2021

HealthCare
KENTUCKY NEUROSCIENCE INSTITUTE

University of Kentucky College of Medicine
Neuroscience Research Priority Area

Neuroscience Clinical Translational Research Sympsoium

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October 8th

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Keynote:
Seth Himelhoch - MD, MPH

2021 Neuroscience Clinical Translational Research Symposium

Agenda

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8:00—9:00	**Poster Hall**	
9:00—9:10	**Welcome & Introduction**	**Larry B. Goldstein, MD** **Linda J. Van Eldik, PhD**
9:10—9:40	**Keynote Presentation**	**Seth Himelhoch, MD, MPH**
9:40—10:50	**Focus on Cognitive/Behavioral Disorders**	**Moderator:** **Frederick A. Schmitt, PhD**
	Thermoneutral Temperature Exposure Increases Slow-wave Sleep in the 3xTg-AD mouse model of Alzheimer's Disease	Jun Wang, MS
	Systemic Amylin Dyshomeostasis Leads to Metabolic Dysfunction and Affects Cognition	Deepak Kotiya, PhD
	Use of Wearable Sensors to Assess the Effects of Performing a Cognitive Task on Sensory Integration of Balance in Healthy Individuals	Geetanjali Gera, PhD
	Vascular Risk Biomarkers are Linked to Frontal Memory-related Neuromarkers	Jeremy Latham
10:50—11:00	**Short Break**	
11:00—12:10	**Focus on Neurodegenerative Disorders**	**Moderator:** **Greg Gerhardt, PhD**
	APOE Genotype Modifies Microglial Immunometabolism	Nicholas Devanney
	Expression and Pharmacologic Modulation of the Ca2+-Permeable Purinergic Receptor P2X4 in Hippocampal Neurons and Astrocytes	Hilaree Frazier, PhD
	A Potential Biomarker for Cerebral Amyloid Angiopathy: Cerebral Blood Flow Low-Frequency Oscillations Method Using Diffuse Correlation Spectroscopy	Ahmed A. Bahrani, PhD
	Effects on NMSS after DBS Plus in Parkinson's Patients	Katherine Manning
12:10—1:30	**Poster Hall with Authors & Lunch Break**	

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1:00—2:00	**Medical School Panel** https://uky.zoom.us/j/84886338061	**Moderator:** **Ima Ebong, MD**
1:30—2:40	**Focus on Neurotrauma**	**Moderator:** **Joe Springer, PhD**
	Time Course of Mitochondrial Bioenergetic Impairment in Mice After Severe Controlled Cortical Impact: Is Sex a Driving Force?	Olivia Kalimon
	Dysregulation of Inflammatory Pathways in miR-223 Knockout Mouse Brain Following Traumatic Injury	Steven Pesina
	Effects of Acute Ethanol Intoxication on Spinal Cord Injury Outcomes	Ethan Glaser
	Distraction - Friend or Foe? Qualitative Inquiry into the Impact of Workplace Distractions for Persons with TBI	DeAnna Pinnow, MA, CCC-SLP
2:40—2:50	**Short Break**	
2:50—4:00	**Focus on Stroke/Neurovascular Disorders**	**Moderator:** **Donna Wilcock, PhD**
	The Impact of Exercise Prior to Stroke in Cathepsin B Knockout Mice	Katherine M. Cotter
	SpeckleFlow: Rapid Spatio-temporal Analysis of Superficial Cerebral Blood Flow Dynamics in High Resolution Laser Speckle Contrast Imaging	Sami Lin Case
	Influence of Body Mass Index on Adenosine Deaminase and Infarct Volume in Mechanical Thrombectomy Subjects	Benton Maglinger, MS
	Enlarged Perivascular Spaces in the Centrum Semiovale Predict MoCA Scores among Cognitively Normal Older Adults	T.J. Libecap
4:00	**Closing Remarks**	**Larry B. Goldstein, MD** **Linda J. Van Eldik, PhD**

Addiction

Holley Allen, MS [1] • Michael Wesley, PhD [2] • Jessica Weafer, PhD [3] • Mark Fillmore, PhD [3]
Clinical Psychology University of Kentucky [1] • Behavioral Science University of Kentucky [2] • Psychology, Behavioral Neuroscience University of Kentucky [3]

Sensitivity to the disinhibiting effect of alcohol: the role of trait impulsivity and sex differences

Student

Higher trait impulsivity is associated with more impulsive responding on behavioral measures of disinhibition. Additionally, behavioral disinhibition is acutely elevated following alcohol consumption. The current study examined the possibility that trait impulsivity may predict individual differences in sensitivity to the disinhibiting effect of alcohol. Specifically, the current study tested the hypothesis that those with elevated trait impulsivity also experience heightened sensitivity to the disinhibiting effect of alcohol, which might further compound their tendency toward impulsive action. To test this hypothesis, data from six studies were aggregated to comprise a sample of 190 young adults. Participants completed the Barratt Impulsiveness Scale-11, and behavioral disinhibition was assessed following consumption of 0.65 g/kg alcohol and a placebo. Alcohol increased disinhibition overall, but higher impulsivity did not predict increased sensitivity to alcohol-induced disinhibition. In men, higher levels of trait impulsivity predicted heightened sober disinhibition following placebo, but this relationship was not present in women. These findings suggest significant sex differences in the relationship between trait impulsivity and disinhibition. This sex difference may explain inconsistent research findings in studies assessing links between trait and behavioral measures of impulsivity. The data also point to trait impulsivity and sensitivity to alcohol-induced disinhibition as independent constructs.

Bethany Jurs, PhD [1]
Neuroscience Transylvania University [1]

E-Cigarette Stroop: Assessing neural correlates of attentional bias in adolescents

Student

Background:

The rapidly rising rates of adolescent (18-25) electronic cigarette (e-cigarette) use represents a public health epidemic with recent reports of serious lung injuries and deaths linked to vaping. Previous research has shown adolescent nicotine use can have serious cognitive consequences. Attentional bias is a well-studied phenomenon in substance use disorders where individuals automatically allocate more attention to features of the environment that are salient to the substance(s) of choice. The current study seeks to examine the behavioral and neural correlates of attentional bias in adolescents who use e-cigarettes.

Method:

Participants (18-22 years old) were recruited in Lexington, KY to be in enrolled in an e-cigarette group or control group. The e-cigarette group (n=16, 6 females) were required to have current e-cigarette use. Controls (n=14, 8 females) were deemed eligible by having no history of nicotine use. All participants completed a demographic questionnaire and a modified addiction Stroop task that consisted of three different word groups: animal words, e-cigarette words, traditional cigarette words. Task design considerations were incorporated from previous research. Electrophysiological (EEG) data was collected using 64-channel Electrical Geodesics Inc. (EGI) HydroCel nets and 300 high-impedance amplifier.

Results:

A 2x3 mixed factors ANOVA with Group and Word Type as factors for both reaction time and accuracy. There were no significant main effects or interactions observed. To investigate any neural effects of e-cigarette use, a similar 2x3 ANOVA was run on P200 amplitude values averaged over 6 clustered electrode sites in the right lateral frontal-parietal lobe area. These electrodes sites were selected as an area of interest for attentional bias based on previous research. While there were no significant interactions, results show a significant main effect of group with overall higher P200 amplitude values for the e-cigarette group vs the control group, $F(1,28)=4.52$, $p=.04$. There was also a significant main effect of word type across groups with e-cigarette related words eliciting overall larger P200 amplitude values compared to the other word conditions, $F(2,56)=5.77$, $p<.01$.

Interpretation:

The results did not fully replicate patterns demonstrated by previous research. However, we did observe a significant difference in P200 amplitude between the groups when performing the modified Stroop task. Although the interpretation of this finding is unclear, future research seeking to understand the potential impacts of adolescent e-cigarette use may benefit from using neural techniques in conjunction with behavioral measures. It remains unclear if e-cigarette treatment requires different approaches to traditional cigarette treatment. Clinicians could benefit from additional research that continues to identify unique targets for treatment in the e-cigarette using population. Study limitations are discussed further.

