health Organization have reported that the spread of COVID-19 can be reduced by individuals engaging in behaviors such as frequent and thorough hand washing, wearing face masks, limiting social contact, and engage in social distancing. States across the nation have encouraged citizens to engage in social/physical distancing in order to reduce the transmission of the virus. While this recommendation promotes physical health, little is known about the effects social distancing on mental health and well-being, specifically anxiety, during a pandemic. The present study investigated the effects of social distancing on Beck Anxiety Inventory (anxiety) scores during the COVID-19 pandemic.

**Methods:** One hundred and forty-two participants (78.2% female), age 18-70 years old ( $M = 35.97$ ,  $SD = 13.98$ ) consented to participate in the study and completed the online questionnaire. A binary logistic regression analysis was conducted to investigate if age, race, gender, currently engaging in social distancing, having recently changed jobs due to COVID-19, being currently employed or going to school, and resiliency (Connor-Davidson Resilience Scale), predict whether participants would have elevated anxiety scores (Beck Anxiety Inventory; BAI).

**Results:** Hosmer and Lemeshow goodness-of-fit analysis was not significant, indicating that the model was a good fit for the data. Controlling for all other variables in the model, participants who were currently employed or going to school were 4 times more likely to reported moderate-severe anxiety ( $CI=1.36, 12.14$ ). Additionally, controlling for all other variables in the model, participants who switched jobs due to COVID were 90% less likely to report moderate-severe anxiety ( $CI= 0.02, 0.49$ ). Finally, controlling for all other variables in the model, for every one unit increase in resilience scores reduced the likelihood of participants reporting moderate-serve anxiety ( $CI= 0.94, 0.99$ ).

**Conclusion:** Results from this analysis suggest that individuals who are currently employed or going to school during the COVID-19 pandemic are more likely to report moderate-severe anxiety, regardless of age, race, gender, and whether or not they are currently social distancing. Whereas individuals, regardless of age, gender, race, or social distancing, who have recently switched jobs due to COVID-19 are less likely to report moderate-severe anxiety during the current pandemic. Additionally, results suggest that participants with greater resilience are less likely to report moderate-severe anxiety symptoms during the COVID-19 pandemic, regardless of age, gender, race, or current social distancing practices.

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### **Machine Learning Based Autism Spectrum Disorder Detection from Videos**

#### ***Student***

Early diagnosis of Autism Spectrum Disorder (ASD) is crucial for best outcomes to interventions. In this paper, we present a machine learning (ML) approach to ASD diagnosis based on identifying specific behaviors from videos of infants of ages 6 through 36 months. The behaviors of interest include directed gaze towards faces or objects of interest, positive affect, and vocalization. The dataset consists of 2000 videos of 3-minute duration with these behaviors manually coded by expert raters. Moreover, the dataset has statistical features including duration and frequency of the above mentioned behaviors in the video collection as well as independent ASD diagnosis by clinicians. We tackle the ML problem in a two-stage approach. Firstly, we develop deep learning models for automatic identification of clinically relevant behaviors exhibited by infants in a one-on-one interaction setting with parents or expert clinicians. We report baseline results of behavior classification using two methods: (1) image based model (2) facial behavior features based model. We achieve 77% accuracy for gaze to face and gaze to object, 70% accuracy for smile and 53% accuracy for vocalization. Secondly, we focus on ASD diagnosis prediction by applying a feature selection process to identify the most significant statistical behavioral features and an over and under sampling process to mitigate the class imbalance, followed by developing a baseline ML classifier to achieve an accuracy of 82% for ASD diagnosis.

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Psychology University of Kentucky<sup>1</sup> • College of Pharmacy University of Kentucky College of Pharmacy<sup>2</sup> • College of Pharmacy University of Texas at Austin<sup>3</sup>

### **The Effects of Adolescent Binge-Like Alcohol Exposure on Adult Alcohol and Nicotine Co-Use in Sprague Dawley Rats.**

#### **Fellow**

Adolescent substance use is associated with an increased occurrence of lifetime substance use disorders. Preclinical studies modeling this phenomenon typically utilize a single reinforcer (e.g. alcohol). However, singular approaches may not capture the treatment seeking population, as alcohol and tobacco are often co-abused. Here, we utilized a model of alcohol and nicotine co-use in rats to assess adult addiction vulnerability following adolescent alcohol exposure. **Methods: In Exp. 1**, adolescent (PND30) male and female Sprague-Dawley rats (n=35) received 25% ethanol (EtOH) in vanilla ensure plus or an isocaloric control solution via oral gavage every 8 hours, for 2 days. A 2-bottle choice between H<sub>2</sub>O and 15% EtOH began in young adulthood (PND 55) in hour-long daily sessions occurring in operant chambers modified to house the bottles; after 7 days, the EtOH solution was sweetened (0.2% saccharin/15% EtOH) for 7 sessions. EtOH and nicotine co-use followed. Active-lever responding was maintained by delivery of 0.03 mg/kg nicotine iv and a paired stimulus light; the 2-bottle choice between H<sub>2</sub>O and sweetened-EtOH was concomitantly available. The nicotine response requirement increased every 3 sessions (FR1, 3, 5, 8, 12, 20, 30, 45, 60). **In Exp. 2**, rats (n=36) underwent the control or EtOH gavage, or no gavage. On PND 47, daily 1-hr 2 bottle choice sessions began in the operant chambers between H<sub>2</sub>O and 15% EtOH, sweetened-EtOH, or 0.2% saccharin. Solution access lasted 7 days, rotated every 7 days, and was presented in a counterbalanced order across treatment groups. **Results: In Exp. 1**, EtOH-gavaged rats consumed less 15% EtOH in adulthood than controls, which persisted in males for sweetened-EtOH. During co-use, devaluation of sweetened-EtOH persisted in EtOH-gavaged rats, evidenced by a rightward shift in the nicotine own- and EtOH-cross price demand functions relative to controls. **In Exp. 2**, we found control-gavaged males displayed significantly higher levels of 15% EtOH than the no-gavage group. This trend was also found for sweetened-EtOH consumption in males, though not significant. **Conclusions:** These results indicate the oral gavage delivery method during adolescence impacts adult alcohol drinking despite the solution administered. **Exp. 1** suggests adolescent EtOH reduced adult drinking in the 2-bottle choice and during co-use, which was evident by the difference in UP50 (~FR5 for control and ~FR8 for EtOH). However, **Exp. 2** indicates the gavage increased adult drinking in the control group, rather than reducing drinking in the EtOH group. The repeated gavage may have served as a stressor significant enough to induce a high drinking phenotype in adulthood, while inclusion of EtOH in the gavage induced either an amnesic or anxiolytic effect for the event. Regardless, these findings suggest preclinical models seeking to assess excessive drinking in adulthood following adolescent alcohol exposure should use a method other than oral gavage.

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Biomedical Engineering University of Kentucky<sup>1</sup> • Sanders-Brown Center on Aging/Alzheimer's Disease Research Center University of Kentucky<sup>2</sup> • Neuroscience University of Kentucky<sup>3</sup> • Molecular & Cellular Biochemistry University of Kentucky<sup>4</sup> • Biology University of Kentucky<sup>5</sup>

### **Effect of Dynamic Thermal Sleep Enhancement in a Mouse Model of Alzheimer's Disease**

#### ***Student***

Alzheimer's disease (AD) is a neurodegenerative disease that is marked by changes in personality, memory impairment, and cognitive deficits. Amyloid plaque buildup and neurofibrillary tangles in the brain are believed to play an integral role in AD pathology and are therefore active topics of research. There is growing evidence that disordered sleep may accelerate pathology. Therapeutic strategies for improving sleep quality may slow disease progression and are therefore desirable. To this end, we have developed a system to accomplish sleep enhancement through simple closed-loop control of ambient temperature ( $T_a$ ), which is known to influence sleep through thermoregulation. After establishing the feasibility of sleep enhancement in wildtype mice (C57BL/6) in a prior study, seven 3xTg-AD mice (12-13 months old; all female) were instrumented for EEG/EMG recording and subjected to four weeks of treatment in which  $T_a$  was manipulated to promote EEG slow wave activity in the delta band (0.5-4 Hz). For each animal thus treated, a sham-treated age and sex-matched control was monitored in an adjacent cage that remained at room temperature. Results from data analysis strongly suggest that slow wave sleep and REM were both elevated in the light period of the 24-hour cycle in the experimental group. The finding on improved sleep performance can be correlated with results from assays performed on brain tissue to detect amyloid beta, tau protein, and inflammatory markers which indicate improvement on AD pathology in the experimental group.

Velmurugan Gopal Viswanathan, PhD <sup>1</sup> • Deepak Kotiya, PhD <sup>1</sup> • Sanda Despa, PhD <sup>1</sup> • Florin Despa, PhD <sup>1</sup>  
Pharmacology and Nutritional Sciences University of Kentucky <sup>1</sup>

**Characterization of conditional human amylin knock-out mouse model to understand the mechanism of amylin-mediated processes in Alzheimer's disease**

**Fellow**

Amylin dyshomeostasis promotes pancreatic amyloid formation in individuals with type-2 diabetes (T2D) and also forms mixed cerebral plaques with  $\beta$ -amyloid (A $\beta$ ) in the brains of patients with Alzheimer's disease (AD). To uncover mechanisms underlying cerebral amylin accumulation, we generated in collaboration with Cyagen a humanized mice model with targeted replacement of floxed human amylin gene (HuAmy-TR<sup>flox/flox</sup>) for mouse amylin and made it conditional by crossing with pancreatic tissue specific Cre mice (Ins-1 CreERT2/HuAmy-TR<sup>flox/flox</sup>). To study amylin-A $\beta$  interaction, we further crossed the Ins-1 CreERT2/HuAmy-TR<sup>flox/flox</sup> mice with APP/PS1 mice to generate Ins-1 CreERT2/ HuAmy-TR<sup>flox/flox</sup>/APP/PS1 mice.

The phenotype of our newly generated humanized mouse model (HuAmy-TR<sup>flox/flox</sup>) replicates some metabolic dysregulation observed in humans with T2D, including hyperinsulinemia, hyperglycemia (fasting and non-fasting), enhanced HOMA-IR, glucose intolerance, insulin resistance and decreased insulin signaling. HuAmy-TR<sup>flox/flox</sup> mice also show decreased novel object recognition (NOR) and increased anxiety-like behavior. Metabolic and neurological deficits are both rescued by conditionally knocking down amylin by injecting tamoxifen in Ins-1 CreERT2; HuAmy-TR<sup>flox/flox</sup> mice. Further studies are ongoing to understand the impact of temporal amylin regulation on AD processes in the brain and peripheral tissues.

### **Resting State Frontal Theta Rhythms are Altered by Working Memory Task**

#### ***Student***

The resting state electroencephalographic (rsEEG) rhythms reflect oscillatory brain mechanisms of neural synchronization that supports neural plasticity and communication, which is relevant to many cognitive functions (e.g. memory). Despite a growing consensus that rsEEG especially during Eyes-closed (EC) is a candidate marker for mild cognitive impairments, it is not clear whether EC rsEEG measures are affected by doing a cognitive task before measurements. The current study examined low- and high- frequency oscillations rsEEG, recorded using a wireless EEG headset before and after a visual working memory task, in 21 cognitively normal older adults (age range 65-80 years old; 65% female) from UK-ADC. After removing the muscle artifacts in the EEG, there were no statistically significant differences found in rsEEG rhythms in delta (1-4 Hz), alpha (8-12 Hz), beta (12-30 Hz), and gamma (30-40 Hz) frequency bands. However, compared to before task baseline, the frontal theta wave during EC rsEEG showed a significant increase in the left frontal site (FC5;  $p < .005$ ) after the task. Our new findings suggest that: (1) A 10-minute working memory task alters the theta rhythms from EC rsEEG measured after the memory task, which has implications for neural plasticity; (2) The best practice for recording rsEEG rhythms as outcome measures for clinical trials should be before neurocognitive tests. Overall, rsEEG has great potential as biomarkers for neural plasticity associated with cognitive functions and risk for dementia.

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**First-in-human studies of MW01-6-189WH, a brain-penetrant, anti-neuroinflammatory, small molecule drug candidate**

**Faculty**

**Background:** Acute brain injuries, such as intracerebral hemorrhage (ICH) or traumatic brain injury (TBI), are major medical problems that cause considerable mortality and morbidity in older individuals. In addition to the initial injury, secondary neuroinflammatory events can further damage the brain and lead to increased risk of neurologic complications, including Alzheimer's disease (AD) and related dementias (ADRD). A specific aspect of neuroinflammation, injury-induced proinflammatory cytokine overproduction from abnormally activated glia, has been linked to subsequent neurological damage and cognitive deficits in both acute and chronic CNS disorders. Despite advances in our understanding of these molecular neuroinflammatory mechanisms, approved therapeutics that target this pathological process are lacking. To address this need, we developed a CNS-penetrant, small molecule drug candidate, MW01-6-189WH (=MW189), that selectively attenuates stressor-induced proinflammatory cytokine overproduction. MW189 is efficacious at low doses in animal models of AD, TBI and ICH. Based on promising drug-like properties, preclinical efficacy, and excellent safety profile, an intravenous formulation of MW189 was developed and tested in phase 1 human studies.

**Methods:** Phase 1 studies were done to evaluate safety, tolerability, and pharmacokinetics (PK) of single and multiple ascending doses of MW189 in healthy adults. A pilot pharmacodynamic study administering low dose endotoxin to induce a systemic inflammatory response was done to evaluate effects of a single dose of MW189 on plasma cytokine levels.

**Results:** MW189 was safe and well tolerated in single and multiple doses up to 0.25 mg/kg, with no clinically significant concerns. No clinically concerning changes were seen in vital signs, ECGs, physical or neurological examinations, or safety laboratory results. PK analysis showed dose-proportional increases in MW189 plasma concentrations after single or multiple doses, with approximately linear kinetics and no significant drug accumulation. In the pilot pharmacodynamic study, MW189 treatment resulted in lower plasma levels of the proinflammatory cytokine TNF $\alpha$  and higher levels of the anti-inflammatory cytokine IL-10 compared to placebo treatment, suggesting engagement of pharmacological mechanism.

**Conclusion:** Overall, the safety profile, PK properties, and pharmacodynamic effect support further development of MW189 for patients with acute CNS injury, AD/ADRD, or other disorders involving dysregulated neuroinflammation as a driver of disease progression.

## **Computer-Assisted Assessment of Attention and Memory Utilizing Ecologically Valid Distractions: A Scoping Review**

### ***Student***

This poster presents a scoping review of computer-assisted assessments have utilized distractions across clinical populations with attention and/or memory deficits. From the literature review we will present a proposed framework for constructing distraction elements into computer-assisted assessments. Databases searched included Pubmed, PsychInfo, Web of Science, Rehabdata, and Scopus databases (1960-March, 2020). Our inclusion criteria included articles: 1) written or available in English; 2) focused on clinical populations with attention and/or memory deficits; 3) and utilized distractions within a computerized simulation. We excluded articles based on: 1) only utilized a healthy population, 2) included second language learners; 3) studied limb movement, pain management, balance, or surgery. We screened five hundred twenty-four titles. Sixteen articles met our criteria. To assess bias we used the Downs and Black 26-item QAT scale, PEDRO Scale, and SCRIBE. We extracted data on population, study design, purpose, distraction modality, distraction characteristics, distraction timing, distraction location, distraction intensity, task/target, primary measurements, program modality, and results. From the data extraction process, common components of distraction emerged including distraction modalities, distraction intensity, distraction timing, and location of distraction. These themes also led to the creation of working definitions for distraction and intensity. From this review, authors formed a conceptual systematic framework for the implementation and dosing of distractions. This framework may guide researchers to design virtual environments that target specific attention or memory processes that resemble real-life functioning for clinical populations. Along with the conceptual framework, authors developed recommendations for both measurement and programming in order for future study designs to support a global approach to ecologically valid simulations.

Deepak Kotiya, PhD <sup>1</sup> • Nirmal Verma, PhD <sup>1</sup> • Florin Despa, PhD <sup>1</sup>  
Pharmacology and Nutritional Sciences University of Kentucky <sup>1</sup>

## **Detection of amylin-A $\beta$ oligomer as a potential biomarker of Alzheimer's Disease**

### **Staff**

### **Introduction**

Elevated levels of blood amylin, an amyloidogenic hormone secreted by the pancreas, is common in individuals with prediabetic insulin resistance and is associated with Alzheimer's disease (AD) via formation of mixed amylin-A $\beta$  deposits in the brain.

### **Hypothesis**

Amylin-A $\beta$  oligomers may form in blood and CSF and could be associated with early AD processes.