Differential Effects of Short and Extended Cocaine Access on Astrocytes in Rat Nucleus Accumbens

Student

Nucleus accumbens is involved in motivational and affective behavioral processing, and plays a significant role in development and maintenance of substance use. The release of dopamine from the ventral tegmental area into the nucleus accumbens regulates incentive salience and facilitates reinforcement and reward-related motor function learning. Recent studies reveal that, in the context of cocaine exposure, dopamine transmission in the ventromedial striatum is pivotal in the control of initial drug use. In addition, astrocyte morphology has been shown to be affected by cocaine, but the functional role of astrocytic signaling in cocaine use remains unknown. The goal of this study is to address astrocytic adaptions within nucleus accumbens under two different cocaine exposure regimes.

We have observed morphological and functional changes under elevated extracellular dopamine in cultured astrocytes. Physiological increases in dopaminergic signaling over the course of cocaine self-administration were shown to alter astrocyte morphology and expression profile of genes.

To characterize the astrocytic effects on cocaine-induced dopamine elevation and how it contributes to neural circuit functions, we used a rat self-administration model. Two groups of animals went through short- (1h/day) and extended- (6h/day) access cocaine self-administration for 14 days. To identify functional effects of cocaine on astrocytes, we imaged astrocytes expressing calcium indicator, GCamp6f and performed whole-cell patch-clamp recordings.

We noted the extended-access to cocaine leads to an elevation of drug taking behavior, but not in the short-access group. Intracellular level of calcium within NAc astrocytes is differentially affected by short- or extended- access cocaine self-administration. Extended exposure significantly decreased the amplitude of spontaneous Ca2+ transients at both plasma membrane and endoplasmic reticulum. Calcium transients at two compartments were differently affected by mGluR or IP3 antagonists, suggested compartmentalized mechanisms of calcium signaling regulation. On-going experiments examine how astrocyte membrane properties are regulated by cocaine exposure regimes and how cocaine may alter coupling within astrocytic networks.

Astrocytes adapt to cocaine exposure differently through short- and extended- access self-administration, both morphologically and functionally. Our results indicate a potential regulatory role of astrocytic signaling in the NAc on neural mechanisms underlying substance use.

Cognitive & Behavioral

Christopher Bauer, PhD [1] • Valentinos Zachariou, PhD [1] • Pauline Maillard, PhD [2] • Arvind Caprihan, PhD [3] • Brian Gold, PhD [1]
Neuroscience University of Kentucky [1] • Neurology University of California at Davis [2] • Neuroscience The Mind Research Network [3]

Intracellular Rather Than Extracellular Diffusion MRI Measures Predict Working Memory Performance in Healthy Older Adults

Staff

Diffusion tensor imaging (DTI) is widely used in MRI research to estimate white matter health in the aging human brain, but little is known about which specific DTI indices are most predictive of cognitive function. While fractional anisotropy (FA) reflects diffusion associated with both intracellular (tissue) and extracellular (Free Water [FW]) compartments, recent multi-compartment models (FW-correction [includes FW-corrected FA and FW] and NODDI [includes intracellular volume fraction {ICVF} and orientation dispersion index {ODI}]) provide more specific intracellular or extracellular compartments. Here, we compared the strength of association between these different DTI metrics to both age and cognition (working memory [WM] performance). Ninety-nine healthy older adults (ages 60-85; 61 women) were recruited from the Lexington community and scanned at the University of Kentucky using a 3 Tesla MRI Siemens Magnetom Prisma with a 64-channel head coil. A 126-direction main DTI sequence using 4 b-values (0, 500, 1000, and 2000 s/mm^2), a reverse phase-encoding direction DTI sequence, and an fMRI sequence were acquired. Participants performed an in-scanner visual WM task (N-back; control, 1-back, and 2-back conditions) during fMRI acquisition with WM performance calculated using D-prime. A network of brain regions showing functional activation during the WM task was defined across participants using a group-level functional contrast (2-Back > [1-Back/2 + Compare/2]/2). White matter tracts connecting the functionally-activated working memory network was reconstructed using probabilistic tractography. Each participant's diffusion measures were extracted from skeletonized white matter tracts connecting WM regions using FSL's tract-based spatial statistics. Linear regression models with diffusion measures as the predictors and age or D-prime (with age as a covariate) as the dependent variables were conducted in SPSS controlling for gender. All diffusion measures except ODI significantly predicted age. However, only ICVF (p = 0.035) and FW-corrected FA (p = 0.061; marginal significance) specifically predicted D-prime. Our results suggest that intracellular-associated (axonal loss and demyelination) rather than extracellular-associated (neurodegeneration, vascular dysfunction, or neuroinflammation) diffusion may be more sensitive to WM performance.

Lawrence Brewer, PhD [1] • Chris Gant, PhD [2] • Katie Anderson, MS [1] • Hilaree Frazier, PhD [2] • Julien Thibault [2] • Adam Ghoweri, PhD[3] • Jessie Hoffman, PhD [4] • Susan Kraner, PhD [2] • Philip Landfield, PhD [2] • Olivier Thibault, PhD [2] • Eric Blalock, PhD [2] • Nada Porter, PhD [2]
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Sexually Dimorphic Effects of Dietary Vitamin D3 Supplementation on Cognition and the Gut Microbiome in Aging Rats

Staff

Increasing evidence suggests that vitamin D plays a role in maintaining cognitive function and that vitamin D deficiency may accelerate age-related cognitive decline. Here, we determined if a long-term enhanced vitamin D (VitD3, cholecalciferol) diet, higher than the standard dietary level, maintains or improves cognitive function in aging male and female rats. We also examined if sex and/or the high VitD3 diet affects the gut microbiome.

Beginning at 12 months of age 20 male and 20 female F344 rats were fed an AIN-93 diet containing either standard (1000 IU/kg diet) or higher (10,000 IU/kg) VitD3 for 6 months. The Morris water maze (MWM) was then used to assess learning and memory. Following the MWM, the gut microbiome from undigested chime collected from the intestinal cecum was identified and taxonomically classified by Argonne National Laboratory using 16S rRNA sequences. ZRT Laboratory determined 25-(OH)VitD3 levels from cardiac blood. A two-way ANOVA and Tukey post-hoc or an ANOVA on Ranks with a Dunn's post-hoc was used to test for statistical significance.

The higher VitD3 diet significantly elevated 25-(OH)VitD3 blood levels in female and male rats. **(MWM)** After 3 days of training the probe test on day 4 indicated that the higher VitD3 diet significantly reduced path length and latency ($P = 0.01$) to the digital platform in females but not males. On day 5 platform location was changed and animals received one day of reversal training. On day 8, three days following reversal training, the reversal probe indicated that higher dietary VitD3 improved performance in males but not females by significantly reducing path length and latency to the digital platform ($P < 0.05$). **(Microbiome)** Analysis of the cecal microbiome content showed sex specific differences and selective effects of the high VitD3 diet. Multivariate analyses found significant distances between the female control group and the high VitD3 female as well as the male control groups. These trends are graphically displayed using a volcano plot of identified bacterial taxa.

These results indicate that an enhanced VitD3 diet may preserve cognitive acuity during aging. Further, VitD3 may have sexually dimorphic effects on memory formation. The present results replicate our previous male only study that a high VitD3 diet preserves cognition in aging male rats (Latimer et al. 2014). The microbiome showed sexually dimorphic differences and the high VitD3 diet appeared to affect the microbiome in a sex specific manner. The significance of these differences in the microbiome are not clear.

Deepak Kotiya, PhD [1] • Velmurugan Gopal Viswanathan, PhD [1] • Nirmal Verma, PhD [2] • Florin Despa, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Pharmacology and Nutritional Sciences University of kentucky [2]

Systemic Amylin Dyshomeostasis Leads to Metabolic Dysfunction and Affects Cognition

Fellow

Introduction: Dysregulation of amylin, a pancreatic hormone, contributes to the development of type-2 diabetes and diabetes-related cognitive decline. Because amylin participates in the central regulation of energy homeostasis, we studied the impact of altered secretion of amylin on brain function.

Method: We developed a conditional human amylin mouse model in which pancreatic mouse amylin was replaced by human amylin and regulated by tamoxifen (TAM) injection, intraperitonially. Wild-type mice were used as controls. Male and female mice (n=7-10/group) from all groups were fed with high fat and chow diet in the presence/absence of TAM treatment at 5 months of age. Glucose tolerance test (GTT), body weight and blood glucose levels were measured. Collected end points (9 months of age mice) included brain function measured by novel object recognition (NOR) and open field tests.