### **Methods**

We have developed a novel sandwich ELISA to detect amylin-A $\beta$  oligomers by using a commercially available anti-A $\beta$  antibody (mouse monoclonal, Biolegend) and an "in house" generated polyclonal anti-amylin antibody (raised in rabbits; against N-terminal of human amylin). The novel amylin-A $\beta$  ELISA was tested on human AD brain homogenates and blood, CSF and brain tissues from APP/PS1 rats transgenic for human amylin in the pancreas (APP/PS1/HIP).

### **Results**

We detected amylin-A $\beta$  oligomers in the blood, CSF and brain tissue homogenates of APP/PS1/HIP rats. Soluble amylin-A $\beta$  oligomers were also identified in brain tissues from patients with AD.

### **Discussion**

It has been established that the cerebral mixed amylin-A $\beta$  deposits contribute to AD pathology. Detection of Amylin-A $\beta$  oligomers in AD could be a potential biomarker of Alzheimer's Disease.

### **Conclusion**

Additional experiments are needed to test the efficiency of the novel amylin-A $\beta$  ELISA on human tissues and laboratory models of AD.

## The Interactive Effects of BDNF Polymorphisms and PTSD Symptoms on Neurocognitive Functioning

### Student

**Background:** Individuals with elevated Posttraumatic Stress Disorder (PTSD) symptoms exhibit a range of mild to moderate deficits in neurocognitive functioning. One hypothesized risk factor that may strengthen the relationship between PTSD and neurocognitive deficits are single nucleotide polymorphisms in the brain-derived neurotrophic factor (BDNF) gene. Only one known study has investigated the interactive effects of the Met allele on neurocognitive functioning among individuals with PTSD. This study seeks to replicate and extend previous findings by examining unexplored neurocognitive domains (i.e., processing speed and attention).

**Methods:** Data was analyzed from European/Caucasian American Veterans who participated in the National Health and Resilience in Veterans Study (NHRVS). A multivariate analysis of covariance (MANCOVA) was conducted to explore the main and interactive effects of PTSD and the Met allele on four domains of cognitive functioning (attention, processing speed, visual learning, and working memory).

**Results:** After controlling for family-wise error rate, there was a significant effect of PTSD ( $F(5,1117) = 7.75, \eta_p^2 = .034, p < .001$ ), which suggests that neurocognitive functioning was poorer for veterans with probable PTSD ( $M$  z-score =  $-.22, SD = .66$ ) than those without probable PTSD ( $M$  z-score =  $.11, SD = .61$ ). There was a significant effect of the Met allele ( $F(5,1117) = 4.77, \eta_p^2 = .021, p < .001$ ), which suggests that neurocognitive functioning was poorer for veterans with the Met allele ( $M$  z-score =  $-.11, SD = .59$ ) than non-Met carriers ( $M$  z-score =  $.01, SD = .64$ ). A significant interaction between probable PTSD and the Met allele ( $F(5,1117) = 8.62, \eta_p^2 = .037, p < .001$ ) was observed. Post-hoc probing revealed that among individuals with PTSD, the Met allele was associated with a poorer neurocognitive functioning than non-Met allele carriers ( $F(5,104) = 4.91, \eta_p^2 = .197, p < .001$ ). This association was most pronounced in attention performance ( $F(1,104) = 17.19, \eta_p^2 = .142, p < .001$ ). There was no effect of the Met allele among individuals without probable PTSD ( $F(1,1009) = 17.19, \eta_p^2 = .012, p = .03$ ).

**Conclusion:** Among individuals with PTSD, the Met allele is associated with deficits in neurocognitive functioning. These findings suggest that phenotypic (BDNF polymorphisms) and psychopathological (PTSD) factors uniquely and interactively influence neurocognitive functioning in U.S. military Veterans.

**Citrullination a potential post-translational modification linked to TDP-43 pathology****Staff**

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TDP-43 is a nuclear RNA/DNA binding protein that in its pathological form, mislocalizes and aggregates into the cytoplasm as insoluble cellular inclusions. TDP-43 inclusions are histological hallmarks of frontotemporal lobar degeneration with TDP-43 (FTLD-TDP) and amyotrophic lateral sclerosis (ALS). Currently, mechanisms responsible for TDP-43 mislocalization and aggregation remain unclear, but it is hypothesized that post translational modifications (PTMs) play an active role. Citrullination is an irreversible PTM in which peptidyl arginine deiminases (PADs) catalyze the conversion of arginine to citrulline. Little is known about PADs in neurodegeneration, but there is evidence of increased PAD4 expression in Alzheimer's Disease (AD) and ALS motor neurons. Through mass spectrometric analyses, we identified that PAD4 induced citrullination of recombinant TDP-43 protein in 11 out of 20 TDP-43 arginine (R) residues. This facilitated the development of several anti-citrullinated arginine (citR) antibodies in our laboratory, two of which were further validated in cellular and animal models of TDP-43. Here, we demonstrate antibody validation using TDP-43 transgenic animals that develop gene dose-dependent TDP-43 pathology (TAR mice). Anterior cortex and hippocampus from TAR mice and non-transgenic (ntg) littermates were immunohistochemically (IHC) labeled with PAD4, citR83 TDP-43 and citR268/272 TDP-43 antibodies. We found that PAD4 expression is increased with pathological TDP-43 progression. Further, PAD4 level expression significantly increased in the neuronal cytoplasm of TAR4/4 mice compared to TAR4 and ntg mice. Within this model, we also compared the affinity of citR antibodies. Though the citR83 antibody appeared more suitable for IHC methods, both citR antibodies demonstrated the occurrence of citrullination as a "bona fide" PTM. Citrullination of TDP-43 was significantly increased within pathological TDP-43 phenotypes, and followed the toxic pattern of neuronal cytoplasmic accumulation. We hypothesize that PAD4-induced citR unfolds TDP-43, leading to an accumulation of unfolded TDP-43 oligomers and soluble aggregates, and from our findings, it is plausible that citrullination may be a factor responsible for TDP-43 aggregation in neuronal cytoplasm. Additionally, PAD4 and both citR antibodies are especially relevant to FTLD-TDP, as these proteins expressed higher abundance within the anterior cortex of diseased brain. Evidence of increased PAD4 levels, combined with increased citR TDP-43 levels, suggests that citrullination is a novel PTM and an attractive therapeutic target for treating TDP-43 proteinopathy.

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### **ASD Diagnosis Prediction in Children from Eye Gaze Data using Machine Learning Methods**

#### ***Student***

As early intervention is highly effective for young children with autism spectrum disorder (ASD), it is imperative to make accurate diagnosis as early as possible. ASD has often been associated with atypical visual attention and eye gaze data can be collected at a very early age. An automatic screening tool based on eye gaze data that could identify ASD risk offers the opportunity for intervention before the full set of symptoms is present.

In this work, we discuss machine learning methods applied to children's eye gaze data collected from free-viewing tasks of natural images for ASD classification. The eye gaze dataset, "Saliency4ASD" is a publicly-accessible collection of 300 images of natural scenes and the associated gaze scan-paths and duration of gaze of 28 children (14 children with ASD and 14 healthy controls) with ages from 5 to 12 years. One approach uses a generative model of synthetic saccade patterns to represent the baseline scan-path from a typical non-ASD individual and combines it with the real scan-path as well as other auxiliary data as inputs to a deep learning classifier. Significant class differences were observed by this method using raw scan-path fixations and duration with ASD prediction accuracy of 61%. Another approach adopts a more holistic image-based approach by feeding the input image and a sequence of fixation maps into a convolutional or recurrent neural network. This experiment, designed to evaluate correlation between scan-path fixations and high-level image semantics extracted through deep learning, predicts ASD diagnosis with accuracy up to 62%.

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### **Arginase 1 Deficiency in Brain Myeloid Cells Activates Amyloid- $\beta$ Plaque Associated Glial Genes in a Mouse Model of Alzheimer's Disease**

#### **Student**

**Objectives:** Brain myeloid cells, including infiltrating macrophages and resident microglia, play an important role in responding to and inducing of neurodegenerative diseases, such as Alzheimer's disease (AD). Genome-wide association studies (GWAS) implicate many AD casual and risk genes enriched in brain myeloid cells. Altered arginine metabolism has been recently proposed as a promising biomarker for AD. Coordinated arginine metabolism through arginase 1 (Arg1) is critical for brain myeloid cells to perform biological functions, whereas dysregulated arginine metabolism disrupts. We previously reported Arg1 deficiency in myeloid cells exacerbated amyloidosis related neuropathology and behavioral impairment. However, it remains unclear how Arg1 deficient myeloid cells impact the whole brain to promote amyloidosis. Herein, we aim to determine how myeloid Arg1 deficiency during amyloidosis affect fundamental neurodegeneration pathways at the transcriptome level.

**Methods/Results:** We extracted posterior cortex mRNA from mouse brains of *nTg/Arg1+/+/LysMcreTg/+*, *nTg/Arg1fl+/+/LysMcreTg/+*, *APP+/-/Arg1+/+/LysMcreTg/+* and *APP+/-/Arg1fl+/+/LysMcreTg/+*. Then we performed a transcriptomic profiling analysis by applying the mRNA samples in nCounter mouse neuropathology panel (770 genes, NanoString Technologies, Inc). From several bioinformatic analyses, we found amyloid- $\beta$  (A $\beta$ ) stimulated autophagy pathway and increased inflammatory response of myeloid cells, whereas myeloid Arg1 deficiency during A $\beta$  stimulation promoted lipid metabolism and myelination pathways and increased migration of myeloid cells. Focusing on neurodegenerative disease-associated glial transcriptomic signatures, we found myeloid Arg1 deficiency up-regulated glial gene transcripts that positively correlated with A $\beta$  plaque burden. We also observed A $\beta$  preferentially activated disease-associated microglial signatures that were phagocytic, while myeloid Arg1 deficiency selectively promoted homeostatic and non-phagocytic microglial signatures.

**Conclusions:** These novel findings suggest that proper arginine metabolism regulated by Arg1 in brain myeloid cells is critical for performing phagocytosis to restrict amyloidosis and neuroinflammation and thus can be a key therapeutic target in AD.

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## **Antimicrobial Protein REG3A and Network Inflammatory Proteins are Predictive of Infarct Volume and Functional Impairment In Ischemic Stroke**

### **Student**

**Introduction:** Regenerating Family Member 3 Alpha (REG3A) is an antimicrobial protein secreted by the intestine and pancreas and is involved in immune-mediated inflammatory responses. Studies have shown an increased expression of REG3A in inflammatory responses. Particularly, pro-inflammatory cytokines such as IL17 and IL6 enhance REG3A expression and activity. These cytokines in addition to immune mediators such as CCL19, IL1 $\alpha$ , and IL15 are observed in the pathogenesis of neuroinflammation sequela of ischemic stroke. The University of Kentucky Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol utilizes thrombectomy to isolate intracranial (i.e. distal to thrombus) arterial blood and systemic (i.e. carotid) arterial blood from thrombectomy procedures to better understand stroke. From the analysis of plasma protein from this study, systemic REG3A is elevated in patients with ischemic stroke, but its role within mediating neuroinflammation during stroke remains unclear. Our aim was to examine the association of plasma REG3A levels with other signaling proteins in systemic plasma during stroke, and its correlation with clinical outcomes of ischemic stroke.

**Methods:** Intracranial and systemic plasma samples from n=25 thrombectomy subjects underwent Proximity Extension Assay via Olink Proteomics. REG3A levels and inflammatory markers were examined using bivariate regressions. Stepwise regression determined the predictability of infarct volume by REG3A. Two-tailed t-tests were used to examine the relationship between the National Institutes of Health Stroke Scale (NIHSS) and levels of REG3A.

### **Results:**

Higher levels of REG3A correlated with increased infarct volume ( $p=.009$ ,  $R=.514$ ). Stepwise regression predicting infarct volume yielded a model including REG3A and systemic proteins such as CCL19, IL15, and IL1 $\alpha$  ( $P<.001$ ,  $R=.980$ ). NIHSS scores corresponding to moderate-severe/severe strokes had higher levels intracranial REG3A ( $p<.05$ ) and higher REG3A expression intracranially relative to systemic REG3A( $p=.05$ ).

### **Conclusions:**

Within a network of proteins, REG3A is predictive of increased infarct volume and decreased function. NF $\kappa$ B is a key transcriptional regulator of neuroinflammatory processes and enhances expression of pro-inflammatory cytokines such as IL6, IL17, CCL19, IL1 $\alpha$ , and IL15. As previously stated, IL6 and IL17 can enhance REG3A, and in models examining systemic inflammatory processes, REG3A appears to interact and alter NF $\kappa$ B activity. This highlights the possible involvement of REG3A in NF $\kappa$ B-driven neuroinflammation. Considering the importance of time in context to the NIHSS score, future studies will examine systemic and intracranial REG3A in relation to the patients last known normal. Examining this relationship will be critical in evaluating the role of REG3A in neuroinflammation and as a potential prognostic and therapeutic target in large vessel occlusive stroke.

# Epilepsy & Brain Metabolism

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### **Effect of Different Electroencephalographic Signal Derivations on Alpha Reactivity to Eye Closure**

#### **Student**

**Introduction:** Electroencephalography (EEG) is a widely used technique for monitoring and analyzing brain activity in experimental, diagnostic, and therapeutic applications. Since EEG is susceptible to many noise and artifact sources, multiple referential signals can be combined in different ways to improve the signal-to-noise ratio and better localize cortical activity. In this investigation, we compared three such signal derivations against referential EEG (eEEG) in terms of their ability to measure “alpha reactivity” to eye closure: common average referencing (CAR), a large Laplacian (LP), and a focal Laplacian (tEEG) estimated using a novel tripolar concentric ring electrode (TCRE). Alpha reactivity refers to the surge in alpha band (8-13 Hz) EEG activity that occurs upon eye closure and is a simple test used to verify signal quality in EEG analysis.

**Methods:** EEG recordings were made from nine healthy adult subjects who were instructed to alternate between opening and closing their eyes for 30 seconds five times in succession. Subjects were seated during the task and electrodes affixed to their scalp at nine locations. All impedances were below 5 kohm. The four signals derived from each TCRE lead were filtered into the alpha band and the Hilbert envelope calculated. The Hilbert envelope was lowpass-filtered down to 1 Hz to reduce noise fluctuation and normalized by the mean value in the 3-second period before eye closure. The maximum value of the scaled envelope in the 10-second period after eye closure was used as an estimate of alpha reactivity.

**Results:** An rmANOVA showed that there were significant differences in the alpha reactivity measured using tEEG, eEEG, and CAR across all 9 electrode locations ( $p < 0.05$ ). Post hoc analysis concluded that CAR showed significantly greater alpha reactivity than either tEEG or eEEG, and that tEEG showed significantly greater alpha reactivity than eEEG. When all four derivations were compared for location Cz alone (since LP could only be computed for this location), the rmANOVA indicated that there were significant differences between these estimates of alpha reactivity ( $p < 0.01$ ). Post hoc analysis concluded that CAR again showed significantly larger alpha reactivity than tEEG, eEEG, or LP; however, none of the other differences reached significance.

**Conclusion:** The choice of EEG measurement locations and their derivations greatly influence the ability to detect dynamic changes in EEG signals associated with mental and physical activity. For the specific case of alpha modulation by eye closure, our results show that CAR maximizes the event-related response better than the other derivations tested here. However, this requires the use of multiple electrodes. When comparing single electrode derivations, the tEEG appears to evince greater alpha modulation compared to the referential eEEG at the same location. This provides a foundation for ongoing analysis of spatial source of variability.

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**Estrogen receptor alpha is required to protect daily metabolic rhythms from disruption by high-fat feeding in female mice**  
**Student**

The circadian system is a critical regulator of obesity in male mice, but its role in females is poorly understood. In our previous studies we found that estrogen regulates daily rhythms in female mice to confer resistance to diet-induced obesity, but the mechanism is unknown. Estrogen signals via the classical estrogen receptor alpha (ER $\alpha$ ) to regulate metabolism and obesity. Therefore, in this study we tested the hypothesis that estrogen regulates daily metabolic rhythms in females via ER $\alpha$ . To do so, we studied daily rhythms in global ER $\alpha$  knockout (ER $\alpha$  KO) PERIOD2::LUCIFERASE female mice fed high-fat diet for 6 weeks. ER $\alpha$  KO female mice became obese and hyperglycemic when fed high-fat diet, while wild-type females were resistant to diet-induced obesity. Chronic high-fat diet feeding also reduced the amplitude of the daily rhythm of eating behavior in ER $\alpha$  KO, but not wild-type, female mice. In wild-type females, the amplitude of the locomotor activity rhythm increased during high-fat feeding. In contrast, high-fat feeding decreased the amplitude of the activity rhythm in ER $\alpha$  KO females. Circadian organization of tissue clocks was disrupted by high-fat feeding in ER $\alpha$  KO females since the phase of the liver PER2::LUC rhythm was advanced 4 hours by high-fat feeding in ER $\alpha$  KO mice compared to wild-type females. Taken together these results show that estrogen signals via ER $\alpha$  to protect daily metabolic rhythms from disruption by high-fat feeding in female mice.

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**Baclofen toxicity masquerading as burst suppression pattern on EEG**

***Other***

We presented a patient with C5/6 tetraplegia secondary to a remote motor vehicle accident (MVA), neurogenic bladder, recurrent UTIs, nephrolithiasis who presented with AMS and worsening clinical status three weeks after an initial bilateral ureteral stent placement at an outside hospital. His EEG demonstrated a burst suppression pattern. This led to the correct diagnosis of baclofen toxicity induced AMS. Brief literature review of baclofen toxicity induced EEG changes were discussed.

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### **Uniform Criteria for Accurate Detection of High Frequency Oscillations in the Epileptic Brain**

#### **Student**

High frequency oscillations (HFOs) are gaining favor in clinical practice as markers of the epileptogenic cortex in patients with epilepsy. Unfortunately, population spikes and sharp transients in the interictal baseline tend to look like genuine HFOs and produce false positive detections when subjected to bandpass filtering, which is invariably the first step in the process of HFO detection. Rejection of such filtering artifacts is critical if HFOs are to be used for diagnostic prediction of the cortical areas to be resected in order for a patient to become seizure-free. To complicate matters, HFOs often co-occur with spikes, and such events may be rejected (false negative outcome) by algorithms that attempt to remove spikes and improve the accuracy of HFO detection. The purpose of this study is to develop an unsupervised detection algorithm that requires no tuning and retains genuine HFOs with and without spikes while rejecting artifacts by applying simple criteria for ripple amplitude, rhythmicity, and ringing artifact. With IRB approval, in nine epilepsy patients admitted for invasive presurgical evaluation, intracranial EEG (iEEG) recordings were acquired with 1000 Hz sampling rate. HFO candidates were first identified using a slightly modified version of well-known algorithm (Staba et al., 2002), which is highly sensitive to HFOs but admits spikes and other artifacts. In addition, a nonlinear method was devised to estimate the transient baseline of candidate HFOs in a way that suppresses spikes and other artifacts in the residual signal without the need for bandpass filtering. A superset of 2700 detections made by the Staba algorithm with relaxed amplitude threshold were selected at random from 1-3 channels in each patient's recording. The new algorithm proposed here was tested on this set and found to distinguish true HFOs from spikes with a sensitivity of 20%, specificity of 93%, and positive prediction value of 90%. This procedure mostly rejected spikes while retaining highly rhythmic HFOs with and without spikes whose site of origin is strongly correlated with the seizure onset zone marked by the physician.

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### **A membrane-palmitoylated protein interacts with dystrobrevin to regulate neural circuit function in *C. elegans***

#### **Student**

Balancing synaptic excitation and inhibition is necessary for proper function of neural circuits. Disruption of this E/I balance is characteristic of multiple neurologic disorders, including epilepsy. We use *Caenorhabditis elegans* (*C. elegans*) as a model to study the regulation of neural circuits *in vivo* at the molecular level. *C. elegans* locomotion is modulated by a precise balance between cholinergic excitation and GABAergic inhibition at the neuromuscular junction. To assess perturbations in this circuit we employed aldicarb, an inhibitor of acetylcholinesterase. MAGU-3 is a membrane palmitoylated protein, which is a subfamily of membrane associated guanylate kinases. MAGU-3 has two mammalian orthologs, MPP2 and MPP6, that function in neurons to regulate postsynaptic density scaffolding and myelin formation respectively. Here we found that when treated with aldicarb, *magu-3* mutant animals become paralyzed at a faster rate than wild type animals, suggesting mutations in *magu-3* cause an E/I imbalance in the motor circuit. Using a MAGU-3::GFP transgene, we were able to restore aldicarb sensitivity to wild type levels. We also determined via GFP fluorescence that MAGU-3 is primarily expressed in neurons including the cholinergic and GABAergic motor neurons. To explore the molecular mechanisms through which MAGU-3 regulates the motor circuit we screened for interacting genes. A yeast two-hybrid SH3 interactome study predicted an interaction between MAGU-3 and DYB-1, which has been shown to exhibit acetylcholine transport and cytoskeletal binding activity. DYB-1 is an ortholog of the mammalian dystrobrevin. Previously null or loss of function mutants of *dyb-1* were shown to be hypersensitive to aldicarb. Investigating a potential loss of function allele of *dyb-1*, we observed that it is slightly more hypersensitive than the *magu-3* mutant. While the *magu-3;dyb-1* double mutant remained hypersensitive as compared to wild type animals, the hypersensitivity was reduced to *magu-3* single mutant levels. This suggests that MAGU-3 and DYB-1 interact *in vivo*; however, the roles of this interaction in regulating neural circuit function remain unknown. Interestingly, we observed that another *dyb-1* mutant did not affect aldicarb sensitivity on its own but reduced the *magu-3* mutant aldicarb sensitivity to wild-type levels, suggesting this may be a potential gain of function mutation. These results highlight an unexplored interaction between *magu-3* and *dyb-1* to regulate neural circuit function. Future studies of this interaction, including analysis of circuit activity, circuit formation, expression patterns, protein localization, and additional participating genes will provide new insights into mechanisms that regulate brain function.

# Movement Disorders – Motor Control

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### **Rapid-Onset Dystonia Parkinsonism: Evolution of Clinical Phenotype 26 Years Post Diagnosis**

#### **Fellow**

#### **Objective:**

To discuss the evolution of the clinical phenotype in a 49-year-old man with Rapid Onset Dystonia Parkinsonism (RDP) 26 years post diagnosis, and the heterogeneity associated with ATP1A3 mutations.

#### **Background:**

RPD is a rare movement disorder characterized by the abrupt onset of parkinsonism and dystonia manifesting over hours to weeks in childhood or early adulthood. Our patient belongs to a family of 15 affected individuals spanning 3 generations who tested positive for a mutation in ATP1A3. This family was previously reported in *Movement Disorders* by Brashear et al.

#### **Methods:**

We report on the evolution of the clinical phenotype in a 49-year-old man 26 years post diagnosis with RPD who was lost to follow up for 12 years and returned with worsening symptoms.

#### **Results:**

At age 23 he reported cramps and tremor of the left hand, followed 2 months later by intermittent spasms of the left fifth toe lasting several weeks before resolving spontaneously. He described stiffness of the left leg while walking and cramping of the right hand. Examination demonstrated cogwheel rigidity at the left elbow, wrist and knee. Dystonic postures were noted in the left hand at rest that increased during walking. There was bradykinesia, but no tremor, postural instability, dysarthria, or dysphagia. A trial of levodopa/carbidopa demonstrated minimal improvement. 12 years later he underwent Deep Brain Stimulation with no benefit. Botulinum toxin injections, however, were helpful. He returned at the age of 49 with complaints of generalized stiffness and dysarthria. He had saccadic pursuit eye movements, facial dystonia, moderate-severe hypophonia with dystonic speech, generalized dystonia (most prominent in posterior neck and upper extremity muscles) and broad-based gait. There was symmetric rigidity, and bradykinesia of all extremities.

#### **Conclusion:**

Dobyns et al. suggested a consistent and unique phenotype consisting of rapid onset dystonia and parkinsonism initially, with slow or no progression. We now know that a spectrum of clinical presentations are possible with the ATP1A3 mutations that include classic RDP, classic RDP with intermittent worsening, slowly progressive Dystonia Parkinsonism, alternating hemiplegia of childhood, alternating hemiplegia with evolution to dystonia parkinsonism, and combinations involving seizures and Cerebellar ataxia, Areflexia, Pes Cavus, Optic atrophy and Sensorineural hearing loss (CAPOS) syndrome. This may suggest that these diseases are not necessarily distinct clinical entities but perhaps different manifestations along a clinical spectrum of disease.

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### **Preliminary Indication of Graded Correlates from Electroencephalography During Motor Planning**

#### ***Student***

Volitional movement is essential for daily life. Brain-computer interfaces (BCIs), which are meant to assist individuals with severely impaired movement, often rely on brain signals associated with actual or imagined movement as control signals. In this ongoing study, we modeled changes in brain activity associated with motor planning at different intensities as a predictor of effort. Specifically, we recorded scalp electroencephalography (EEG) in healthy human subjects (n = 6) performing isometric hand contractions of varying forces in response to a cue. A Wilcoxon signed-rank test showed significant trends ( $p < 0.05$ ) in 8-30 Hz EEG signal power prior to the cue correlated with the target force to be generated subsequently in the task in 5 out of 6 subjects. This demonstrates that signal features that anticipate motor effort can be extracted from the EEG and potentially harnessed for BCI control.

## Role of *Caenorhabditis elegans* RapGEF *pxf-1* in Regulation of Neural Motor Circuit Function

### Student

Mutations within the human RAPGEF2 and RAPGEF6 genes have been previously linked to schizophrenia. When knocked out in the mouse brain, the animals were found to have reduced anxiety and fear behaviors, increased LTP, but no changes in gross brain morphology. In order to further understand the role of RAPGEF proteins in neuronal circuit function and regulation, we used the motor circuit of *Caenorhabditis elegans* as our model system. *C. elegans* has one RAPGEF2 homologue named PXF-1. To determine the role of PXF-1 in regulating neural circuit function, we used the acetylcholinesterase inhibitor, aldicarb, to measure synaptic activity in the motor circuit. Treatment with aldicarb causes paralysis over time that directly correlates with levels of synaptic activity. We found that two independent mutant alleles of *pxf-1* caused the animals to become resistant to aldicarb as compared to wild type animals. In previous studies, PXF-1 has been shown to interact with MAGU-2, a membrane associated guanylate kinase, via yeast two-hybrid. We have recently shown that MAGU-2 modulates synaptic connectivity in the motor circuit. Based on its interaction with MAGU-2, we hypothesized that PXF-1 could be interacting with cell polarity pathways to modulate synaptic activity or development and that disruption of this pathway reduces synaptic connectivity or function similar to schizophrenia. To determine if MAGU-2 and PXF-1 act together to regulate motor circuit function, we measured aldicarb sensitivity in double mutant animals. While *magu-2* and *pxf-1* mutant strains displayed resistance to aldicarb, mutations in *magu-2* produced a higher level of resistance as compared to *pxf-1* mutants. The *pxf-1; magu-2* double mutants displayed resistance to aldicarb similar to *magu-2* single mutant levels. These results suggest that MAGU-2 may function upstream of PXF-1 and additional signaling pathways to regulate synaptic function. Next, we used fluorescent synaptic markers to investigate whether *pxf-1* mutants displayed alterations in synapse morphology. We observed a reduction in active zone size in *pxf-1* mutants as compared to wild type animals. Additionally, *magu-2* mutants and *pxf-1; magu-2* double mutants also showed smaller active zones. Based on these findings, PXF-1 may function within the same pathway as MAGU-2 to coordinate synapse development. Overall, our current work suggests that cell polarity signaling pathways govern synapse development and disruption of these pathways may underlie synaptic connectivity defects observed in schizophrenia. Future cellular and molecular studies will elucidate the molecular mechanisms through which PXF-1 and MAGU-2 promote synaptic function.

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Neurology University of Kentucky <sup>1</sup>

### **Controlled release of hydrogen sulfide protects cells overexpressing A53T mutant of alpha-synuclein**

#### **Fellow**

**Title:** Controlled release of hydrogen sulfide protects cells overexpressing A53T mutant of alpha-synuclein.

E. Ostrakhovitch, E. Song, and T.Yamasaki

**Objective:** This study investigates the effect of hydrogen sulfide donors, NaHS and GYY 4137, in human HEK-293 cells overexpressing the A53T mutant of  $\alpha$ -synuclein.

**Background:** Parkinson's disease is characterized by progressive loss of dopaminergic neurons. Its neuropathological hallmark is the accumulation of aggregated form of alpha-synuclein ( $\alpha$ Syn). Hydrogen sulfide, a gasotransmitter and potent antioxidant, plays an important role in many physiological and pathological processes. Recent studies revealed that impairment in hydrogen sulfide synthesis leads to Parkinson's disease. Administration of NaHS (an H<sub>2</sub>S donor) reverses the progression of movement dysfunction in 6-OHDA-induced PD model. However, the mechanism underlying this action of hydrogen sulfide remains unknown.

**Methods:**  $\alpha$ Syn aggregation in cells overexpressing a-syn A53T CFP/YFP was seeded by lipofectamine aided-transduction of 1 and 10nM of preformed  $\alpha$ Syn fibrils (PFF). Cell lysates and isolated mitochondria were analyzed by western and dot blotting. The real-time quaking-induced conversion (RT-QuIC) assay was performed to examine effect of NaHS on formation of a-syn fibrils.

**Results:** Pathogenic  $\alpha$ Syn, indicated by serine 129 phosphorylated  $\alpha$ Syn and conformation specific- $\alpha$ Syn measured by immunoblot, was associated with mitochondria. PFF induced permeability transition pore opening, loss of nicotinamide phosphoribosyl-transferase (NAMPT), which plays an important role in cellular bioenergetics and metabolism, and ultimately cell death. Remarkably, treatment with H<sub>2</sub>S donors restored level of NAMPT, mitochondrial membrane potential and consequently increased cell viability. However, NaHS triggered  $\alpha$ Syn phosphorylation and  $\alpha$ Syn assembly. NaHS-induced aggregation was observed in A53T mutant and not in wild type  $\alpha$ Syn.

**Conclusions:** These data suggest that conformational ensemble in the presens of H<sub>2</sub>S donors prevents PFF cytotoxicity. Our findings also designate H<sub>2</sub>S donors as a viable clinical avenue for neuroprotection in PD

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**Clinical trial platform combining delivery of investigational therapeutics with deep brain stimulation surgery in patients with Parkinson's disease**

**Faculty**

**Introduction:**

Over the last seven years, we have been evaluating the safety and feasibility of investigational cell therapy delivered to the substantia nigra (SN) in participants (n=63) with Parkinson's disease as part of Phase I open-label, single center, clinical trials (NCT01833364 and NCT02369003). The source of our cell therapy material is autologous peripheral nerve tissue obtained from the sural nerve. Schwann cells are abundant in peripheral nerve tissue and transdifferentiate after injury into "repair cells".

**Objectives:**

To address several challenges of clinical trials focusing on disease modifying therapies for PD by using a combined surgical deployment of the investigational therapy to the SN at the time of DBS (we termed, DBS Plus).

**Methods:**

Tissue grafts were harvested and implanted into the SN, unilaterally or bilaterally, during DBS surgery directly following the placement of the stimulating electrodes. Safety, feasibility, and tolerability were assessed.

**Results:**

To date, in our experience, DBS Plus has an overall adverse event profile to that of DBS alone. Sixty-three out of 63 participants scheduled to undergo DBS Plus received graft. Five of 55 (8 participants are ongoing) participants have missed final study visit. Trial costs were greatly reduced because DBS surgery was standard of care and covered by insurance.

**Conclusions:**

A major ethical advantage of DBS Plus is that participants do not have to forego the therapeutic benefits of DBS to be involved in the study. Overall, DBS Plus provides an excellent platform for exploratory, interventional, disease modifying clinical trials.

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### **Acquired Movement Disorders Secondary to Tumefactive Virchow Robin Spaces**

#### **Student**

**Introduction:** Perivascular spaces (PVSs), commonly known as Virchow-Robins spaces, are interstitial cystic spaces filled with cerebrospinal fluid (CSF). Traditionally, these spaces are asymptomatic and benign, but in rare instances, these spaces can expand into giant tumefactive PVSs (GTPVSs) compressing on nearby structures causing neurologic conditions and hydrocephalus (3).

**Case Presentation:** We present a case of a 17-year-old male with a history of congenital hypothyroidism, that presents to the movement disorders clinic with chronic, nonprogressive left upper limb tremors. He describes the tremor as an unchanging jerky movement of his left shoulder. He also uses orthotic insoles to correct a chronic, nonprogressive left-sided gait imbalance.

The patient first experienced symptoms at age 11. At that time, he experienced excessive drowsiness and developed left upper extremity tremors and abnormal extraocular movements with a compensatory head tilt. MRI revealed a large cystic lesion at the right midbrain and thalamus. Biopsy revealed a GTPVS with mild hydrocephalus. There was no evidence of an infectious etiology and no further neurosurgical interventions were pursued.

Annual head MRIs have demonstrated no evolution of the GTPVS. The patient attempted a trial of Sinemet 25/100mg TID for 2 years without resolution or improvement of the tremor.