Results: Conditional expression of human amylin induced glucose dysregulation and behavior deficits in male mice independent on high fat diet ($P<0.05$).

Conclusion: Systemic amylin dyshomeostasis leads to metabolic dysfunction and affects brain function. Further studies are needed to describe the mechanisms by which altered secretion of pancreatic amylin affects brain function.

Jeremy Latham [1] • Tiffany Sudduth [2] • Baoxi Wang, PhD [3] • Justin Barber [2] • Margaret Kelly [2] • Katherine Snyder [2] • Barbara Martin [2] • Gregory Jicha, MD, PhD [4] • Donna Wilcock, PhD [5] • Yang Jiang, PhD [6]
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Vascular Risk Biomarkers are Linked to Frontal Memory-related Neuromarkers

Student

We reported longitudinal evidence that left frontal memory-related signals predict risk of amnestic Mild Cognitive Impairment (MCI) about 5 years before clinical diagnosis (Jiang et al., 2021) in healthy cognitively intact older adults. MCI is a prodrome of dementia. There is limited understanding of MCI due to mixed Alzheimer's disease (AD) and vascular pathologies. Identifying cause-specific MCI risk pre-symptomatically increases the ability to slow cognitive decline. The relationship between subtypes of MCI induced by vascular dementia was not clear. Here we test the hypothesis that increased vascular risk alters brain signals during a cognitive task in older adults.

20 cognitively intact healthy older adults (median age 68, 9 females) from the University of Kentucky Alzheimer's Disease (AD) Research Center participated in the study. Each volunteer was assessed with the Montreal Cognitive Assessment (MoCA) and vascular/AD plasma markers were collected. Brain activity and memory performance were recorded during the 15-min Bluegrass working memory test using a 14-channel, wireless electroencephalography (EEG) headset (eMOtiv; Borhani et al., 2021). Pearson correlations were used to assess associations between frontal EEG sites and plasma markers for Vascular risk factors.

Increased frontal F7 or F3 "difference brainwaves" (Diffs: Nontarget > target) were associated with reduced ability to hold the memory target. We found that frontal F3 Diffs are negatively correlated with MoCA scores (R= -0.457) while diastolic and systolic blood pressure were positively correlated with F7 Diffs (R=0.431 and R=0.463). Left frontal signals were also significantly correlated with multiple serum biomarkers. The most significant biomarker was serum A1C (a known risk factor for developing vascular MCI-like states), which was positively correlated with mean Diffs at F3/F7 sites, F3, and F7 (R=0.645, 0.528, and 0.313).

We report that a variety of plasma biomarkers for vascular risks, specifically changes in hemoglobin and blood pressure, were associated with decreased working memory-related neural functions. Our findings directly link increased vascular risk factors with reduced neural functions of holding items in working memory in pre-symptomatic older adults.

DeAnna Pinnow, Other [1]
Rehabilitation and Health Sciences University of Kentucky [1]

Distraction, Friend or Foe? Qualitative Inquiry into the Impact of Workplace Distractions for Persons with TBI

Student

BACKGROUND: Persons with traumatic brain injuries (TBIs) who return to work often struggle with managing environmental distractions due to residual cognitive impairments. Previous literature has established that environmental distractions impact persons with TBI, yet, the extent to which distractions impact workplace performance is unknown. This qualitative, phenomenological study explored the experiences of seven individuals with TBIs and how they perceived workplace distractions to impact their productivity.

METHODS: Data was collected using semi-structured interviews with seven participants who were diagnosed with mild, moderate, and severe TBIs. Interviews were transcribed and analyzed using thematic analysis.

RESULTS: Main findings centered around what environmental distractions impacted work performance, the farther-reaching consequences of distraction beyond task outcomes, strong emotional feelings and worry about perceived work performance associated with distractibility, mitigating distractibility through "gaming the attentional system," and utilizing music as a distraction masker to enhance task performance.

CONCLUSIONS: In light of this study's findings, researchers, and clinicians are encouraged to consider the wider impact of distractions on persons with TBI. The real-life accounts documented in this study will assist in the effort of both researchers and clinicians to account for the impact of environmental distractions in rehabilitation. Inquiries about distraction experiences may guide the development of individualized treatment plans and compensatory strategies to support employment for persons with TBI.

Geetanjali Gera, PhD [1]
Physical Therapy University of Kentucky [1]

Use of wearable sensors to assess the effects of performing a cognitive task on sensory integration of balance in healthy individuals

Student

Purpose/Hypothesis: We investigated the effects of performing a cognitive task on sensory integration of balance in healthy individuals. Our goal was to identify quantitative postural sway measures that could assess differences in cognitive and non-cognitive tasks. To be able to standardize the postural sway measures, we assessed 1) test-retest reliability and 2) the order effect of performing cognitive and non-cognitive tasks. We hypothesized that there will not be any order effect and the results for the postural sway measures will be similar for the test-retest.

Materials/Methods: We recruited 11 healthy individuals but report the findings for 10 subjects (one subject was excluded because of issues with reliable data). Ten healthy subjects, (5F/5M; 21.5±2.17 years; Caucasian), without known balance issues, performed the instrumented Modified Clinical Test of Sensory Interaction on Balance (mCTSIB). This test involved four balance task conditions: eyes opened or closed on firm or foam surface with (cog) or without a cognitive task (non-cog). Postural sway was assessed using an inertial sensor (IMU) placed around the waist close to lumbar4-5 region. We assessed five postural sway variables: sway area, jerk, root mean square (RMS), mean velocity (MV), and centroidal frequency (CF). The study randomized whether the subjects performed the non-cognitive or cognitive task first. The subjects were also retested for the four conditions later using the same order of the first test.

Results: Inertial-sensor based assessment showed that postural sway variables were different for non-cognitive and cognitive tasks. In general, postural sway was higher for the cognitive task as compared to the non-cognitive task. Postural sway jerk and CF were consistently higher for the cognitive task compared to the non-cognitive task ($p<0.01$). RMS approached significance for this comparison (0.052). The measures of sway area and MV did not show significance between the non-cognitive and cognitive task. Our results revealed no-order effect, i.e. the postural sway measures were similar whether the subjects performed the non-cognitive task first or vice-versa. Also, there was no difference in the postural sway measures when we repeated the assessment after a 30-minute break.

Conclusions: These findings show that jerk and CF are promising measures to differentiate postural sway performance between non-cognitive and cognitive tasks in healthy individuals.

Clinical Relevance: Subtle differences in postural sway performance during quiet stance might get unnoticed by observation in healthy individuals. Thus, we recommend the use of quantitative measures of postural sway using inertial-sensors.

Valentinos Zachariou, PhD [1] • Christopher Bauer, PhD [1] • Brian Gold, PhD [1]
Neuroscience University of Kentucky [1]

Cortical Iron Disrupts the Anatomical Connectivity of Working Memory Networks by Damaging Proximal White Matter Microstructure in Older Adults.

Staff

Aging is associated with accumulation of non-heme brain iron, which has been linked with oxidative stress, demyelination and cognitive decline. For instance, several previous studies have reported negative associations between iron concentration in the brain and working memory performance. However, the mechanisms by which excess brain iron interferes with working memory are not well understood. One possibility could be that increased non-heme iron concentrations in brain regions that support working memory, increase oxidative-stress-related injury to proximal white matter (WM) which can disrupt the anatomical connections between them. Here, we explored this possibility using quantitative susceptibility mapping (QSM), an in-vivo MRI technique for measuring iron concentration in brain tissue, in conjunction with fMRI, diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI). A cohort of 113 cognitively normal older adults (age range: 60-86) performed an N-Back visual working memory task inside a 3T Siemens Prisma MRI scanner (64-channel head coil). Using whole-brain fMRI activity from this task, we identified a network of bilateral brain regions in which the magnitude of activity in response to the N-Back task was significantly stronger than a control task. These regions included the anterior and posterior cingulate cortex, the dorsolateral prefrontal cortex, the middle frontal gyrus and the inferior parietal lobules. Then, using each of these regions as a seed, probabilistic tractography was conducted to map the WM connectivity between the seeds. In addition, QSM values were extracted from each of the seed ROIs as a measure of iron concentration. Voxelwise, linear regressions were then conducted between QSM values and (1) neurite density (ND), (2) radial diffusivity (DR), both of which are measures of WM microstructure integrity. All voxelwise regressions were constrained within a mask of the WM network we identified and controlled for participant age. We found that iron concentration extracted from the frontal-lobe seed ROIs predicted lower ND and higher DR in frontal but not parietal lobe regions of the WM network (Figure 1A). Conversely, iron concentration extracted from the parietal-lobe seed ROIs predicted lower ND and higher DR in parietal but not frontal lobe regions of the WM network (Figure 1B). Based on these findings, we conclude that in older adults, non-heme iron in brain regions supporting working memory can disrupt their anatomical connectivity by damaging proximal WM microstructure.

Figure1: https://tinyurl.com/NCTR2021

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Biomedical Engineering University of Kentucky [1] • University of Kentucky University of Kentucky [2] • Spinal Cord and Brain Injury Research Center University of Kentucky [3] • Dept. of Mol. & Cell. Biochem University of Kentucky [4] • Biology University of Kentucky [5] • Neuroscience University of Kentucky [6]

Thermoneutral temperature exposure increases slow-wave sleep in the 3xTg-AD mouse model of Alzheimer's Disease

Student

There is growing evidence that disordered sleep, which is known to be associated with Alzheimer's disease (AD), may accelerate neuropathology, thus promoting a vicious cycle. Strategies for improving sleep quality may slow disease progression. Here we investigate the feasibility of sleep enhancement through ambient temperature regulation and examine the effect on amyloid-pathology. Female 3xTg-AD mice (~12 m.o.) were instrumented for EEG/EMG monitoring. After a week-long baseline, one half of the mice (n=9, EXPT; one animal did not survive for analysis) were exposed to stepwise diurnal increases in ambient temperature (Ta) to reach 30°C (thermoneutral for mice) during the light phase while the rest (n=8, CTRL) remained at room temperature (22°C). Vigilance state – i.e., Wake, REM, NREM, and slow wave sleep (SWS) within NREM – was scored in 4-second epochs and sleep metrics computed. SWS and REM were significantly greater ($p<0.05$) in the light phase for EXPT mice. These effects suggest better sleep consolidation and greater sleep depth with thermoneutral warming. After four weeks of treatment, the animals were euthanized, and the brains removed to assay amyloid pathology by ELISA. We found that thermoneutral warming caused a significant reduction in both Aβ40 and Aβ42 in the hippocampus, but not in the cortex. These data imply that thermoneutral warming might have some regional specificity in its effects, the effects appear to be specific to some brain areas more than others, with implications for the cognitive and neuropathologic changes found in AD. Furthermore, since SWS and REM support memory, future studies should investigate the effects of thermoneutral enhancement of SWS and REM on cognition.

Funding: R01AG068215; seed funds UK Department of Neuroscience

Epilepsy

Diane Iradukunda, MS [1] • Dillon Huffman, PhD [1] • Pacifique Rukundo, Other [1] • Jasmine Perdeh, MS [2] • Maxwell Lavin, MS [1] • Sydney Daniels, Other [3] • Kevin Donohue, PhD [4] • Bruce O'Hara, PhD [5] • Bjoern Bauer, PhD [6] • Sridhar Sunderam, PhD [1]
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A home-cage monitoring solution for non-invasive seizure screening in rodent models of epilepsy

Student

Rationale: There is an urgent need for research into treatment options for epilepsy and other seizure disorders. Animal models are increasingly used to understand disease mechanisms and to screen potential therapeutic approaches. However, such investigations typically require validation via expensive and labor-intensive methods (i.e., EEG measurements) – limiting the pace and scale of experimentation. Previously, we have shown that piezoelectric technologies show great utility in non-invasive seizure detection. Here, we present further developments in this effort where monitoring is accomplished through an external sensor platform located beneath the housing cage.

Methods: Adult Wistar rats (female; 2 months aged) were treated with pilocarpine i.p. to induce acute status epilepticus (SE). Three months after induction, rats were monitored in individual standard housing cages via piezoelectric *Adapt-a-Base* sensors (Signal Solutions, LLC) for 10 weeks alongside a continuous video record. Week-long recordings were processed to derive a seizure detection statistic time series, to which a threshold was applied so as to identify the 20 most intense events (20 dets/week × 8 cages = 160 detections/week). Timestamps of threshold crossings were written to a spreadsheet for inspection through the video record and were annotated according to observed behavior – providing a reference of true and false positive detections. Detection precision was evaluated according to the incidence of true positive detections relative to all detections.

Results: Piezoelectric sensors captured the essential dynamics of seizures, as well as pre- to post-ictal changes in physiological parameters (i.e., breathing). Derivation of a simple detection statistic was successful in discriminating seizure events with reasonable precision (15-30%) – offering great labor-saving for identifying seizures compared to exhaustive video review.

Conclusions: Overall, this approach shows great utility in identifying spontaneous seizures in chronic epilepsy monitoring. The signals generated by the piezo- sensors not only capture tonic-clonic behavior during seizures, but also other relevant behaviors such as breathing, freezing, and tremor. Moreover, as this technology has been well validated for use in discriminating sleep-wake activity, it opens the door to investigating seizures in the context of sleep/circadian rhythms, which may be a key aspect to consider when evaluating potential treatment strategies. Further development of this method will focus on capitalizing on the temporal evolution of the piezo- signal during seizures, with the hopes of increasing detection sensitivity/specificity, as well as discriminating between seizure types.

This work was supported by NIH Grant No. NS107148.

Julie Youssefi, MD [1] • Jason Chisolm, MD [2] • Shweta Kamat, MD [3] • Christopher McLouth, PhD [4] • Farhan Mirza, MD [5] • Thomas Pittman, MD [5] • Meriem Bensalem-Owen, MD [3] • Sally Mathias, MD [3]
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Epilepsy Surgical Outcomes Between Patients Age 50 and Older versus Younger Patients

Faculty

Introduction:

- Epilepsy surgery is a safe and effective treatment for refractory epilepsy. There are few studies reporting outcomes specific to patients age 50 and older and these studies have conflicting results. Older patients are not referred for surgical evaluation as often as younger patients. Older patients may have more medical comorbidities and are more likely to have longer duration of epilepsy. These factors may limit favorable outcomes. This study was designed to compare surgical outcomes at a single institution for patients age 50 and older versus younger patients.

Methods:

- This is a retrospective study of adult patients who had brain resection for the treatment of refractory epilepsy from February 2006 through September 2019. Hospital and clinic records were reviewed to determine patient demographics, location of surgical intervention, length of follow up, Engel outcomes, and complications of surgery. The older patient group was defined as patients age 50 and older. Patients were excluded for age less than 18 or for surgeries that were not planned to treat refractory epilepsy.

Results:

- 193 records were reviewed and 116 met inclusion and exclusion criteria.
- 23 surgeries were older adults and 93 were in younger adults.
- The older patients were more likely to be female (57% vs 45%) and have a duration of epilepsy greater than 20 years (61% vs 35%).
- Surgeries in older patients were more often on the right side of the brain.
- There was no statistical difference in Engel outcomes or complications between the older and younger patients.
- 60.9% of older patients and 60.2% of younger patients had Engel class I outcome.
- 91.3 % of older patients vs 93.6% of younger patients achieved meaningful (Engel class I-III).
- Complications were similar among both groups.

Conclusions:

- In this study there was no significant difference between Engel outcomes for elderly patients as compared to younger patients. Complication rates and type were similar in older and younger adults. Age above 50 should not be a barrier to epilepsy surgery.

Sally Mathias, MD [1] • Meriem Bensalem-Owen, MD [2] • Paige Sutton, MD [3]
Neurology University of Kentucky [1] • Neurology University of kentucky [2] • Neurology Duke University [3]

Efficacy, Safety and Need for Dosing Change of Zonisamide During Pregnancy in Women with Epilepsy

Faculty

Objective:

To determine the efficacy, fluctuations in anti-epileptic drugs (AED) level per trimester, tolerability and outcomes in women with epilepsy (WWE) treated with zonisamide (ZNS) during pregnancy.

Background:

Among the newer generation AEDs, the most commonly prescribed during pregnancy are lamotrigine and levetiracetam. There is insufficient data regarding ZNS. For routine use of ZNS during pregnancy, information about the efficacy, need for titration and safety for patient and fetus is necessary.