Physical examination showed a well-developed, awake, alert, and oriented male with readily understandable speech and a leftward head tilt. CN III-XII were tested and intact with the following abnormalities: Patient's left eye had an elevated gaze when compared to the right, with skewed lateral left and right gaze. Intermittent horizontal and rotary nystagmus was appreciated. Patient's muscle tone, strength, and deep tendon reflexes were normal. Light touch was intact with a negative Romberg sign. Left side showed no resting adventitious movements, but a proximal, 4-6hz tremor in the left arm and hand is appreciated when arms were placed in a winged position with fingertips juxtaposed without touching. Dystonic posturing in the left hand was noted and finger-to-nose testing showed ataxia without dysmetria. While holding his left forearm with his right hand the finger-to-nose testing showed a marked decrease in ataxia. Mild bradykinesia in the left upper and lower extremity was elicited with rotating hand movements and left heel tapping. Ataxia was noted with heel tapping, heel-to-shin testing, and tandem gait.

**Discussion:** This case highlights that dilated perivascular spaces are not always benign entities and can result in complex neurologic presentations.

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**Retro-orbital injections of microR-223 containing liposomes downregulate pro-inflammatory signaling following traumatic brain injury**

**Fellow**

Traumatic brain injury (TBI) results in a significant and prolonged phase of neuroinflammation that is thought to significantly impact neuronal tissue repair. Currently, there are no effective treatments for TBI due, in part, to the highly complex biochemical and pathophysiological events that occur at different time points following the initial insult. One major secondary injury event is the synthesis and release of pro-inflammatory factors from microglia/macrophage cell types. We and others have previously shown that the levels of several inflammatory-related microRNA (miRNA) are significantly elevated in the rat hippocampal formation following a severe controlled cortical impact injury. MicroRNAs (miRNAs) are short, non-coding RNA sequences that provide post-transcriptional regulation of widespread biochemical and molecular events including inflammation. In this study, we report the differential expression of a subset of inflammatory miRNAs in CD11b<sup>+</sup> cells isolated from bone marrow and mouse brain at 3hr, 24hr, 7 days and 14 days following TBI. We also measured the expression levels of several pro- and anti-inflammatory macrophage/microglia marker genes at these same time points. TaqMan Low Density Array (TLDA) analysis of macrophage/microglia phenotypic gene expression suggested a high activity of these genes in CD11<sup>+</sup> cells at 24 hours following TBI, with a subsequent reduction at 7- and 14-days post injury. We then investigated the ability of intravenous liposomal delivery of the anti-inflammatory miRNA, miR-223, to downregulate pro-inflammatory target genes following TBI. We found that retro-orbital injections of liposome+miR-223 at 4-6 hours following severe TBI resulted in a 2-fold increase in miR-223 in the injured brain and down-regulation of the miR-223 pro-inflammatory targets CxCl10, NLRP3, and IKK-a. The manipulation of secondary neuroinflammatory responses using a specific miRNA targeting pro-inflammatory signaling may provide an effective strategy for treating TBI pathogenesis at acute stages.

# Neuro Trauma

Alyssa Day <sup>1</sup> • Michael Williams, PhD <sup>1</sup> • Hamsini Ajjan <sup>1</sup> • Amanda Glueck, PhD <sup>2</sup> • Dan Han, PhD <sup>2</sup>  
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### **Disruptive Behaviors and Headache in Adolescents after Mild Traumatic Brain Injury**

#### **Other**

**Introduction:** Nearly one in five adolescents between grades 8-12 report at least one diagnosis of concussion/mild traumatic brain injury (mTBI) in their lifetime. Headache is among the most common post-injury complaint. Disruptive behaviors are associated with increased difficulties with peer relationships and decreased academic achievement, making identification and treatment of these behaviors essential. Increased disruptive behaviors have been reported in both non-traumatic and post-traumatic headache (PTH) populations. This study examined differences in disruptive behaviors among adolescents with and without PTH, hypothesizing higher disruptive behaviors among adolescents with PTH compared to those without.

**Method:** Participants included 122 adolescents (grades 8-12; age 13-17 years) who received care at an ambulatory mTBI clinic. As part of routine care, each participant completed the Beck Disruptive Behavior Inventory for Youth-II (BDBI). Headache status (yes or no, within past week) was recorded at the time of the visit. An independent samples t-test was conducted to assess for potential differences in disruptive behaviors between adolescents with ( $n = 101$ ) and without PTH ( $n = 21$ ).

**Results:** There were no significant differences among demographic and injury related variables except for gender. Girls reported headache more often than boys,  $p = .047$ . Gender was not used as a covariate since BDBI T-scores (outcome) are adjusted for age and gender. Adolescents with PTH reported significantly greater disruptive behaviors compared to adolescents without PTH;  $t(118) = -2.22$ ,  $p = .029$ ,  $d = .54$ . Levene's test for equality of variances was not significant.

**Conclusions:** Findings suggest that adolescents with PTH have a greater risk for disruptive behavior after mTBI and a higher proportion of girls report PTH relative to boys. Future research should explore neurological and psychiatric mechanisms for the relationships among headache, mTBI, and disruptive behaviors in adolescents. This study suggests an ongoing need for intervention research on PTH among adolescents.

**Using vessel painting to investigate brain vascular structure changes after a mild traumatic brain injury in mice*****Student***

Traumatic brain injury (TBI) is a major health concern globally, affecting millions of people. These brain injuries lead to many different pathologies which can affect individuals for years following a single event. One major issue following TBI is alterations to the brain vasculature. Changes to vascular structure and function can have major consequences on brain functioning and thus greatly affect the person's recovery. Research has shown that following TBI there are changes to the cell types that make up the brain vasculature including brain endothelial cells, pericytes, astrocytes, and microglia. However, less work has described changes to overall structure, specifically in mild TBI. Mild TBI accounts for up to 90% of all TBIs and many of these individuals never fully recover. We were interested in determining structural changes following a single mild TBI. Vessel painting is a technique that can be used to label the intact vasculature of the mouse brain. Using this technique, we were able to demonstrate that 24 hours following a single, mild closed head injury that there was a significant decrease in vessel surface area and volume.

# Neurorehabilitation

Jackie Tierney <sup>1</sup> • Stephen Dundon <sup>1</sup> • Chase Haddix <sup>1</sup> • Sridhar Sunderam, PhD <sup>1</sup>  
Biomedical Engineering University of Kentucky <sup>1</sup>

### **Development of an Online Training Paradigm for Mobile Brain-Computer Interface Applications**

#### ***Student***

Previous brain-computer interface (BCI) studies in our lab have demonstrated the ability to detect movement onset and predict exerted force in a handgrip task from the electroencephalogram (EEG). However, this required stationary equipment and offline analysis limiting the BCI from being used outside the lab or hospital and does not allow the subject to receive real time feedback on their performance. As part of our lab's ongoing research, we tested the feasibility of replicating previous sensorimotor rhythm detection and analysis capabilities in real time using a relatively inexpensive and portable open source EEG platform, OpenBCI. Initial development and testing involved simultaneous EEG and forearm EMG recordings from a healthy adult male subject. The subject responded to visual cues by squeezing a dynamometer. A detection algorithm for EEG modulation associated with the task was coded in MATLAB (MathWorks). The algorithm initially tracks the mean-squared signal power in a 5-s moving window and computes a dynamic threshold that optimizes the weighted sum of detection sensitivity and specificity as determined by the state of the EMG (active vs. inactive). A second trial was performed with a function generator that mimicked oscillations from the sensorimotor cortex during motor tasks. Trials of the online training and detection algorithm were assessed by computing the detection sensitivity as the percent time spent in active handgrip that was below the mu-beta power threshold and the detection specificity as the percent time spent in the relaxed state that was above the threshold. The completed analysis yielded a sensitivity and specificity of 45 and 75%, respectively. Our experiments show that real-time analysis of EEG in a movement-based task on a mobile platform is feasible for BCI applications using our novel approach. This will bring us closer to our goal of being able to provide rehabilitation or support for patients with impaired movement.

This material is based on work supported by the National Science Foundation under Cooperative Agreement No. 1849213.

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Biomedical Engineering University of kentucky <sup>1</sup> • Biomedical Engineering University of kentucky <sup>2</sup>

### **Design of a Sensor Glove for Movement Feedback and Rehabilitation Using a Brain-Computer Interface**

#### ***Student***

Individuals who have suffered from a stroke or a spinal cord injury may no longer have the ability to open or close their hand depending on the nature of the insult. The purpose of this research is to design a closed loop system that can detect when a human subject intends to squeeze their hand using electroencephalography (EEG) and quantify their actual hand movement using physical sensors to provide useful feedback. We are experimenting with a system designed to track and verify hand movement based on two different sensors: 1. Piezoelectric sensors that change resistance when they are flexed (Adafruit); and 2. Photovoltaic sensors that measure the amount of light passing through an optic fiber, which changes when the fiber is bent or constricted by applied pressure. A custom-designed sensor glove will incorporate both the flex and optical sensors so that they work in tandem and cross-check each other for accuracy. The purpose of designing the custom glove is to capture hand movements, which have a high number of degrees of freedom, more accurately and in real time. Flexion of each finger will be tracked independently. A commercial motion capture glove (VRFree, Sensoryx) will be used to check the accuracy of our custom design and tracks three-dimensional coordinates of multiple locations on the glove. To monitor the person's intent in simple manual tasks, EEG signals will be recorded in real-time; synchronous measurements from the glove will help assess actual versus intended movement. We anticipate that this closed loop system will enable versatile protocols to improve and better understand motor control in patients with impaired function.

Justin Huber, MD <sup>1</sup>

Department of Physical Medicine & Rehabilitation University of Kentucky <sup>1</sup>

### **Applying 3D Printable Elastics to Rehabilitation: A Case Series of Stroke Survivors**

#### ***Student***

In neurorehabilitation, orthoses serve many important roles in patient care—therapeutic intervention (e.g. Bioness systems), reduction of disease sequela (e.g. resting hand brace), and functional restoration (e.g. tenodesis splint). A growing demand for rehabilitation devices is prompting new approaches to orthotic fabrication. Additive fabrication with 3D printing offers many benefits including unique geometries, rapid prototypes, and personalized devices. Research on 3D printable orthoses has primarily focused on rigid constructs such as arm and ankle splints. This study investigates non-rigid, 3D printable elastic materials and explores their application to compliant orthoses. We designed a compliant hand orthosis featuring an interchangeable finger component. This component of the orthosis was duplicated in five 3D printed elastic materials. By interchanging this finger component, each elastic material underwent usability testing by volunteer participants. In a case series, two participants with chronic hand impairment secondary to stroke performed the usability tests. Outcomes included qualitative participant feedback and quantitative metrics—pincer force, pincer aperture, and the Box and Block Test. This study demonstrates 3D printable elastic materials in a compliant orthosis, which may inspire future applications in soft robotics and wearable technologies.

Key Words: Hand Orthosis, Compliant Design, Additive Manufacturing, Assistive Technology, Hemiparesis, Upper Extremity, Hand

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### **Transient fecal microbiome changes after traumatic brain injury in mice**

#### **Student**

Over 2.8 million people suffer a traumatic brain injury (TBI) annually in the United States. It has long been recognized that TBI can induce gastrointestinal dysfunction. Even in the absence of polytrauma, TBI patients have an increased incidence of intestinal inflammation, ulceration, fecal incontinence, and gastrointestinal-related mortality. The gut microbiome serves as a key regulator of gastrointestinal health, and recent findings have suggested that experimental TBI is capable of inducing microbiome changes, and human TBI patients have microbiome changes years after their original injury. However, the timeline of early changes to the microbiome after TBI are not well understood. Conventionally raised male C57Bl/6J mice underwent a microbiome transplant to minimize differences in their starting microbiomes prior to undergoing a controlled cortical impact (CCI) or sham injury. To assess changes to the fecal microbiome both at acute and chronic timepoints, fecal samples were collected prior to injury, 1, 2, 3, 7, 14, and 28 days post injury. DNA extracted from fecal samples were used for 16s rRNA gene sequencing and subsequent analysis. Alpha and beta diversity were assessed, and differential abundance analysis was conducted using ANCOM-BC within R. Findings suggest that the phylum *Verrucamicrobiota* is differentially abundant in CCI mice compared to their sham counterparts at 1, 2, and 3 days post injury. The phylum *Verrucamicrobiota* plays an important role in GI function as well as promoting normal immune function systemically. This bacterial phyla has been implicated in other neurological conditions, and has been explored for its potential therapeutic potential.

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Department of Physical Medicine & Rehabilitation University of Kentucky <sup>1</sup>

**Applying 3D Printable Elastics to Rehabilitation: A Case Series of Stroke Survivors**

***Student***

In neurorehabilitation, orthoses serve many important roles in patient care—therapeutic intervention (e.g. Bioness systems), reduction of disease sequela (e.g. resting hand brace), and functional restoration (e.g. tenodesis splint). A growing demand for rehabilitation devices is prompting new approaches to orthotic fabrication. Additive fabrication with 3D printing offers many benefits including unique geometries, rapid prototypes, and personalized devices. Research on 3D printable orthoses has primarily focused on rigid constructs such as arm and ankle splints. This study investigates non-rigid, 3D printable elastic materials and explores their application to compliant orthoses. We designed a compliant hand orthosis featuring an interchangeable finger component. This component of the orthosis was duplicated in five 3D printed elastic materials. By interchanging this finger component, each elastic material underwent usability testing by volunteer participants. In a case series, two participants with chronic hand impairment secondary to stroke performed the usability tests. Outcomes included qualitative participant feedback and quantitative metrics—pincer force, pincer aperture, and the Box and Block Test. This study demonstrates 3D printable elastic materials in a compliant orthosis, which may inspire future applications in soft robotics and wearable technologies.

Key Words: Hand Orthosis, Compliant Design, Additive Manufacturing, Assistive Technology, Hemiparesis, Upper Extremity, Hand

# Stroke & Vascular

# Abstracts

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## Characterizing NMDA Receptor Subunits On B Cells

### Student

**Background:** N-methyl-D-aspartate (NMDARs) play a critical role in neuronal excitotoxicity after stroke. The actions of NMDARs have been shown mostly in obligatory GluN1 subunits on neurons and not GluN2A/B subunits. In B cells, these subunits have not been highly characterized though the presence of NMDARs has been shown. The function of the GluN2A/B subunits can be neuroprotective or pro-death in neurons, respectively. We hypothesized that GluN2A and GluN2B subunit presence on B cells would be affected by exposure to extracellular glutamate. **Methods:** Splenic B cells were isolated from 3-4mo-old C57BL/6 male mice via magnetic separation and treated with physiologic levels of L-glutamate (glu; 1uM) in the presence or absence of 5ug/mL LPS. B cell cytopins were stained for B220, GluN2A, and GluN2B, imaged using confocal microscopy, and quantified in FIJI. An average of 10.7 B cells were quantified per image at 80-157x magnification. RGB channels of the z-stacks were quantified to identify positive B220 expression. The z-stacks were split into 2D images and quantified plane-by-plane to identify GluN2A/B subunit clusters. Each cluster of subunits was recorded per cell in view across all planes of the original z-stack to yield total subunit count. Groups included 14-43 B cells quantified, and the number of subunits per cell were analyzed via ordinary two-way ANOVA, Sidak post-hoc test (Graphpad Prism). Significance was  $p < 0.05$ . **Results:** There was an average of  $19.3 \pm 7.2$  GluN2A subunits and  $19.0 \pm 5.0$  GluN2B subunits per cell for unstimulated, untreated B cells. Neither glu treatment ( $p = 0.23$ ) nor LPS stimulation ( $p = 0.10$ ) impacted the number of GluN2A subunits per B cell. LPS decreased GluN2B subunits when compared to unstimulated B cells ( $11.1 \pm 5.1$  subunits;  $p = 0.02$ ). Glu treatment normalized GluN2B subunits per B cell near untreated baseline levels ( $18.2 \pm 11$  subunits per cell;  $p = 0.01$ ), resulting in an interaction between LPS stimulation and glu treatment in B cells ( $F(1, 86) = 6.180, P = 0.015$ ). **Conclusions:** Our data suggests activated B cells downregulate GluN2B-containing NMDARs following LPS stimulation. This downregulation mimics that of NMDAR activity on neurons upon excitotoxicity (PMID: 24361499) but future studies should confirm GluN2B internalization.

## The B cell response to extracellular glutamate in the ischemic brain

### Faculty

**Background:** Neuronal networks require significant neurotrophic support for functional plasticity after stroke. We showed that B cells exhibit a cell-specific migration pattern in the post-stroke brain. Post-stroke B cell depletion impedes neurogenesis, increases anxiety, and exacerbates memory deficits in mice; deficits generally mediated by brain regions occurring outside the initial infarct. **We hypothesize that the post-stroke microenvironment can enhance neurotrophic capacities of B cells to promote plasticity.**

**Methods:** Splenic B cells were isolated from 3-5 mo-old male C57Bl/6J mice. B cell N-methyl-D-aspartate receptor (NMDAR) subunits were identified by confocal microscopy. The acute (8 min)  $Ca^{2+}$  response to 1uM glutamate (glu) +/- NMDAR antagonists (10uM DAPV (competitive NMDAR inhibitor), 30uM ifenprodil (ifen., GluN2B subunit inhibitor), and 10uM TCN201 (GluN2A subunit inhibitor)) was assessed via flow cytometry in B cells (+/- 5ug/mL LPS). B cell viability and neurotrophin (NT)-related genes were assessed by flow cytometry and qPCR, respectively, in B cells (+/- LPS) treated with glu +/- NMDAR antagonists for 24h. Data were analyzed in Graphpad Prism.

**Results:** B cells express functional GluN2A- and GluN2B-containing NMDARs that influx  $Ca^{2+}$  in response to extracellular glu (\* $p < 0.05$ ). While LPS did not impact NMDAR-dependent  $Ca^{2+}$  influx in most B cell subsets,  $Ca^{2+}$  influx was significantly reduced by NMDAR antagonists in LPS-stimulated B cells (Effector B cells (DAPV \* $p < 0.05$ , ifen \*\* $p < 0.01$ ), Bregs (DAPV \* $p < 0.05$ , Ifen \* $p < 0.05$ ), B220<sup>+</sup> antibody-secreting cells (ifen \* $p < 0.05$ , TCN201 \* $p < 0.05$ )). Furthermore, a 24h glu treatment increased NT (BDNF: 2.28-fold, IL-10: 27.16-fold), NT receptor (TrkB: 1.33-fold) and NMDAR (GluN2A: 2.01-fold, GluN2B: 1.27-fold) expression in LPS-stimulated B cells (vs. untreated controls).

**Conclusions:** Our studies show that B cells respond to glu via NMDARs. Our data suggests that exposure to physiologic levels of glu enhance NMDAR-dependent signaling and upregulate NTs and NT receptors. These results are the first to indicate a glu-induced neurotrophic role for B cells in the ischemic brain. Future studies will determine whether B cell-derived NTs can protect neurons after stroke.

**An evaluation of blood gases in blood specimens immediately proximal and distal to occlusive thrombi in patients undergoing mechanical thrombectomy.**

**Staff**

**INTRODUCTION:** Ischemic stroke is a prevalent, devastating disease with high morbidity and mortality. Despite extensive research using animal models, there remains significant gaps in understanding processes of stroke in human patients. To address this, we developed a protocol to obtain and to analyze blood immediately proximal in systemic circulation and distal to a thrombus in patients undergoing mechanical thrombectomy ([www.clinicaltrials.gov](http://www.clinicaltrials.gov) NCT03153683). Our goal for this project was to evaluate blood gas changes and acid/base balance during stroke and how these changes are affected by patient factors.

**METHODS:** We analyzed blood samples from the first 62 patients in the BACTRAC registry. Bicarbonate, pO<sub>2</sub>, and pCO<sub>2</sub> values of intracranial (distal) and systemic (proximal) arterial blood relative to the occlusive thrombus were analyzed. Changes were compared in patients according to vascular collateralization as measured by CTA collateral scores.

**RESULTS:** Mean age was 68.9 years (25 – 95 years). 29 were male, 33 were female. 15 were current smokers (24%), and 47 were non-smokers (no smoking within the last 6 months; 76%). Overall, intracranial gas values differed significantly from systemic. Compared to systemic, mean intracranial pO<sub>2</sub> was decreased (211.39 vs. 246.91,  $p < 0.001$ ), pCO<sub>2</sub> was decreased (32.19 vs. 38.12,  $p < 0.001$ , and bicarbonate was decreased (18.90 vs. 22.20,  $p < 0.001$ ). Collateralization did not significantly affect distal blood gas values.

**DISCUSSION AND CONCLUSION:** A compensated metabolic acidosis is present in arterial blood gas samples immediately proximal and distal to thrombi in large vessel occlusive stroke. Vascular collateralization may not significantly affect the acid-base environment immediately distal to a large vessel occlusion.

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**White Matter Hyperintensity Volume and Location: Contributions of WM Microstructure, Brain Iron and Cerebral Blood Perfusion**

**Fellow**

White matter hyperintensities (WMHs) on T2-weighted magnetic resonance imaging (MRI) are a well-established marker of advanced cerebral small vessel disease (cSVD). However, more subtle MRI markers of cSVD are not well-established. Here we explored associations between three potential markers of early cSVD [white matter microstructure, iron concentration and cerebral blood flow (CBF)] and total WMH volume. Associations between early markers and regional WMH volumes were also explored, focusing on periventricular (PV) and deep (peripheral) regions. Eighty healthy older adults (ages 60-86) were scanned at 3 Tesla MRI using fluid attenuated inversion recovery (FLAIR), diffusion tensor imaging (DTI), multi-echo gradient-recalled echo (GRE) and pseudo-continuous arterial spin labeling (pCASL) sequences. In a stepwise regression model, DTI-based radial diffusivity (DR) contributed significant variance in predicting total WMH volume (adjusted R<sup>2</sup> change=0.136). In contrast, iron concentration (adjusted R<sup>2</sup> change=0.043) and CBF (adjusted R<sup>2</sup> change=0.027) made more modest improvements to the variance accounted for in total WMH volume. However, there was an interaction between iron concentration and location on WMH volume such that QSM predicted peripheral ( $p=0.034$ ) but not PV ( $p=0.414$ ) WMH volume. Our results suggest that WM microstructure may be a better predictor of WMH volume than either brain iron or CBF but also draws attention to the possibility that some early cSVD markers may be location-specific.

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### **Noncontact Optical Assessment of Spontaneous Low-Frequency Fluctuations of Cerebral Blood Flow in Neonatal Intraventricular Hemorrhage**

#### **Student**

Intraventricular hemorrhage (IVH) is the most common neurological complication of prematurity. The incidence of IVH reaches up to 45% in premature infants. IVH is a bleeding inside or around ventricles, spaces in the brain containing the cerebrospinal fluid, which occurs as a result of the fragility and immaturity of blood vessels in premature brains. Severe IVH disrupts development of structural and functional connectivity networks, leading to impairments of cerebral development and neurologic deficits. Preterm infants with IVH are prone to alterations in cerebral blood flow (CBF) and associated spontaneous low-frequency fluctuations. However, there are no established noninvasive imaging methods for continuous monitoring of CBF alterations at the bedside in neonatal intensive care units (NICU). Functional near-infrared spectroscopy (fNIRS) and diffuse optical tomography (DOT) are portable research tools for continuous monitoring of cerebral oxygenation in newborn infants at the cot side. However, fNIRS and DOT systems use a cap to hold the sources and detectors on the head for contact measurements. Adjusting and maintaining a stable optical coupling of numerous sources and detectors to a small fragile neonatal head is labor-intensive and poses significant challenges on head cap design and safety concerns. An innovative CCD/CMOS based speckle contrast diffuse correlation tomography (scDCT) technology has been recently developed in our laboratory, enables noncontact, noninvasive, and high-density 3D imaging of CBF distributions in deep brain cortex. In this study, we demonstrated for the first time, using our innovative scDCT technique to detect alterations in CBF and associated spontaneous low-frequency fluctuations in a neonatal piglet model of IVH, which closely replicate the head anatomy and brain pathology of human neonatal IVH. Relying on the neurovascular response, low-frequency fluctuations in neural activity can be evaluated by analyzing CBF data. Evaluation of low-frequency fluctuations enables understanding of functional brain activity, which is important when the neurovascular coupling is either unknown (as in infants) or altered (as with brain injury). In this study, we showed that IVH resulted in a CBF decrease in deep brain cortex. Resting-state spontaneous low-frequency fluctuations after inducing IVH showed attenuations in all frequencies (0.009–0.08 Hz) compared to the baseline before inducing IVH, representing hemodynamic disruptions by inducing IVH. We will also explore the use of scDCT to map resting-state functional connectivity networks to evaluate how brain lesion impacts the functional relationship between different brain regions. This pilot preclinical study is part of an ongoing study that moves one more step forward towards translation of our innovative scDCT technique to NICUs for continuous monitoring and rapid management of cerebral pathologies and interventions in human neonates with perinatal diseases.

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### **Uromodulin Protein Expression During Ischemic Stroke**

#### **Student**

**Introduction:** Uromodulin, also known as Tamm-Horsfall protein, is a glycoprotein that is expressed by the epithelial cells of the thick ascending limb of Henle's loop in the kidney. Uromodulin is the most abundant urinary protein in the healthy individual and has important roles in ion transport, water and electrolyte balance, and prevention of urinary tract infections. Research has shown that increased uromodulin expression may be associated with lower risk of cardiovascular disease in adults. Utilizing the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) (clinicaltrials.gov NCT03153683), a continuously enrolling tissue bank, we aimed to examine the associations between serum uromodulin, age, and high BMI (BMI>25) and its relationship to stroke in patients.

**Methods:** Arterial blood distal and proximal to the thrombus was collected during a thrombectomy procedure using the BACTRAC protocol and sent to Olink (Boston, MA) to determine proteomic expression via proximity extension assay. Arterial samples were taken from age and diseased matched control patients undergoing diagnostic angiograms but not experiencing a large vessel occlusion. Uromodulin expression was recorded and analyzed using Welch's two tailed T-tests and linear regressions.

**Results:** The relationship between systemic and intracranial uromodulin, high BMI and age were assessed. Systemic uromodulin expression increased with BMI > 25(P=0.0062). Systemic and intracranial uromodulin decreased with age (P=<0.0001, P=0.0416). The relationship between systemic and intracranial uromodulin expression and the presence of acute ischemic stroke were assessed. Systemic and intracranial uromodulin expression decreased in the stroke group compared to the control group, P=0.0126 and P=0.0013 respectively.

**Conclusions:** In our study uromodulin was found to be increased significantly in overweight patients and decreased significantly in older patients and in those that experienced a stroke. The increase in uromodulin in people with high BMI could be a protective reaction of the kidney to worsening conditions that make ischemic stroke more likely, with a goal of delaying dangerous outcomes. People with high BMI experience strokes on average 10 years earlier than those with normal BMI. The decreased expression of uromodulin in older adults could be associated with the decline of general kidney function that accompanies aging. Kidney dysfunction caused by the stroke could lead to the decrease in expression of uromodulin. Further analyzes of clinical data is needed to understand the role of uromodulin after ischemic stroke.

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### **Amylin deposition in skin capillaries as a marker for cerebral small vessel disease**

#### **Staff**

Amylin is a  $\beta$ -cell hormone that forms pancreatic amyloid. Individuals with prediabetes, type-2 diabetes and obesity have aggregated amylin in pancreatic, brain, heart and renal microvessels. We previously showed that circulating aggregated amylin attaches to red blood cells and capillary endothelium, which induces hypoxia and microcirculatory disturbances. Given the amyloidogenicity of human amylin and its adverse effects on microvasculature, we hypothesized that amylin deposition in skin capillaries could be a marker for cerebral small vessel disease. Using rats with pancreatic overexpression of human amylin (HIP rats), we show that accumulation of human amylin in skin capillaries and brain microvasculature correlated with the development of cerebral small vessel disease and the activation of hypoxia signaling pathways. Co-staining for amylin and collagen IV, a component of the basement membrane structure, showed amylin deposition in skin and brain capillaries of HIP rats. Capillaries isolated from diabetic HIP brains showed elevated levels of incorporated aggregated amylin, and the accumulation of amylin in capillaries was associated with depletion of both caveolin-1 and collagen. The levels of claudin, occludin, and ZO adapter proteins were also lower in capillaries from HIP rats compared to WT littermates suggesting altered structural integrity of tight junctions in HIP rat capillaries. The immunoreactivity signal of HIF-1 $\alpha$  was also higher in skin tissue from HIP compared to WT rats. Pharmacologically increased levels of endogenous epoxyeicosatrienoic acids (EETs) lowered amylin deposition in brain capillaries and improved capillary stability. In conclusion, detection of amylin in a skin biopsy could be a biomarker of cerebral small vessels disease. A skin biopsy to detect capillary amylin deposition can complement brain and may provide a molecularly based approach for the identification of individuals at risk of cerebral small vessel disease. Blocking amylin dyshomeostasis may be a novel approach for reducing small vessel type ischemic brain injury.

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**Isolation and Identification of Leukocyte Populations in Intracranial Blood Collected During Mechanical Thrombectomy**  
**Student**

Using standard techniques during mechanical thrombectomy, the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol (NCT03153683) isolates intracranial arterial blood distal to the thrombus and systemic blood proximal in the carotid artery. We augmented the current collection protocol to study the distribution of leukocyte subpopulations both distal and proximal to thrombus during human stroke (n=14 patients). We isolated leukocytes for flow cytometry from small volume (<1 mL) intracranial blood and systemic blood (5-10 mL) to identify B cells, T cells, dendritic cells, NK cells, neutrophils, and myeloid cells, in addition to platelets and endothelial cells. Intracranial blood isolated between 2.7 and 27.8 hours of last known normal exhibited significant increases in T cell representation and decreases myeloid/macrophage representation compared to within-patient arterial blood from the carotid artery. Intracranial blood showed minimal changes in platelets. This novel protocol successfully isolates leukocytes from small volume intracranial blood samples of stroke patients at time of mechanical thrombectomy and can be used to confirm preclinical results, as well as identify novel targets for immunotherapies.

Chong Huang, PhD <sup>1</sup>

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### **Noncontact Optical Imaging of Deep Brain Hemodynamics in Preterm Infants**

#### **Faculty**

**Background:** Hemodynamic instability places preterm infants at high risk for brain injury. Currently there are no established bedside methods to continuously monitor cerebral hemodynamics in the neonatal intensive care unit (NICU).

**Methods:** An innovative speckle contrast diffuse correlation tomography (scDCT) device was developed and used for noncontact, high-density, 3D imaging of cerebral blood flow (CBF) in preterm infants. The scDCT delivers coherent near-infrared point light to multiple source positions for deep tissue penetration and controls an EMCCD camera to achieve a high-density sampling.

**Results:** The scDCT was first verified for transcranial brain imaging against an established diffuse correlation spectroscopy (DCS) in an infant-head-simulating phantom with varied optical properties. The insignificant influence of transparent incubator wall on scDCT measurements was then confirmed by comparing adult forearm blood flow responses to artery cuff occlusions measured inside and outside the incubator. Finally, two preterm infants were imaged by the scDCT through transparent incubator wall in the NICU. Infant #1 with no major organ deficits showed little CBF fluctuation over the first 3 weeks of life. Infant #2 showed an acute CBF increase after the 2-hour pharmacotherapy for patent ductus arteriosus (PDA) closure.

**Conclusions:** While these CBF variations meet physiological expectations, the fact that no significant changes are noted with peripheral monitoring of blood oxygen saturation suggests necessity of direct cerebral monitoring. This feasibility study is an important step towards larger clinical studies with more subjects to further validate it for continuous monitoring and instant management of cerebral pathologies and interventions in the NICU.

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**Ishemic Injury In A Dmcao Model of Stroke Demonstrates Long-term Immune Cell Diapedesis Into The Brain Parenchyma**  
**Student**

**Background:** Stroke injury following a middle cerebral artery occlusion (MCAo) induces a rapid migration of leukocytes into the injured brain that lasts for weeks. The current study focuses on whether a focal cortical stroke using the distal MCAo (dMCAo) model induces similar long-term immune cell diapedesis into the brain parenchyma as seen following transient (t)MCAo stroke.

**Methods:** Cells were isolated from spleens and brain hemispheres of adult 1-year old male C57BL/6J (B6; Jackson Labs) mice 30 days after a dMCAo (n=10). Sham animals (n=5) received a craniotomy without the distal MCA ligation. Peripheral migration was assessed in the spleen and brain using flow cytometry (FACSymphony) to identify viable (Ghost dye 780) CD3, CD4, CD8b, CD11b, Ly6C/Ly6G, CD19, CD45, and NK1.1 leukocytes. Populations were analyzed with FlowJo and assessed via repeated measures two-way ANOVA, Sidak post-hoc test (Graphpad Prism). Significance was  $p < 0.05$ .

**Results:** CD45<sup>+</sup> leukocytes were elevated in the ipsilesional (ipsi) hemisphere compared to the contralesional hemisphere ( $p=0.01$ ) after dMCAo, though a group hemispheric effect ( $F(1,13)=5.4$ ;  $p=0.04$ ) suggests long-term inflammation in sham-treated mice. Hemispheric effects also occurred for CD8<sup>+</sup> T cells ( $p=0.046$ ), B cells ( $p=0.03$ ), monocytes ( $p=0.01$ ), and macrophages ( $p=0.06$ ), with elevations in both dMCAo and sham-treated mice in the ipsi vs. contralesional hemispheres. Only monocyte populations were significantly elevated ( $p=0.03$ ) in dMCAo vs. sham mice.