Design/Methods:

Retrospective chart review of pregnant women aged 18-45 years old seen at the University of Kentucky Epilepsy Clinic. WWE prescribed ZNS during pregnancy between 1/1/2013-12/30/20 were included. We reviewed seizure frequency before and during pregnancy, analyzed ZNS levels during pregnancy, investigated reported side effects and monitored for congenital malformations in children born to these subjects.

Results:

274 pregnant WWE were treated during this time period. 29 of these patients were treated with ZNS during pregnancy. 13 met the inclusion criteria. These included starting ZNS prior to conception or during the first trimester and having at least 3 serum ZNS levels checked during pregnancy. ZNS levels throughout pregnancy were analyzed using a concentration/dose (C/D) ratio by gestational age revealing a decrease in C/D ratio over the course of pregnancy. The rate of decline of the C/D ratio also slowed over the course of pregnancy indicating highest rates of decline in the beginning of gestation. Seizure frequency was evaluated prior to pregnancy and during pregnancy. 33.3% of subjects had a reduction in frequency of seizures during pregnancy while 66.7% had no change in seizure frequency. Reported side effects were investigated and separated by monotherapy and polytherapy. 1/6 (16.7%) on monotherapy reported dizziness and blurry vision while 2/7 (28.6%) on polytherapy reported nausea and fatigue. The majority of monotherapy (83.3%) and polytherapy (71.4%) subjects reported none. Fetal outcome was discoverable in 8 of 13 subjects. 5 were on polytherapy. 1 on polytherapy (20%) had congenital malformations while 4 (80%) had normal fetal outcomes. 3 on monotherapy had no congenital malformations.

Conclusions:

Although the sample size was small, this study showed some consistent results. Our findings indicate that ZNS levels decrease during pregnancy with the highest rate of decline in the beginning of pregnancy and may require dose adjustments. Only 1 subject with congenital malformations may be clouded by polytherapy. The remaining subjects on monotherapy had normal fetal outcomes. There were minimal side effects with ZNS during pregnancy. All subjects maintained the same level of seizure frequency or had improved seizure frequency during pregnancy. These results indicate that ZNS may be a safe, effective and well tolerated treatment for pregnant WWE. Further larger studies are needed to confirm our results.

Movement Disorders – Motor Control

Elizabeth Wallace, MS [1] • Jorge Quintero, PhD [2] • Craig van Horne, MD, PhD [3]
Psychology University of kentucky [1] • Neurosurgery University of Kentucky [2] • Neurosurgery University of kentucky [3]

Neurocognitive changes post-bilateral globus pallidus interna DBS for Parkinson's disease with autologous sural nerve graft to the substantia nigra or

Faculty

Objective: To determine cognitive safety of globus pallidus interna (GPi) deep brain stimulation (DBS) in Parkinson's disease (PD) patients receiving autologous sural nerve grafts to either the substantia nigra (SN) or nucleus basalis of Meynert (NBM)

Background: DBS is a standard of care treatment for PD resulting in improved motoric symptoms and quality of life. Relative to subthalamic nucleus (STN) DBS, GPi DBS typically results in better outcomes for cognitively comprised patients. Typical post-surgery changes include reduced verbal fluency, memory, and verbal processing speed. The current pilot study examined whether dopaminergic neurons can be regenerated via sural nerve graft implantation during GPi DBS. Current results assess the safety of this novel procedure and examine postoperative cognitive decline for two target sites: SN and NBM.

Methods: Pre- and post-surgical cognitive data were analyzed for 14 patients with bilateral GPi DBS plus unilateral SN graft and 7 patients with bilateral GPi DBS plus unilateral NBM graft. Notably, GPi plus SN evaluations were on average 22.31 months post-surgery (SD = 4.57) while GPi plus NBM evaluations were on average 12.4 months post-surgery (SD = 3.05). Paired sample t-test and Cohen's *d* effect sizes were calculated.

Results: GPi DBS plus SN patients showed moderate declines in phonemic fluency (FAS $t = 2.95$, $p < .05$; $d = 0.50$) and working memory (WAIS-IV Digit Span, $t = 3.46$, $p < .01$; $d = 0.76$) and small declines in verbally mediated processing speed (Stroop Color, $t = 2.24$, $p < .05$; $d = 0.41$). No significant changes were noted in GPi DBS plus NBM; only phonemic fluency neared significance (FAS $t = 2.295$; $p = 0.061$).

Conclusions: GPi plus SN showed worsened phonemic fluency, auditory working, and verbal processing speed consistent with STN DBS literature but somewhat larger than standard GPi DBS. GPi NBM patients did not evidence significant cognitive changes. Overall, results indicate initial safety evidence as sural nerve grafts do not likely add incremental cognitive change compared to standard DBS. NBM results show particular robustness and should be further examined for longitudinal change, as results suggest stabilization of cognitive abilities.

Implantation of a Nerve Graft to Treat Parkinson's Disease

Student

Parkinson’s disease is a neurodegenerative disorder that causes progressive symptoms including bradykinesia, tremor, gait and balance problems, and a variety of other non-motor symptoms. The dopaminergic neurons in the substantia nigra are believed to be the neurons effected in the progression of the disease. There is currently no cure for Parkinson’s disease, but there are various treatment options that have alleviated the severity of symptoms in some Parkinson’s patients. There are medicinal options that that use L-dopa, and then surgical procedures that are presented as options as the disease progresses if the patients were responsive to levodopa therapy. Our research is focused more specifically on the non-motor symptoms presented with Parkinson’s. Researchers have taken a nerve graft from the ankle of patients undergoing DBS and implanted them into different areas of the substantia nigra or the nucleus basalis of Meynert. Surveys such as the NMSS, UPDRS I and II, and PDQ8 were done at enrollment and then 12-month or 24-month post operation. So far, the data suggests that the patient’s symptoms are remaining stable in certain areas or even getting less severe. This is promising, as patient’s symptoms are not getting more severe.

Neuromuscular Disorders

Salvatore Cherra, PhD [1]
Neuroscience University of Kentucky [1]

Role of C. elegans RAPGEF in Synapse Development at the Neuromuscular Junction

Student

Guanine Exchange Factors (GEFs) are a family of proteins that activate GTPase signaling cascades. They accelerate the rate of GDP dissociation and subsequent GTP binding. The RAPGEF subfamily are associated with multiple neurological disorders such as schizophrenia and myoclonic epilepsy. In murine models, disruption of RAPGEF6 function resulted in reduced anxiety behaviors, increased long term potentiation, but no changes in gross brain morphology. Although these studies suggest that RAPGEFs modulate synaptic function, the exact mechanism is currently unknown. To determine how RAPGEFs regulate synapses, we used the *Caenorhabditis elegans* neuromuscular junction. PXF-1 is the only *C. elegans* RAPGEF6 orthologue. We hypothesized that PXF-1 contributes to neural circuit function by promoting synapse development at the neuromuscular junction. To determine how PXF-1 modulates neural circuit function, we used aldicarb, an acetylcholinesterase inhibitor, to measure changes in motor circuit activity. Treatment with aldicarb induces paralysis that directly correlates with levels of cholinergic activity. We found that two independent mutant alleles of *pxf-1* caused the animals to become resistant to aldicarb in comparison to wild type animals. To reveal the expression pattern of PXF-1, we used CRISPR/Cas9 genome editing to create a PXF-1::3xFLAG knock-in animal. In the nervous system, we detected PXF-1 in cell bodies of neurons and in processes near synapses. We then used fluorescently tagged synaptic proteins to investigate whether *pxf-1* mutants displayed alterations in synapse number or morphology. We detected no differences in the number of cholinergic synapses between wild type and *pxf-1* mutant animals, suggesting that PXF-1 does not regulate synapse formation. We then measured the intensity of synaptic vesicle markers in developing animals. We found that *pxf-1* mutant animals displayed a decrease in the fluorescence intensity of synaptic vesicles. Since the actin cytoskeleton is essential for synaptic vesicle clustering, we measure the levels of actin filaments using a fluorescent actin binding protein. We found that *pxf-1* mutants displayed a decrease in actin filaments at synaptic terminals. Based on our findings, PXF-1 may promote the development and function of neuromuscular junctions by modulating actin dynamics in motor neurons. Overall, our work provides a new insight into how RAPGEFs regulate nervous system function and how disruption of RAPGEF function may contribute to the development of certain neurological disorders.