**Conclusions:** Our study shows that immune cells remain elevated in the injured hemisphere at 30 days after a focal stroke confined to the neocortex, but inflammation occurred in both sham and dMCAo-treated animals. Only monocytes were differentially affected by dMCAo, and infiltrating cell numbers are not as robust as after tMCAo. This demonstrates a long-term injury response from the craniotomy in the dMCAo model that should be considered for long-term studies using this model.

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### **Antimicrobial Protein REG3A and Network Inflammatory Proteins are Predictive of Infarct Volume and Functional Impairment In Ischemic Stroke**

#### **Student**

**Introduction:** Regenerating Family Member 3 Alpha (REG3A) is an antimicrobial protein secreted by the intestine and pancreas and is involved in immune-mediated inflammatory responses. Studies have shown an increased expression of REG3A in systemic inflammatory responses. Particularly, pro-inflammatory cytokines such as IL17 and IL6 enhance REG3A expression and activity. These cytokines in addition to immune mediators such as CCL19, IL1 $\alpha$ , and IL15 are observed in the pathogenesis of neuroinflammation sequela of ischemic stroke. The University of Kentucky Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol (clinicaltrials.gov NCT03153683) utilizes thrombectomy to isolate intracranial arterial blood (i.e. distal to thrombus) and systemic arterial blood (i.e. carotid) to better understand the pathophysiology of stroke. From the analysis of plasma protein from this study, systemic REG3A is elevated in patients with ischemic stroke, but its role within neuroinflammatory processes during stroke remains unclear. Our aim was to examine the association of plasma REG3A levels with other signaling proteins in systemic plasma during stroke, and its correlation with clinical outcomes of stroke.

**Methods:** Intracranial and systemic plasma samples from n=25 thrombectomy subjects underwent Proximity Extension Assay via Olink Proteomics. REG3A levels and inflammatory markers were examined using bivariate regressions. Stepwise regression determined the predictability of infarct volume by REG3A. Two-tailed t-tests were used to examine the relationship between the National Institutes of Health Stroke Scale (NIHSS) and levels of REG3A.

#### **Results:**

Higher levels of REG3A correlated with increased infarct volume ( $p=.009$ ,  $R=.514$ ). Stepwise regression predicting infarct volume yielded a model including REG3A and systemic proteins such as CCL19, IL15, and IL1 $\alpha$  ( $P<.001$ ,  $R=.980$ ). NIHSS scores corresponding to moderate-severe/severe strokes had higher levels intracranial REG3A ( $p<.05$ ) and higher REG3A expression intracranially relative to systemic REG3A ( $p=.05$ ).

#### **Conclusions:**

Within a network of proteins, REG3A is predictive of increased infarct volume and decreased function. NF $\kappa$ B is a key transcriptional regulator of neuroinflammatory processes and enhances expression of pro-inflammatory cytokines such as IL6, IL17, CCL19, IL1 $\alpha$ , and IL15. As previously stated, IL6 and IL17 can enhance REG3A, and in models examining systemic inflammatory processes, REG3A may alter NF $\kappa$ B activity. This highlights the possible involvement of REG3A in NF $\kappa$ B-driven neuroinflammation. To take into account the difference of time when the NIHSS score was obtained and when patient plasma samples were collected, future studies will include statistical examination of the patients last known normal. Examining this relationship will be critical in evaluating the role of REG3A in neuroinflammation and as a potential prognostic and therapeutic target in large vessel occlusive stroke.

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**Cerebral Autoregulation Assessment in Older Adults using Diffuse Optical Spectroscopy Technique**

*Fellow*

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**# Headache: When a Common Problem Becomes a Neurological Emergency in Acute Stroke Patients**

**Other**

# Headache: When a Common Problem Becomes a Neurological Emergency in Acute Stroke Patients

**BACKGROUND:**

Headache is frequently reported in acute stroke patients, yet remains poorly understood. The International Classification of Headache Disorders Third Edition lists over 50 types of headaches and the most fundamental classification is to distinguish between primary and secondary headache. The challenge of making this distinction when managing acute stroke patients, is, at its core, determining when a headache problem is a neurological emergency; most notably, when headache pain is a manifestation of a neurological change signifying another acute ischemic event or an acute hemorrhage. Thus, the purpose of this project was to assess the knowledge of current neurology residents regarding the problem, and to develop an algorithm to assist neurology residents in the evaluation and management of headache in the acute stroke patient.

**METHODS:**

We conducted a survey of neurology residents at the University of Kentucky, to evaluate the level of understanding of primary and secondary headaches, to assess current practices, and to determine whether a structured protocol would aid in the management of these patients.

**RESULTS:**

Greater than 93% of neurology residents (14 of 15 respondents) surveyed at the University of Kentucky reported having a clear or fairly clear understanding of the difference between primary and secondary headache, and 80% reported understanding the mechanisms that drive headache pain in stroke patients. However, only 60% have a process to further characterize headache in acute stroke patients. Only 46.7% (n=7) reported "always" going to bedside to evaluate new headache in the stroke patient, and 33.3% (n=5) felt that this problem could be handled by phone. More than 90% reported "always" or "often" avoiding sedating medications that might impact subsequent neurological assessments.

**CONCLUSIONS:**

While the majority of neurology residents seem to have adequate knowledge regarding the difference between primary and secondary headache disorders, there appears to be a practice gap in the need for a bedside evaluation process to determine possible neurological deterioration. Based on these data, an algorithm could be helpful in distinguishing between headache that may be a harbinger of neurological complication and those that are secondary to the acute stroke itself.

Further, while the majority of residents would appropriately avoid sedating medications, a consensus regarding medical management has not been established. A future aim of this project is to develop an algorithm for evaluation and a protocol for treatment of acute stroke-related headache.

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## Colocalization analysis of genome-wide association and eQTL signals detects target genes for brain arteriolosclerosis

### Student

### Background

Brain arteriolosclerosis (B-ASC) is a cerebrovascular pathology characterized by dysmorphic arteriolar wall thickening. B-ASC is common in elderly people and is observed in over 80% of autopsied individuals over 80 years of age. B-ASC pathology is associated with other neuropathologies, including Alzheimer's disease and limbic-predominant age-related TDP-43 encephalopathy (LATE), and is independently associated with cognitive decline. However, despite its frequency and clinical importance, B-ASC remains understudied. In this study, we perform the first genome-wide association study of B-ASC. We then perform colocalization analysis using quantitative trait loci (QTL) data to locate gene targets for B-ASC pathology to investigate potential molecular functional pathways by which identified variants may affect B-ASC risk..

### Methods

We used neuropathology data from participants in the National Alzheimer's Coordinating Center (NACC) Neuropathology Dataset linked to genotype data in the Alzheimer's Disease Genetics Consortium (ADGC) for our GWAS. Traditional GWAS quality control (QC) procedures were performed on both participants and variants used. We used a case-control logistic regression model, assigning participants with no or minor B-ASC as controls and those with moderate or severe B-ASC as cases, and an ordinal regression model. Variants exceeding a suggestive p-value threshold of  $1 \times 10^{-5}$  in either model were then compared to expression QTL (eQTL) and splicing QTL (sQTL) data from the Genotype Tissue Expression Project (GTEx). We performed colocalization analysis using a Bayesian-based approach to compare loci identified in GWAS and QTL analyses, designating a posterior probability of colocalization (PP) of at least 50% as evidence of colocalization.

### Results

In total, 3318 participants and 4.9 million variants passed QC and were included in GWAS. One locus on chromosome six achieved the genome-wide significance p-value threshold of  $5 \times 10^{-8}$  (rs2603462, odds ratio = 1.5, p-value =  $1.4 \times 10^{-8}$ ). Of 19 independent loci meeting the suggestive threshold, four were also significant QTL in GTEx. Three of these loci colocalize with at least one QTL with a posterior probability of 50%, indicating that these GWAS loci share signals with QTL. rs2603462 colocalizes with *ELOVL4* expression with a PP of 93.3%; rs2352974 colocalizes with *DALRD3*, *FAM212A*, and *MST1R* expression as well as multiple *RNF123* sQTL with PP > 50%; and rs34349961 colocalizes with *SPRED2* expression with PP of 66%.

### Discussion

Our study entailed the first GWAS of autopsy-proven B-ASC. We then followed our initial study with colocalization analysis, a technique that can identify shared genetic association signals between phenotypes. We identified one genome-wide significant locus that colocalizes with *ELOVL4* gene expression in the brain and several other suggestive loci that colocalize in GTEx. These findings provide gene targets for future studies of B-ASC pathophysiology.

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### **Uromodulin Protein Expression During Ischemic Stroke**

#### **Student**

**Introduction:** Uromodulin, also known as Tamm-Horsfall protein, is a glycoprotein that is expressed by the epithelial cells of the thick ascending limb of Henle's loop in the kidney. Uromodulin is the most abundant urinary protein in the healthy individual and has important roles in ion transport, water and electrolyte balance, and prevention of urinary tract infections. Research has shown that increased uromodulin expression may be associated with lower risk of cardiovascular disease in adults. Utilizing the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) ([clinicaltrials.gov NCT03153683](https://clinicaltrials.gov/NCT03153683)), a continuously enrolling tissue bank, we aimed to examine the associations between serum uromodulin, age, and high BMI (BMI>25) and its relationship to stroke in patients.

**Methods:** Arterial blood distal and proximal to the thrombus was collected during a thrombectomy procedure using the BACTRAC protocol and sent to Olink (Boston, MA) to determine proteomic expression via proximity extension assay. Arterial samples were taken from age and diseased matched control patients undergoing diagnostic angiograms but not experiencing a large vessel occlusion. Uromodulin expression was recorded and analyzed using Welch's two tailed T-tests and linear regressions.

**Results:** The relationship between systemic and intracranial uromodulin, high BMI and age were assessed. Systemic uromodulin expression increased with BMI > 25 (P=0.0062). Systemic and intracranial uromodulin decreased with age (P=<0.0001, P=0.0416). The relationship between systemic and intracranial uromodulin expression and the presence of acute ischemic stroke were assessed. Systemic and intracranial uromodulin expression decreased in the stroke group compared to the control group, P=0.0126 and P=0.0013 respectively.

**Conclusions:** In our study uromodulin was found to be increased significantly in overweight patients and decreased significantly in older patients and in those that experienced a stroke. The increase in uromodulin in people with high BMI could be a protective reaction of the kidney to worsening conditions that make ischemic stroke more likely, with a goal of delaying dangerous outcomes. People with high BMI experience strokes on average 10 years earlier than those with normal BMI. The decreased expression of uromodulin in older adults could be associated with the decline of general kidney function that accompanies aging. Kidney dysfunction caused by the stroke could lead to the decrease in expression of uromodulin. Further analyzes of clinical data is needed to understand the role of uromodulin after ischemic stroke.

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## **Cancer history associates lower burden of dementia and Alzheimer's-type neuropathology in autopsied research volunteers** **Student**

### **Background**

Cancer and Alzheimer's disease (AD) are common chronic diseases in aging populations. Intriguingly, prior research has reported a lower incidence of AD among individuals with a history of cancer compared with cancer-free population controls. The current study was conducted to investigate the association of cancer history with neuropathological findings.

### **Methods**

Data was analyzed from elderly, longitudinally evaluated participants in a community-based cohort study of aging and dementia who had undergone autopsy at the UK Alzheimer's Disease Center (UK-ADC). The UK-ADC data was linked to the Kentucky Cancer Registry, which is a population-based cancer surveillance system for the state, to obtain cancer-related data. We examined the association between cancer and neuropathological findings adjusted for sex, age at death, years of education, and *APOE*ε4 genotype using inverse probability of censoring weights (IPCW) to address missing data.

### **Results**

Included participants (n=810) had a mean [SD] age of death of 83.7 [8.6] years, and a majority (59.6%) were female. The mean [SD] follow-up in the UK-ADC was 8.6 [6.1] years. Cancer history was KCR-confirmed in 190 participants. Participants with cancer history had significantly lower odds of dementia at the last UK-ADC visit (odds ratio [OR], 0.39; 95% confidence interval [CI], 0.27-0.57). At autopsy, participants with cancer had significantly lower odds of Braak Neurofibrillary Tangle (NFT) III/IV stages OR=0.56 (95% CI: 0.36-0.90) and V/VI NFT stages OR=0.41 (95% CI: 0.28-0.59) vs. 0/I/II NFT stages. Cancer history was also associated with reduced odds of moderate/frequent neuritic plaques, OR=0.56 (95% CI: 0.40-0.80), of moderate/frequent diffuse plaques, OR=0.53 (95% CI: 0.36-0.78) and of moderate/frequent cerebral amyloid angiopathy, OR=0.57 (95% CI: 0.37-0.89). TDP-43 proteinopathy, α-synuclein pathology, and cerebrovascular pathologies were not significantly associated with cancer history.

### **Conclusion**

In this sample of autopsied, longitudinally followed research volunteers, history of cancer was associated with lower odds of dementia as well as lower burden of AD pathology. These findings are in line with prior epidemiological research reporting a protective association between cancer and AD dementia and provide an additional basis of support.

**Other**

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### **Two Cases of COVID-19 Encephalitis: case series**

#### ***Other***

Background: Coronavirus (COVID-19) presents as a febrile illness with a myriad of symptoms ranging from anosmia, generalized weakness, gastrointestinal upset, to severe hypoxemia with respiratory distress, and neurologic complications. Neurological manifestations of COVID-19 are becoming more prevalent, including encephalitis.

Case 1: A 20-year-old female with history of systemic lupus erythematosus diagnosed with COVID-19 two weeks prior to acute worsening of her symptoms including encephalopathy, lower extremity weakness and one reported episode of urinary incontinence. MRI brain with contrast showed findings consistent with infectious versus post-infectious encephalitis. CSF studies revealed a normal glucose, elevated protein, negative gram stain, marked pleocytosis (lymphocytic predominance). Despite no detection of COVID-19 on CSF PCR, a diagnosis of postinfectious COVID-19 encephalitis was made given absence of other underlying etiologies. Patient's symptoms of encephalopathy, upper motor neuron signs, and bilateral lower extremity weakness gradually improved with supportive care.

Case 2: 42-year-old male with history of hypertension and gout was diagnosed with COVID-19 presented with focal seizure without secondary generalization or loss of awareness. Initially patient had fever, chills, cough and backache prior presentation. Patient progressively worsened requiring intubation and pressures and renal failure. Seizure was reported as twitching of face that lasted for 1 minute. Patient was started on sedation and was severely encephalopathic. MRI brain without contrast showed findings consistent with infectious versus post-infectious encephalitis with deep white matter/subcortical hyperintensities. CSF studies revealed a mild elevation in glucose, normal protein, negative gram stain, no nucleated cells, one RBC. Despite no detection of COVID-19 on CSF PCR, a diagnosis of postinfectious COVID-19 encephalitis was made given absence of other underlying etiologies and imaging findings. High dose glucocorticoids and Remdesivir were started. Patient's symptoms of encephalopathy, gradually improved with supportive care.

Discussion and conclusions: These cases illustrate one of the major late neurologic complications of COVID-19—postinfectious encephalitis. Symptoms can manifest as fever, headache, confusion, and focal neurologic deficits. Review of the literature reveals multiple cases of COVID-19 involving the CNS; in those with encephalitis, the majority have CSF demonstrating inflammation, pleocytosis, and lymphocytic predominance with no isolated COVID-19 via PCR. Recognition of COVID-19 as a potential pathogen in the setting of acute encephalitis is imperative, and necessitates that routine testing of CSF for COVID-19 be implemented for suspected COVID-19 encephalitis in the future.

## Analyzing the Gene Expression Profile and Networking in Humans and Rats Following Stroke

### Student

**Introduction:** Ischemic stroke has lent itself as one of the most ubiquitous and detrimental causes of death and disability in the United States. Although thrombectomy is a prevalent treatment of stroke, an adjuvant therapeutic is warranted to further improve clinical outcomes post procedure. In effort to target such therapeutics, we created a model using aged rats of both sexes that mirrors thrombectomy. The gene expression profile of rats was analogized against comparable systemic and intracranial samples in humans during ischemic stroke or control patients during diagnostic angiograms. Our goal for this project was to exhibit parallel changes in gene expression between rat and human subjects in order to propose a potential application of our existing animal model to human condition.

**Methods:** Human samples were collected using the previously established Blood and Clot Thrombectomy Registry and Collaboration ([clinicaltrials.gov NCT03153683](https://clinicaltrials.gov/NCT03153683)), a continuously enrolling tissue bank. Arterial blood distal and proximal to the thrombus was collected during a thrombectomy and expression of 84 genes were measured using a Qiagen (Germantown, MD) Human Chemokine and Receptor Array. Control arterial blood was taken from age and disease matched control patients during a diagnostic angiogram and underwent identical gene expression analysis. For rat samples, blood was taken from the jugular vein pre-surgery, post-surgery (following reperfusion of the artery 5 hours after filament is placed), and 3 days post operation during a 5-hour transient middle cerebral artery occlusion (5t-MCAO) surgery. The Qiagen array was utilized again to measure gene expression in rats. Sham rats underwent the 5t-MCAO procedure, but the filament was not placed to incur stroke. Standard two tailed t-tests were performed to determine significance between control and stroke gene expression in both human and rat samples.

**Results:** Upon statistical inquiry, we identified six genes that exhibited a significant increase ( $p < 0.05$ ) in expression post ischemia across rat and human models via two tailed t-tests: CCR3, CCR5, IL15, IL21, LTA, and TNFSF11. Expression of these genes was not only increased but sustained amongst our rat and human samples. String analysis confirmed the network of these genes in humans and rats.

**Discussion:** It is pertinent that the animal models we are using can be rightfully implemented into human research. Our protocol behind this study achieves just that, in which the methods are commensurable enough biologically to foster relevant, scientific gene expression analyses. We found correlation in gene expression profiles of rats and humans in respect to the following genes: CCR3, CCR5, IL15, IL21, LTA, and TNFSF11. Because of the matched relationship between two chemokine receptors, two interleukins, and two members of the tumor necrosis factor (TNF) superfamily, we believe this gene network could potentially target such adjuvant therapeutics successfully.

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### **Piglet Model of Neonatal Intraventricular Hemorrhage**

#### **Faculty**

Intraventricular hemorrhage (IVH) poses great threat to extreme preterm babies. To improve our understanding of the IVH-related brain pathology and to validate a bedside cerebral blood flow (CBF) monitoring technique, namely **Speckle Contrast Diffuse Correlation Tomography (scDCT)**, we established a piglet model of IVH that more closely mimics human babies. Neonatal piglet (9 days after birth) were anesthetized with Isoflurane and their heads were secured on a customized stereotactic frame. After the hair on the scalp was removed with hair cream, focused-point near-infrared laser beam was projected in a matrix pattern to the region of interest, and the reflected signal from deep tissue (~10mm) were captured with a CMOS camera. After surgical preparation of the skin, a small incision was cut on the temporal scalp, and a 3mm hole (P: -5 mm, D: -5mm from bregma) was drilled on the temporal skull, through which an intracranial pressure (ICP) probe was inserted under the guidance of Ultrasound Doppler until the tip of ICP probe entered the right lateral ventricle and clear cerebrospinal fluid (CSF) was withdrawn. The animal vitals and ICP were measured simultaneously with clinical devices. To induce IVH, 1-ml heparinized autologous blood was gradually injected through the ICP probe catheter in 5 minutes, followed by a 6-ml saline injection at 0.1 ml/min in 60 minutes to keep the ICP elevated to ~4 mmHg above its baseline (1mmHg). Animals were sacrificed at 2 hours after the IVH and variations of CBF were recorded with scDCT throughout the study. Piglet brains were collected and sliced at 5mm thickness, and characteristic pathologies of IVH, including blood clots in the ipsilateral ventricle and ventriculomegaly, were observed. More importantly, CBF variations detected with scDCT correlated well with the changes of ICP and vitals. All these results indicate success of establishing a piglet model of IVH and validating the accuracy and feasibility of scDCT for potential translational application.

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### **Healthy dietary-intake moderates the effects of age on cerebral iron concentration and working memory performance**

#### **Staff**

Age-related brain iron accumulation has been linked with oxidative stress, neurodegeneration and cognitive decline. Certain nutrients, such as antioxidants and iron chelators appear to reduce brain iron concentration in animal models. However, this association is not well established in humans and importantly, it remains unknown if healthy dietary intake moderates the effects of age on brain iron concentration and/or cognition. Here, we explored these issues in a sample of seventy-five healthy, older adults (61-86 years old). Quantitative susceptibility mapping (QSM) was used for assessment of non-heme brain iron concentration and participants performed an N-Back paradigm to evaluate working memory performance. Nutritional-intake was assessed via a validated questionnaire detailing dietary-intake over the preceding month. Nutrients were grouped into three distinct nutrition factors based on previous literature and factor analysis. One of these factors (factor two), comprised of vitamin E, lysine, DHA omega-3 and LA omega-6 PUFA, representing food groups such as nuts, healthy oils and fish, moderated the effects of age on both brain iron concentration and working memory performance (Figure 1; D-prime; all analyses controlled for age, gender and years of education). Our results suggest that incorporating nutrients primarily derived from nuts, healthy oils and fish in daily dietary patterns may slow the rate of brain iron accumulation and working memory declines in aging.

Figure 1:

<https://i.postimg.cc/90SZ6L7w/Figure1.jpg>

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### **MOYAMOYA-LIKE VASCULOPATHIES OBSERVED IN A NOVEL MOUSE SURGICAL MODEL**

#### **Faculty**

**Objectives:** Moyamoya is an unusual chronic cerebrovascular condition that is characterized by the progressive stenosis of the internal carotid arteries (ICA) and their major branches. The vascular stenosis is accompanied by the formation of an abnormal vascular network of collaterals at the base of the brain, all of which can result in ischemic and hemorrhagic strokes. Current treatments include antiplatelet therapy and surgical bypass, which provides additional collateral flow to the ischemic tissue. However, to determine the etiology of moyamoya and possibly develop novel therapies, experimental animal models are needed. Recently, investigations have linked a susceptibility gene (RNF213) and developed a transgenic model for idiopathic moyamoya disease, which occurs primarily in East Asian children. However, there is no animal model which mimics the acquired vasculopathy of moyamoya syndrome, which occurs in adults in their 20's -40's and is associated with underlying conditions such as auto-immune disease or coagulopathy. We report here, the characterization of a novel surgical model of moyamoya, using the placement of microcoils on the ICA of mice.

**Methods:** Male C57Bl/6J mice (4 months old) underwent surgery for the unilateral placement of a microcoil (0.16 mm) onto the proximal ICA or sham control. After 30 days (N = 6-8/time point), the blood vessels were examined for changes in diameter, number of anastomoses, and development of new collaterals using an injection of Dil. Brain tissue was examined for hemorrhage using Prussian blue stains and cross-sections of blood vessels were examined for intimal thickening using H&E. Expression of VEGF, which is associated with angiogenesis and moyamoya syndrome, was quantified by qPCR.

**Results:** After 30 days, the distal ICA and anterior cerebral artery (ACA) had significantly decreased diameters at the Circle of Willis, with an initial decrease in the number of cortical anastomoses. Histology demonstrated changes in the various layers of the blood vessels, indicating possible intimal thickening of affected blood vessels. There was also a significant increase in the number of microbleeds, suggesting compromised vascular integrity. This may be due to a significant upregulation in VEGF gene expression within the striatum.

**Conclusions:** We report the development of an animal model with vasculopathies which mimic those observed in patients with moyamoya syndrome. While further characterization of this model is needed, it provides a critical novel tool to test new therapies for this cerebrovascular disease.

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### Knockout of microglial p38<sup>MAPK</sup> in a mouse model of Alzheimer's disease

#### Fellow

**Background:** The p38a MAPK signaling pathway is a well-established regulator of neuroinflammation, and pharmacological inhibitors of this pathway can protect against cognitive impairment in animal models of Alzheimer's disease (AD). This protection by p38a inhibitors might be mediated via protective effects on neurons, anti-inflammatory effects on glia, or some combination thereof. To assess whether reduction of p38-dependent microglial proinflammatory responses is beneficial in this context, we generated AD model mice with microglial knockout (KO) of p38a.

**Method:** The APP<sup>swe</sup>/PS1<sup>dE9</sup> (MMRRC #34832) mouse AD model was crossed with p38a<sup>fl/fl</sup> mice (Jax #031129) to generate AD model mice homozygous for the floxed p38a allele. These were subsequently crossed with CX3CR1<sup>CreERT2</sup> mice (Jax #020940) that express a tamoxifen-inducible promoter allowing for removal of p38a from microglia. This breeding scheme generated four groups of mice, used in a 2 x 2 study design: WT and AD mice with floxed p38a, with or without a copy of the myeloid-specific Cre allele. All mice were placed on tamoxifen diet (400 ppm) for 4 weeks beginning at 5 months of age, near the beginning of amyloid plaque deposition in this AD model. After administration of tamoxifen, mice were returned to normal chow for several months, allowing turnover of peripheral myeloid cells. Mice underwent behavioral testing in open field and novel spatial recognition y-maze (8 months of age), and radial arm water maze (11 months of age). Microglial isolation via fluorescence-activated cellular sorting and subsequent RNAseq analysis was performed on a subset of 4 mice per group.

**Results:** Microglial p38a KO had no effect on the hyperlocomotive phenotype associated with amyloid overexpression in this model; however, p38a KO increased errors in the RAWM test of spatial learning and memory in the AD model but not WT mice.

**Conclusions:** The microglial p38a signaling pathway is important in restricting amyloid-associated cognitive decline. We are currently characterizing the effects of microglial p38 KO on amyloid pathology, neuroinflammation, and microglial gene expression.

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### **Estradiol Regulates the Daily Activity Rhythm and Inhibits Diet-Induced Obesity in Male Mice**

#### **Student**

Male mice fed high-fat diet become obese, but female mice are resistant to diet-induced weight gain. We previously found that circulating estrogen in females protects their daily rhythms from disruption by high-fat feeding to prevent diet-induced obesity. The goal of this study was to determine the effects of estrogen on daily metabolic rhythms in male mice. Male C57BL/6J PERIOD2::LUCIFERASE mice were implanted with Silastic tubing containing either 17 $\beta$ -estradiol (E2) or vehicle (sesame oil) at 7 weeks of age. The mice were fed low-fat diet (10% kcal fat) for 1 week and then high-fat diet (45% kcal fat) for 2 weeks. We measured the effects of high-fat diet feeding on daily rhythms of eating behavior and locomotor activity, and on the phases of PER2::LUC rhythms in central and peripheral tissues. We found that males treated with E2 gained less weight on high-fat diet than males treated with vehicle. E2 treatment decreased the total number of calories of high-fat diet consumed, but did not affect total activity levels, compared to vehicle-treated males. Males treated with E2 had higher amplitude locomotor activity rhythms during high-fat diet feeding compared to control males. There was no effect of E2 on the daily rhythm of eating behavior as it was low-amplitude or arrhythmic during high-fat diet feeding in both vehicle- and E2-treated males. Likewise, the phases of PER2::LUC rhythms in the SCN, liver, muscle, and other peripheral tissues were not altered by E2 treatment. These results suggest that estradiol may inhibit diet-induced obesity in male mice by reducing energy intake and increasing the amplitude of the locomotor activity rhythm. These results will inform studies of metabolic changes in transgender women using exogenous estrogen during cross-sex hormone therapy.

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**Temporal characterization of central and peripheral immune responses following complete high thoracic spinal cord injury**  
*Fellow*

**Temporal characterization of central and peripheral immune responses following complete high thoracic spinal cord injury**

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Traumatic spinal cord injury (SCI) at or above upper thoracic spinal levels leads to profound sensory, motor and autonomic dysfunction. The disruption of supraspinal sympathetic pathways results in severe secondary complications such as autonomic dysreflexia and immunosuppression, and the onset of inflammatory responses further exacerbates these conditions. Systemic immunosuppression is reported to be correlated with spleen atrophy in rodent models of high thoracic SCI, which are both associated with the incidence and severity of autonomic dysreflexia. While inflammatory cytokine expression in the injured spinal cord and spleen have been investigated in the mouse model and the more widely used rat upper thoracic (T4) SCI model, the temporal-spatial correlation between spleen atrophy and the development of autonomic dysreflexia has not been well characterized in the rat. We assessed spleen wet weights systematically at multiple time points after T4 SCI in adult female Wistar rats and subsequently evaluated the expression of pro-inflammatory and immunomodulatory genes using qRT-PCR. Despite exhibiting similar dynamic increases in TNF $\alpha$  and IFN $\gamma$  in both spleen and spinal cord tissues, we found that spleen atrophy was quite varied overtime but not significantly different than uninjured controls chronically (8 weeks). The acute increases in TNF $\alpha$  levels in the spleen and both spinal cord segments were followed by delayed peaks after 2-4 weeks. In parallel, IFN $\gamma$  expression occurred at more delayed time points in all tissues, likely reflecting persistent immune activation elicited by infiltrating leucocytes and monocytes. In particular, the lumbosacral cord showed TNF $\alpha$  peaking at 2 weeks followed by IFN $\gamma$  peaking at 4 weeks and remaining elevated at 8 weeks. This is indicative of persistent immune activation thought to contribute to maladaptive plasticity in the lumbosacral spinal cord underlying autonomic dysreflexia.

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### **Time-course of pathological changes during progression of hyperhomocysteinemia induced cerebrovascular pathology.**

#### **Student**

#### **Background**

Vascular contributions to cognitive impairment and dementia (VCID) is one of the leading causes of dementia. High levels of plasma homocysteine or hyperhomocysteinemia has been characterized as a risk factor for VCID however, the mechanism underlying the connection between hyperhomocysteinemia and development of VCID pathology remains elusive. I hypothesize that hyperhomocysteinemia initiates a pro-inflammatory cascade that increases the activity of MMP9 causing perivascular astrocytes to dissociate from their vessels, leading to blood brain barrier dysfunction and the progression toward VCID pathology.

#### **Method**

For *in vivo* studies, C57BL6 WT mice were placed on a control diet or a diet deficient in folate and vitamins B6 and B12 and enriched in methionine to induce hyperhomocysteinemia for 6, 10, 14, or 18 weeks. For *in vitro*, experiments, astrocytes were treated with a 50 $\mu$ M homocysteine solution in serum free media for 24, 48, 72, or 96 hours. Immunohistochemistry and gene expression analysis were used to determine neuroinflammatory changes while histology was used to identify changes in astrocytic end-feet proteins and microhemorrhages. Gel zymography was used to assess proteinase activity of matrix metalloproteinases. Behavior was assessed using the 2-day radial arm water maze.

#### **Result**

After 6 weeks of diet administration in WT mice, we saw a significant increase in gene expression of TNF $\alpha$ , IL-1 $\beta$ , IL-6 and IL-12 $\alpha$ . This was followed by increases in matrix metalloproteinase transcription and proteinase activity seen at 10 weeks on diet though this was the only timepoint at which this endpoint was quantified. Also, beginning at the 10-week time point, cognitive deficits became detectable, which coincided with significant disruptions between astrocytic end-feet and the cerebrovasculature. Finally, there was a significant increase in the number of microhemorrhages at 14 weeks on diet. *In vitro*, astrocytes showed decreased levels of several potassium channels and Aqp4 with up to 72 hours of homocysteine treatment and an increase in matrix metalloproteinase 9 after 48 hours of homocysteine treatment.

#### **Conclusion**

Collectively, our findings suggest that astrocytic MMP9 may play an integral role in the mechanism associating homocysteine induced neuroinflammation with vascular pathogenesis leading to VCID, highlighting this pathway as an important subject for future study.

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### **Functional Imaging and Neuro-Diagnostics for Epilepsy and Resective Surgery (The FINDERS Alliance)**

#### **Faculty**

**Introduction** In the US, approximately 3.5 million individuals suffer from epilepsy. Roughly 40% of these are refractory to medical management. Refractory epilepsy has serious physical and psycho-social sequelae. Neurosurgical treatment in the form of resection, disconnection, or neuromodulation can be a life changing measure for these patients. The challenge lies in successfully localizing the seizure onset zone in preparation for surgery. New creative multidisciplinary approaches are needed to improve seizure localization in epilepsy.

**Goals of The Alliance** The FINDERS Alliance brings together clinicians (Radiologists, Neurologists, Neurosurgeons, Neuropsychologists), MRISC scientists (Neuroimaging Researchers, Physicists), Engineers (Biomedical, Electrical), as well as external expertise from Montreal Neurological Institute and Cleveland Clinic. A key priority of this alliance is to equip clinicians with the technological resources to answer clinical questions, and nurture mentoring partnerships with proven scientists, leading to extramural grants. The principal aim of the alliance is to improve seizure localization through the development of advanced neuroimaging and signal analysis that are relatively new and underutilized in epilepsy.