Neuro Opthalmology

Single cell transcriptome analysis of periocular mesenchyme during anterior segment development

Fellow

Periocular mesenchyme (POM) is a subgroup of neural crest cells, important for forming the anterior segment (AS) of the eye. Despite their importance for eye development, our knowledge of the molecular background of these cells is limited. The purpose of this study is to characterize the transcriptomic regulation of POM cells as they form the zebrafish AS. We employed scRNA analysis over the course of zebrafish AS development. Larval eyes of transgenic zebrafish Tg(*Foxc1b:GFP*) and Tg(*Lmx1b*:GFP) were collected every 24 hours between 48hpf and 144hpf. GFP+ cells were isolated and processed with the 10x genomics chromium single cell transcriptome kit. The resulting Illumina sequencing single cell transcriptomes were processed with the Cell Ranger pipeline. Analysis was done with the Cell Loupe Browser 5.1 and Monocle3. We collected over 40,000 Foxc1b+ and Lmx1b+ cells, including one biological replicate for each time point. Clustering analyses showed the cells were organized in re-occurring clusters, representing specific tissues within the developing AS. Pseudotime analyses revealed gene regulatory progression during early development. We identified several genes, previously not associated with the AS, with specific AS expression patterns and importance for eye development, as proven by genetic knockout. These genes include *hgd*, *si:ch211-251b21.1*, *slc22a7a* and s*tmn1a*. The genes *hgd* and *si:ch211-251b21.1* were chosen for further characterization by means of drug induced manipulation. Specifically, inhibition of the former caused severe phenotypes, suggesting an important role during AS formation. Our data reveal not only previously unknown genetic markers, but also give a first insight into potential genetic interactions necessary for AS development.

Meet Patel [1] • Jakub Famulski, PhD [1]
Biology University of Kentucky [1]

Examining the absence of a Cone Rod Dystrophy candidate gene, CDHR1 – a Photoreceptor specific cadherin in larval zebrafish

Student

Inherited retinal blindness affects millions of people worldwide. Cone rod dystrophy is a type of retinal disorder caused by the degeneration of photoreceptor cells (PRCs) in the retina. Over 30 genes have been found to be associated with cone-rod dystrophy. CDHR1, a photoreceptor-specific cadherin has been found to be associated with the incidence of cone-rod dystrophy. Functionally, Cdhr1 has been shown to localize at the junction of the Inner segment and the Outer segment (OS) of Photoreceptors (PRCs). Although studies on mice and work on zebrafish from our lab have shown effects of Cdhr1a (a homolog of CDHR1 in zebrafish) on PRCs, the fundamental role of CDHR1 in development and its function in photoreceptors and retinal progenitor cells remains unknown. As such my primary goal intends to uncover the role of Cdhr1a during early photoreceptor development in larval zebrafish. Using Whole Mount In Situ Hybridization (WISH), wildtype spatial-temporal patterning of Cdhr1a was analyzed. The expression pattern of Cdhr1a initiates around 62hpf (hours post fertilization), which is preceded by the emergence of the first few nascent outer segment discs at around 60hpf. Furthermore, expression of the Prph2a (Peripherin-2) gene which is involved in the assembly of nascent OS discs begins at the same time as that of Cdhr1a. This timepoint could be crucial as based on the localization of Cdhr1a, its absence may play a crucial role on Prph2a and likely affect the OS disc assembly. Affected OS assembly could eventually dismantle the Phototransduction process of the OS discs and induce apoptosis in PRCs. To test this hypothesis, an Alt R CRISPR zebrafish resulting in a cdhr1-null line has been established and mutant embryos have been collected. Including Prph2a, I also decided to test the effects of Cdhr1a on various metabolically and developmentally important genes of PRCs such as Crx and Nr2e3, and thus continuing work aims at using WISH, Immunohistochemistry, and various histological stains to examine OS discs maintenance/ formation in the absence of Cdhr1a function.

Jakub Famulski, PhD [1]
Biology University of Kentucky [1]

Expression and involvement of fmoda, lum, adcyap1b and tgfbi in the development of the anterior segment and migration of the periocular mesenchyme.

Student

The formation of the eye begins early in embryonic development when the neural ectoderm begins to invaginate forming a spade shaped structure. This structure consists of the early stages of the forebrain and two optic vesicles. During the invagination of the optic vesicles, neural crest (NC) cells migrate around the optic vesicles and the surface ectoderm. A specific subset of multipotent NC cells migrates to the areas around the optic vesicles and aids in the development and formation of the eye are called periocular mesenchyme (POM) cells. As the optic vesicles continue to invaginate they will eventually encounter the surface ectoderm. Once in contact with the surface ectoderm, the optic vesicles will develop into the retina and related structures, while the surface ectoderm and POM cells will continue to differentiate and develop into the anterior segment (AS) of the eye. The AS of the eye is made up of the structures of the eye that are visible without special tools, such as the iris and pupil, as well as the cornea, lens, aqueous humor, and ciliary zonules and body. Several genes, such as pax2, sox10, foxc1 and pitx2 are known to be involved in the development of the AS, but there is not much known about the genes that are involved in the regulation and differentiation of POM cells from NC cells. Through scRNA clustering, several genes were identified as potential novel markers involved in the development and/or function of the POM. Of these genes, the most interesting are lum, fmoda, adcyap1b, and tgfbi as they all showed strong expression in the developing AS at 48 hours post fertilization (hpf) and 72 hpf.

In wild type zebrafish embryos, we have observed that fmoda and lum are expressed in the head and eye region with the expression increasing around the iris as the embryo continues to develop. Tgfbi and adcyap1b are both expressed in the head and eye regions during the early stages of development but as the embryos continue developing, the expression levels drop to just the head. This suggests that fmoda and lum are involved during the earlier and later stages of development while tgfbi and adcyap1b are utilized during the earlier stages of development but not the later stages. To determine if these four genes are indeed required for AS development and POM migration ¬the expression of the four genes are examined in Tfap2a and FoxD3 Δ537 mutant lines. Tfap2a and FoxD3 are NC cell specifiers and involved in the development of NC cells. By observing the expression of lum, fmoda, adcyap1b, and tgfbi in homozygous mutant Tfap2a and FoxD3 Δ537 embryos, conclusions about the involvement of lum, fmoda, adcyap1b, and tgfbi in POM migration and AS development will be determined. By examining the expression of these four genes in Tfap2a and FoxD3 Δ537 mutant embryos, the origin of the genes involved in the development of the AS can be determined to be from the NC or the ectoderm.

Neurophysiology

The effect of calcium ions on mechanosensation and neuronal activity in proprioceptive neurons

Student

Proprioception in all animals is important for coordinated locomotion. Stretch activated channels (SACs) are responsible for transducing mechanical forces into electrical signals at sensory endings. Among sensory neurons in animals, the types of SACs vary and are defined by their pharmacological, physiological and molecular identity. Chordotonal organs within insects and crustaceans offer a unique ability to investigate proprioceptive function because the sensory nerves are easily accessible and viable in minimal saline. This allows for ease in experimentation to determine the effects of the extracellular environment on neuronal activity as well as on the function of associated SACs. By using the chordotonal organ in crab limbs as a model, the impact of extracellular [Ca^{2+}] on membrane properties can be readily addressed. Physiologically, Ca^{2+}–induced changes in membrane properties influence the voltage-sensitivity of ion channels and action potential threshold and refractory periods. Results showed that low Ca^{2+} increased both basal and joint movement-associated activity, suggesting these SACs may be Na^{+} channels rather than Ca^{2+} channels. Ba^{2+} substitution for Ca^{2+} allowed the activity to be maintained and slightly increased nerve activity. Mn^{2+} exposure depressed joint movement activity suggesting Mn^{2+} blocked the SACs. It is proposed that axonal excitability may be independently affected from the SAC activity due to the presence of calcium activated potassium channels ($K_{(Ca)}$) and the ability of Ca^{2+} to block voltage gated Na^{+} channels in the axons. Separating the role of Ca^{2+} on the function of the SACs and the excitability of the axons in chordotonal organ nerves is addressed. These experiments may aid in understanding the mechanisms of neuronal hyperexcitability during hypocalcemia within mammals.

The effect of optogenetically activating glia on neuronal function

Student

Glia, or glial cells, are considered a vital component of the nervous system, serving as an electrical insulator and a protective barrier from the interstitial (extracellular) media. Certain glial cells (i.e., astrocytes, microglia, and oligodendrocytes) within the CNS have been shown to directly affect neural functions, but these properties are challenging to study due to the difficulty involved with selectively-activating particular glia. To overcome this hurdle, we selectively expressed light-sensitive ion channels (i.e., channel rhodopsin, ChR2-XXL) in glia of larvae and adult *Drosophila melanogaster.* Upon activation of ChR2, both adults and larvae showed a rapid contracture of body wall muscles with the animal remaining in contracture even after the light was turned off. During ChR2-XXL activation, electrophysiological recordings of evoked excitatory junction potentials within body wall muscles of the larvae confirmed a train of motor nerve activity. Additionally, when segmental nerves were transected from the CNS and exposed to light, there were no noted differences in quantal or evoked responses. This suggests that there is not enough expression of ChR2-XXL to influence the segmental axons to detect in our paradigm. Activation of the glia within the CNS is sufficient to excite the motor neurons. We are also examining behaviors with evoked neural circuits while depolarizing and hyperpolarizing glia through light-sensitive channels. There is hope in the future to be able to implement such glia stimulation in the intact mammalian CNS to modulate disorders related to glial dysfunction.

The Effect of TEA, 4-AP and in Combination on Primary Sensory Neurons in a Crustacean Model

Student

The channels for transmitting electrical activity along neurons is similar from squid to humans with sodium currents accounting for the upstroke of an action potential and potassium channels for the rapid and delayed ionic flux for the repolarization. Pharmacological agents tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) block different subsets of voltage gated potassium (K+) channels. The chordotonal organs in crab limbs are a model of proprioceptive sensation and have rapidly- and slowly-adapting sensory neurons. Since 4-AP is used clinically for amyotrophic lateral sclerosis (ALS) and Multiple sclerosis (MS) treatments, a better understanding of its action on proprioceptive models can aid in understanding the potential effects in mammalian systems. To assess the action of these blockers on the function of proprioceptive sensory neurons, the neurons were evoked by movements associated with the joint while applying these compounds individually as well as in combination. The evoked compound action potentials in isolated nerves as well as with intact to the sensory endings can be examined. Both 4-AP and TEA decreased activity individually, as well as when combined. Potassium channels are sensitive to both blockers in this marine crustacean model. It appears the action is on electrical induction and conduction within the axons. This crab crustacean proprioceptive model can be used for future intensive investigations, building on the results presented, in the pharmacology of the mechanosensitive channels and neuronal activity.

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Mice Expressing Human APOE4 Genotype Exhibit Greater Baseline Respiratory Rate and Less Robust Hypoxic Ventilatory Response Than APOE3 Mice as Measure

Student

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Breathing is vital for life and is both uniquely complex and vulnerable to disruption. Disorders of breathing such as those that occur in sleep apnea and even high cervical spinal cord injury (SCI) can impair quality of life and potentiate the ill effects of comorbidities like Alzheimer's disease (AD). Indeed, studies have shown greater cognitive and cardiovascular impairments in subjects with sleep apnea and AD. Apolipoprotein E (ApoE) is a ubiquitous protein found throughout the body and CNS which aids in the regulation and transport of cholesterol and lipids. Moreover, the *E4* allele confers a higher risk of early onset AD than either the *E2* or *E3* alleles. Previous research in our lab has shown the interaction between ApoE genotype and sex differentially affects breathing motor plasticity following SCI in mice. However, it is unknown how baseline respiratory pattern and capability during hypoxic challenge may differ between uninjured individuals expressing *APOE4* and *E3* alleles. Thus, with *APOE* genotype as our independent variable, we measured the respiratory parameters of male *E3* and *E4* targeted replacement mice using whole body plethysmography (WBP), hypothesizing that *E3* animals would display a more adaptive, heightened response to hypoxic exposure than those expressing *E4*. Preliminary data comparing *E3* animals (n=9) and *E4* animals (n=8) demonstrated differences across several key measurements. At baseline, *E4* animals were shown to breathe in an energy inefficient manner as exemplified high measures in tidal volume and frequency. During sustained hypoxia, frequency, tidal volume, and minute volume initially increased across all animals, but *APOE3* animals mounted a much more robust and sustained response with *APOE4* values falling well below baseline measures prior to termination of hypoxia. These preliminary results suggest a critical role of the *APOE* genotype in regulation of baseline breathing and in response to hypoxic conditions and that possessing the *APOE4* allele may confer vulnerabilities in respiratory function and during environmental challenges leading to cognitive decline. Future directions include examining anatomically critical CNS respiratory regions and neural cell function.

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Department of Pharmacology and Nutritional Sciences University of kentucky [1] • Department of Neurology University of Kentucky [2] • Department of Pharmacology and Nutritional Sciences University of Kentucky [3]

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

Faculty

Amylin is a **β**-cell hormone that forms pancreatic amyloid. Individuals with prediabetes, type-2 diabetes and obesity have aggregated amylin in pancreatic, brain, heart and renal microvessels. Aggregated amylin attaches to RBCs and capillary endothelium, which induces hypoxia and microcirculatory disturbances. Using human amylin overexpressing (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, showed amylin deposition in skin and brain capillaries of HIP rats. Capillaries from 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**α** 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.

Neurorehabilitation

Chase Haddix, Other [1] • Elizabeth Powell, MS [2] • Lumy Sawaki, MD, PhD [2] • Sridhar Sunderam, PhD [3]
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Discrimination of Different Levels of Finger Extension from the EEG in Hemiparetic Stroke

Student

Assistive technologies such as brain-computer interfaces (BCIs) offer individuals with neural injury the means to interact with external devices by monitoring and interpreting their mental commands using electroencephalography (EEG). However, the ability to extract EEG features associated with fine motor control is limited, especially in stroke victims who may have large cortical lesions. Here we test the feasibility of predicting graded motor effort associated with finger extension from the EEG.

We conducted an IRB-approved study on three stroke patients with left hand paresis and three age-matched controls. Subjects were prompted by a visual display to extend their fingers outward from rest every few seconds to one of four levels—Low, Medium, High, or "No-Go"—and then return to rest. This task was performed for six runs of 16 cues each, switching hands after every run. Hand movement and muscle activity (extensor digitorum communis) were monitored using a motion capture glove and bipolar EMG, respectively. The glove recorded each phalange's position in Cartesian space, to track extension of each digit relative to the wrist. The angle of extension in response to each cue was thus measured and averaged over index, middle, and ring fingers. EEG was simultaneously recorded over 32 locations and the mean-squared power in a moving window estimated in the 8–30 Hz band during each cue relative to a reference period preceding the cue. A quadratic classifier was trained on samples of this feature vector and the accuracy of prediction of test samples assessed using four-fold cross-validation.

For controls, the mean accuracy of the EEG classifier was 55-60% on each hand over the four levels of finger extension, much greater than the chance level of 25%; confusion between low and medium extension was the major source of error. Accuracy was strikingly similar—about 60%—on either hand for the stroke subjects, despite there being no measurable extension with the motion capture glove on the impaired hand. An EMG classifier predicted graded finger extension with only 40% accuracy on the stroke-impaired hand but 80% in the unimpaired hand and in controls.

Our findings show that the EEG can predict gradations in finger extension related to both actual and intended movement in individuals with hemiparetic stroke. This offers BCIs a measure of fine control for interactive rehabilitation protocols. Future work will focus on closing the loop using these distinguishable signals to provide real-time feedback, which can be incorporated into existing regimens to promote functional recovery.

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Disclaimer: This work was performed while Lumy Sawaki was employed at University of Kentucky. The opinions expressed in this article are the author's own and do not reflect the view of the National Institutes of Health, the Department of Health and Human Services, or the United States government.