**Proposed Study Methods** The aim is to determine the value for seizure localization of two MRI techniques: simultaneous EEG-fMRI and arterial spin labeling (ASL). Phase 1 will focus on proof-of-concept for: 1) proficiency in EEG-fMRI and ASL, and 2) establishing a workflow for the seamless transfer of subjects from epilepsy monitoring unit (EMU) to MRI. This workflow is a novel approach that involves using the same electrode system for both EEG recordings in the EMU as well as during MRI scans. This approach allows for: 1) acquiring immediate postictal imaging because the patient can be transported immediately after the seizure is identified in the EMU, which introduces a new protocol for seizure imaging; 2) the exact time delay after termination of the seizure will be accurately known, which allows us to establish a relationship between imaging findings and a seizure's propagation and evolution; 3) a battery of imaging can be performed while concurrently continuing to take EEG recordings, which allows us to relate imaging findings to what the seizure is actually doing in real-time. Such a workflow will create the optimal conditions for exploring and evaluating the localization value of advanced neuroimaging techniques. Phase 2 will focus on acquiring prospective preliminary data.

**Anticipated Impact** The FINDERS Alliance lays a foundation to propel the UK Comprehensive Epilepsy and Epilepsy Surgery Program to become best-in-class in management of epilepsy, advancement of imaging techniques, and understanding of seizure localization. This outcome-driven research can potentially expedite treatment pathways, defer unnecessary tests, cut healthcare costs, enhance surgical management, and improve patient outcomes.

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### **A Wearable Fiber-Free Optical Sensor for Continuous Monitoring of Cerebral Blood Flow in Freely Moving Mice**

#### **Student**

**Background:** Wearable technologies for functional brain imaging in freely behaving animals would advance our understanding of cognitive processing and adaptive behavior. However, most current wearable techniques used in conscious rodents require invasive craniotomy and/or probe implantation due to limited penetration/detection depths.

**Methods:** We have developed a wearable, fiber-free, near-infrared diffuse speckle contrast flowmetry (DSCF) probe for continuous monitoring of cerebral blood flow (CBF) in freely behaving animals and humans. The DSCF uses small laser diodes as focused point sources for deep tissue penetration and a tiny CMOS camera as a high-density 2D detector array to detect spontaneous spatial fluctuations of diffuse laser speckles, resulting from movement of red blood cells in the deep brain (i.e., CBF).

**Results:** After calibrating the DSCF against established technologies in tissue-simulating phantoms and *in-vivo* human tissues, a miniaturized probe was fixed on the mouse skull using super glue and dental cement for continuous monitoring of CBF variations in mice during anesthesia, awake, and freely behaving. We noted a small surge when the animal woke up, a mild decrease after the isoflurane washed off, a  $+37 \pm 9\%$  increase during 10%CO<sub>2</sub> inhalation (n = 3), and mild elevations during grooming and walking. These CBF variations are consistent with clinical observations when recovery from anesthesia and impacts by isoflurane, hypercapnia (CO<sub>2</sub>), and activity-induced cortical excitations.

**Conclusions:** We are currently testing noninvasive DSCF in both animals and humans to validate a unique, multiscale, multimodal brain functional monitoring tool for both basic neuroscience research and clinical applications.

## Test Submission

### Other

**Lorem Ipsum** is simply dummy text of the printing and typesetting industry. Lorem Ipsum has been the industry's standard dummy text ever since the 1500s, when an unknown printer took a galley of type and scrambled it to make a type specimen book. It has survived not only five centuries, but also the leap into electronic typesetting, remaining essentially unchanged. It was popularised in the 1960s with the release of Letraset sheets containing Lorem Ipsum passages, and more recently with desktop publishing software like Aldus PageMaker including versions of Lorem Ipsum.

## Why do we use it?

It is a long established fact that a reader will be distracted by the readable content of a page when looking at its layout. The point of using Lorem Ipsum is that it has a more-or-less normal distribution of letters, as opposed to using 'Content here, content here', making it look like readable English. Many desktop publishing packages and web page editors now use Lorem Ipsum as their default model text, and a search for 'lorem ipsum' will uncover many web sites still in their infancy. Various versions have evolved over the years, sometimes by accident, sometimes on purpose (injected humour and the like).

## Where does it come from?

Contrary to popular belief, Lorem Ipsum is not simply random text. It has roots in a piece of classical Latin literature from 45 BC, making it over 2000 years old. Richard McClintock, a Latin professor at Hampden-Sydney College in Virginia, looked up one of the more obscure Latin words, *consectetur*, from a Lorem Ipsum passage, and going through the cites of the word in classical literature, discovered the undoubtable source. Lorem Ipsum comes from sections 1.10.32 and 1.10.33 of "de Finibus Bonorum et Malorum" (The Extremes of Good and Evil) by Cicero, written in 45 BC. This book is a treatise on the theory of ethics, very popular during the Renaissance. The first line of Lorem Ipsum, "Lorem ipsum dolor sit amet..", comes from a line in section 1.10.32.

The standard chunk of Lorem Ipsum used since the 1500s is reproduced below for those interested. Sections 1.10.32 and 1.10.33 from "de Finibus Bonorum et Malorum" by Cicero are also reproduced in their exact original form, accompanied by English versions from the 1914 translation by H. Rackham.

## Effects of prebiotic supplementation on chronic mild traumatic brain injury recovery

### Student

**Background:** Mild traumatic brain injury (mTBI) currently affects 1.6-3.8 million people in the US annually, including the hospitalization of 100-300 per 100,000 young adults. Recently, it was found that gut dysbiosis occurs acutely after traumatic brain injury and literature suggests that manipulation of the gut microbiome may be actionable to reduce long term symptoms. Prebiotic fibers are known to beneficially alter the gut microbiome and increase metabolites such as short chain fatty acids (SCFA) which play a role in metabolism and inflammation. It is also known that the gut microbiome and associated metabolites play a role in the regulation of brain vascular, structural and metabolic integrity which are all important in recovery following mTBI.

**Method:** Midline injury was administered at 4 months of age. A group of animals undergo the procedure but not receive the injury (sham). For the gut microbiome analysis, fecal samples were collected at 5 m.p.i. Shotgun metagenomic sequencing was done by CosmosID. Targeted metabolomics for SCFA analysis was done by Metabolon, Inc. Quantitative cerebral blood flow (CBF) was measured using MRI-based pseudo-continuous arterial spin labeling (pCASL). Diffusion tensor imaging (DTI) was used to characterize microstructural changes in the brain. MRI sequences were conducted at 9 months of age (5 m.p.i).

**Results:** For the gut microbiome, we see trends to indicate that inulin is increasing putatively beneficial bacteria such as *Bifidobacterium pseudolongum*, *Akkermansia muciniphila* and *Eubacterium spp*. We also see decreases in putatively harmful bacteria such as *Dorea sp. 5-2*. SCFA levels reflect these increases as *B. pseudolongum* and *A. muciniphila* both produce acetate, which we see higher levels of in the cecum and blood of mice fed inulin. Butyrate and propionate are also higher in the cecum of mice fed inulin and butyrate is produced by *Eubacterium spp*. Inulin also proved to be beneficial in the brain showing increased CBF in both the thalamus and hippocampus, as well as decreased Glycerophosphocholine (GPC) in the hippocampus.

**Conclusion:** The increase in beneficial bacteria and SCFA may tie back to the improved cerebral blood flow and reduced GPC. Butyrate is known to increase tight junction protein expression in the intestine and the blood brain barrier (BBB). This could be indicative of changes in CBF as more intact BBB is protective of CBF levels. SCFA are also known to decrease inflammation system wide. The literature shows that in chronic time points that increases in choline related metabolites may indicate glial proliferation. As inulin decreases the GPC levels this could be indicative of reduced glial proliferation. Inulin also is decreasing harmful bacteria such as *Dorea sp. 5-2* which has been positively correlated with intestinal permeability. It is clear that even when administered in the chronic phase of injury, inulin exerts beneficial effects that aid recovery.

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### **Preclinical model for blast-induced traumatic brain injury**

#### ***Fellow***

Many soldiers and Veterans are exposed to explosive blast waves as well as occupational low-level blast (LLB) during normal training operations, including but not limited to breaching activity. These military personnel are at increased risk for persistent neuropsychological impairment due to repeated LLB exposure over several deployments with limited time for recovery between exposures. The extent of the long-term consequences after cumulative LLB exposure is unknown, though reports show that deficits can be present late in life. Furthermore, the resultant post-traumatic stress disorder (PTSD)-related behavioral deficits are more pronounced in soldiers and Veterans with a history of chronic blast exposure. There is no clear understanding of which pathological mechanisms drive this chronic PTSD phenotype after LLB exposure. While preclinical models replicate the chronic depressive, anxiogenic, and PTSD-related traits observed in Veterans, there are many knowledge gaps in what contributes to these chronic deficits. By utilizing a multimodal McMillan blast device (MBD) at the University of Kentucky, we can experimentally replicate the blast waveform typically observed following chemical explosions. The MBD can deliver varying levels of blast peak overpressure based on controlled parameters, such as diaphragm thickness and driver pressure. In general, blast exposure causes acute blood-brain barrier and neurovascular unit abnormalities that can persist over time. Our short-term goals are to determine the timing of acute neurovascular dysfunction and the profile of longitudinal behavioral traits after a single LLB. This research will drastically improve our understanding of the effects of LLB as well as potentially identify novel, clinically-relevant biomarkers. In addition, this line of investigation can lead to better therapeutic targeting of neurovascular dysfunction to improve neurological outcome in soldiers and Veterans.

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**Phenelzine improves hemoglobin-induced oxidative stress and mitochondrial dysfunction in oligodendrocyte progenitor cells**  
**Staff**

Oligodendrocyte progenitor cell (OPC) dysfunction in the newborn brain leads to white matter injury and persistent neurodevelopmental delays. OPCs are vulnerable to oxidative stress that occurs after hypoxia or ischemia, which are common events in preterm infants. Another pathological event that injures white matter in infants is intraventricular hemorrhage (IVH) of prematurity. Although blood products released into the CSF after IVH contain the powerful oxidant hemoglobin, oxidative stress-mediated OPC injury has not been studied in IVH. If oxidative stress from hemoglobin injures white matter after IVH, pharmacological compounds that reduce oxidative stress could preserve white matter after IVH.

In the current study, we utilized an cell culture system to investigate hemoglobin-induced OPC injury and the therapeutic effect of the antioxidant phenelzine (PLZ) on hemoglobin-induced oxidative stress and mitochondrial dysfunction. OPCs were isolated from Sprague Dawley rat pups and exposed to of hemoglobin with and without PLZ. Outcomes assessed included intracellular reactive oxygen species (ROS) levels using 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) fluorescent dye, oxygen consumption using the XFe96 Seahorse assay, and OPC proliferation by BrdU incorporation assay. Hemoglobin induced oxidative stress and impaired mitochondrial function in OPCs, and reduced OPC proliferation. PLZ treatment reduced hemoglobin-induced oxidative stress and improved OPC mitochondrial bioenergetics. The effects of hemoglobin and PLZ on OPC proliferation were not statistically significant, but showed trends towards hemoglobin reducing OPC proliferation and PLZ increasing OPC proliferation. Collectively, our results indicate that phenelzine may be protective in white matter diseases mediated by hemoglobin-induced oxidative stress, such as IVH of prematurity.

## **The Influence of Age on Microglia Degeneration in the Human Brain**

### ***Student***

Microglia activation—typically described in terms of hypertrophic appearance—is a well-established feature of aging. However, recent studies have suggested that microglia dystrophy, not activation, that may lead to increased propagation of progressive neurodegenerative diseases such as Alzheimer's disease. Yet, a clear understanding of cause and consequences of dystrophic microglia is lacking. Although frequently observed in diseased brains, the appearance of dystrophic microglia in the hippocampus of individuals free of cognitive impairment suggests that microglia may be undergoing senescence with age, leading to dystrophy. Therefore, we hypothesized age could be a significant contributor to senescence and the presence of dystrophic microglia. To investigate this relationship, we employed stereological counts of total microglia, hypertrophic, and dystrophic microglia across the decades of the human lifespan. The microglia counts were completed in the frontal cortex grey matter and the CA1 subregion of the hippocampus in individuals without known neuropathology or neurodegenerative disease. We also compared these cases to previously generated data of individuals with confirmed neurodegenerative disease. Consistent with our hypothesis, in aging individuals, we found a significant increase in the number of dystrophic microglia in the CA1 region and frontal-cortex grey matter related to age. However, only in the frontal cortex was the growth of dystrophic microglia significantly greater than the increase in other microglia morphologies. In the presence of disease pathology, the percentage of microglia observed to be dystrophic was significantly greater than in normal aging. These results provide evidence that normal aging is only associated with a modest increase in dystrophic microglia and suggest that dystrophic microglia may be a disease-associated microglia phenotype. Future work is required to understand the link between the increase in dystrophic microglia and neurodegenerative disorders.

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### Targeting Bruton Tyrosine Kinase for Treatment of Spinal Cord Injury

#### Faculty

**Objective:** Early microglia and subsequent B cell/astrocyte responses to injury are major contributors to the secondary injury progression after traumatic spinal cord injury (SCI) in human and rodents. Unfortunately, no approved therapies targeting these mechanisms for SCI are available. Bruton tyrosine kinase (BTK) activation mechanistically links many of these B cell and glial inflammatory mechanisms. Our recent study showed that SCI resulted in increases in spinal BTK activation (phospho-BTK) and total BTK upregulation at 4 weeks postinjury in a rat model of SCI. We hypothesized that inhibiting BTK activation and upregulation will improve pathological and functional outcomes by attenuating multiple neuroimmune/inflammatory responses after SCI. Our objective is to use FDA-approved BTK inhibitor Ibrutinib to inhibit the early microglial inflammatory and delayed B cell autoimmune/astrocyte injury cascade thereby improving recovery of locomotor

/bladder function after SCI in rats.

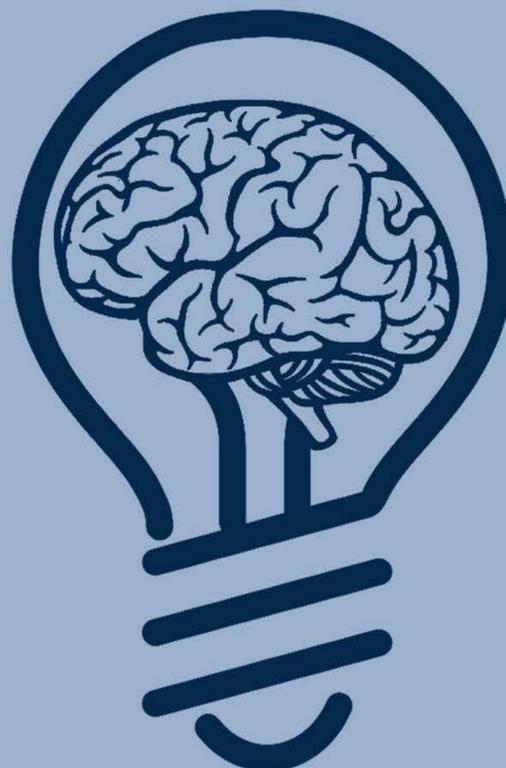
**Methods:** Moderate contusive SCI (180 kdyn, T10) was produced using an Infinite Horizons (IH) Impactor in female Sprague-Dawley (SD) rats, age 3 months. Both BTK expression and phosphorylation were measured at the lesion site at 2, 7, 14, and 28 days after SCI or sham operation using Western blot analysis. Ibrutinib treatment (6 mg/kg/day, intraperitoneally, IP) started at 3 h post-injury for 7 or 14 days. Locomotor, bladder and anatomical recovery was evaluated for up to 28 or 77 days post-injury. Microglial/astroglial activation, plasma cell marker, and IgG antibody production were evaluated at 7 and 14 days post injury.

**Results:** Both BTK expression and phosphorylation were increased at the lesion site at 2, 7, 14, and 28 days after SCI. Ibrutinib treatment (6 mg/kg/day, IP, starting 3 h post-injury for 7 or 14 days) reduced BTK activation and total BTK protein after SCI in rats compared with vehicle-animals. Acute Ibrutinib treatment (6 mg/kg/day, IP, starting 3 h post-injury) for 7 days post-injury attenuated the injury-induced elevations in Iba1, GFAP, CD138, and IgG at 7 days post-injury and improved locomotor function (BBB scores) up to 4 weeks post-injury. Sustained Ibrutinib treatment for two weeks also reduced the levels of CD138 and IgG at the injury site 14 days after SCI in female rats. The 2-week Ibrutinib treatment improved long-term locomotor outcomes (BBB scores) and bladder recovery, and resulted in a trend towards improved tissue sparing by 21%, which did not reach statistical significance, at 11 weeks post-injury.

**Conclusion:** These results indicate that Ibrutinib exhibits neuroprotective effects against locomotor deficits and bladder dysfunction by blocking excessive neuroinflammation through BTK-mediated microglia/astroglial activation and B cell/antibody response in rat model of SCI. Further, these data identify BTK as a critical therapeutic target/marker for SCI.

**Thank you for joining us.  
If you have any questions, please contact  
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