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Neuroscience University of Kentucky [1] • Neurosurgery and Pediatric Surgery University of Texas Health Science Center at Houston [2]

Intermittent Hypoxia, Sustained Hypoxia, and Normoxia Treatments Induce Differential HIF-1α and VEGF expression in the CNS of Uninjured Adult Rats

Student

Spinal cord injuries (SCIs) most commonly occur at the cervical level and often render injured individuals unable to breathe. Mechanical ventilation can preserve life despite respiratory motor paresis, but detrimentally affects quality of life and can lead to long-term complications including lung damage and respiratory muscular atrophy. Promisingly, moderate intermittent hypoxia (mIH) treatment can be used to induce increased respiratory output known as long term facilitation (LTF) after SCI, but is currently unlikely to accomplish complete recovery of breathing once lost. Seemingly subtle modifications of hypoxia treatment paradigm activate distinct cellular signaling pathways and thus respiratory motor response, even as originally demonstrated in uninjured laboratory animals. A better understanding of these signaling pathways will likely contribute to development of hypoxic dosing patterns more adaptive for achievement of full respiratory motor recovery after SCI. One key pathway involves hypoxia inducible factor 1**α** (HIF-1**α**) and vascular endothelial growth factor (VEGF) activity. HIF-1**α** is a transcription factor induced by cellular hypoxia and increases in neurons after mIH treatment while VEGF is downstream and can induce LTF-like plasticity by exogenous application to the spinal cord both alone and combined with mIH treatment. However, it is unknown how endogenous HIF-1**α** and VEGF levels compare between animals treated with mIH and moderate sustained hypoxia (mSH). Our prediction is that a moderate level of HIF-1**α** and VEGF expression is adaptive for LTF and that both the low and high protein levels likely induced by normoxia and mSH treatments will be outside the ideal range induced by mIH. Thus, with hypoxia treatment type as our independent variable, we hypothesize that mSH will induce the most HIF1**α** and VEGF expression in the spinal cord and brainstem, followed by mIH and normoxia, in descending order. We treated uninjured adult Sprague-Dawley female rats with 5 consecutive days of either normoxia, mIH, or mSH (n=3 per treatment group) and harvested fresh whole central nervous system tissue (CNS) immediately following final treatment, measuring HIF-1**α** and VEGF via MSD and ELISA assays, respectively. Interestingly, results demonstrated that mSH induced the least expression of HIF-1**α** across CNS regions while paradoxically inducing the greatest VEGF expression of the three treatments. To understand this, future experiments should explore whether VEGF exerts feedback inhibition on HIF-1**α** expression following mSH by use of pharmacological inhibition or genetic knockout of VEGF prior to treatment with mSH and measurement of HIF-1**α**.

Neurotherapeutics

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Exploring cell signaling pathways engaged by two novel small molecule drug candidates that selectively modulate stressor-induced neuroinflammation

Student

Neuroinflammation plays an important role in many different brain pathologies and injuries and therefore may represent a beneficial therapeutic target across neurological conditions. Two brain-penetrant, anti-inflammatory small molecule drug candidates, MW151 and MW189, are in or have completed Phase 1 clinical trials for safety (clinicaltrials.gov ID NCT04120233 and NCT02942771, respectively). MW151 and MW189 are structural analogs, and both ameliorate neuroinflammatory effects across a broad range of preclinical animal models, such as reducing glial activation and pro-inflammatory cytokine production. However, their molecular target(s) are not defined. To maximize the likelihood of eventual clinical success for one or both drugs (or similar compounds), we have started exploring potential target pathways *in vitro*. For these studies, we are utilizing the BV-2 mouse microglial-type cell line. It is already known that these drugs do not inhibit the p38 MAPK pathway, so other major inflammatory pathways such as NFkB, STAT3, JNK, and ERK are currently being explored. We found that MW151 and MW189 inhibit lipopolysaccharide (LPS)-induced iNOS stimulation and LPS-induced cytokine secretion, which may occur in part via inhibition of NFkB signaling. Both drugs also block extended IL-6 induced STAT3 phosphorylation (1 to 4 hours post-stimulation), with no effect on the early phase (30 minutes or less). While these findings offer intriguing clues, an unbiased proteomic exploration of BV-2 cells exposed to MW151 and MW189 is being undertaken to more clearly identify the molecular mechanism(s) and target pathway(s) of these drugs. Successful identification of the signaling pathways and proteins modulated by MW151 and MW189 *in vitro* will provide the biological rationale for design of future clinical trials, and possibly open new avenues of exploration in future drug development programs.

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Progesterone Pretreatment Decrease Acute Stress Effect on Cognition and Impacts Downstream Expression

Fellow

Behavioral stress is prevalent, sexually dimorphic, and has negative health consequences associated with action in multiple tissues, including the brain. Glucocorticoids are key stress-signaling hormones with enriched hippocampal receptor expression, and stress-driven expression of immediate early genes such as serum-and-glucocorticoid kinase 1 (Sgk1) is considered indicative of glucocorticoid receptor-based central action. While glucocorticoids have anti-inflammatory actions, stress exacerbates neuroinflammation, possibly through myelin fragmentation and resulting stimulation of microglia phagocytosis. Previous work has shown that progesterone may ameliorate stress effects, but whether that effect is exerted at the HPA-axis, on downstream targets, or both, remains unclear. To address this knowledge gap, we hypothesize that progesterone pretreatment would reduce acute stress response. Eighty-eight intact adult Sprague-Dawley rats (50 males / 38 females) were trained in the Morris Water Maze. The male and female rats were placed into one of four groups (n = 9-13): 1) control + vehicle; 2) control + progesterone; 3) stressed + vehicle; 4) stressed + progesterone. Oral progesterone-pretreatment (10 mg/ kg) was administered daily for 3 days after each Morris water maze training session. On day 4, a 3-hour restraint was applied immediately prior to the probe trial, and blood and brain were collected within fifteen minutes of probe trial completion. In both sexes, progesterone pretreatment alleviated stress-induced behavioral deficits but did not alter stress-induced corticosterone levels. In males, progesterone also attenuated stress-induced hippocampal Sgk1 mRNA increases, while progesterone, but not stress, increased Iba1 expression in the stratum oriens and Iba1/ Mbp overlap in the alveus. Females showed multiple baseline-level differences compared to males, including increased: maze training path length, blood corticosterone, pyramidal layer Iba1, and reduced Sgk1 mRNA in the hippocampus. Unlike males, female Sgk1 mRNA was unaffected by stress or progesterone, and Iba1 levels in stratum oriens (and Iba1/Mbp overlap in the stratum oriens and pyramidal layer) were increased by both progesterone and stress, but in the stress condition progesterone blunted Iba1 increases. Overall, although results do not support a myelin-fragment driven effect, results do support sexual dimorphism of stress responses and indicate that progesterone pretreatment blunts stress effect through actions downstream of the HPA-axis activation.

Neurotrauma

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Meningeal Lymphatic Deregulation Following Traumatic Brain Injury

Student

The meninges are made up of three membranes; the pia mater, arachnoid mater, and dura mater, that surround the brain and spinal cord. These specialized layers protect the central nervous system by adding a layer of cushion to the brain along with cerebrospinal fluid (CSF), removing waste products from the brain and acting as a physiological barrier between the central nervous system and the periphery. The meningeal lymphatic system is a specialized group of vessels that lie within the meninges that assist in the flow of fluid and waste products from the brain. If the meningeal lymphatic system malfunctions, issues arise such as toxins not being removed from the brain, for example amyloid beta. Interleukin-1 (IL1) is a cytokine that initiates a cascade of inflammatory pathways through the interleukin-1 receptor (IL1R1). If IL1R1 is localized within the meninges, it may play a role in secondary TBI cascades of hemorrhages, as well as in the signaling of myeloid cells from the dura mater to the brain. The goal of this study is to establish a meningeal extraction protocol and to establish whether and where IL1R1 resides in the meninges. To do so, the skullcaps were extracted from the mice following transcardial perfusion. The mice used for this study were IL1R1 reporter mice. This line has been genetically altered to express red fluorescent protein (RFP) in any cell that also expresses IL-1R1, allowing us to visualize the expression pattern in the brain. The leptomeninges were pulled from the skull cap and stained using immunohistochemistry for glial fibrillary protein (GFP). In conclusion, the GFAP staining did reveal astrocytes within the confluence of sinus. While it is currently unclear why astrocytes localized to the confluence of sinuses, further work will be done to investigate these cells in the meninges. Staining for RFP, which shows what cell types of IL1R1 is expressed in revealed that there is IL-1R1 expression within the meninges. Next steps include determining what cell type within the meninges are expressing IL1R1.

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High-Low training to induce long-term motor and respiratory plasticity after SCI

Student

"Living high, training low" is a concept already used by professional athletes. This "High-Low" approach (H-L) refers to engaging in normal lifestyle activities such as sleeping at lower oxygen levels, mimicking living at a high altitude, accompanied by performing regular exercise routines at oxygen levels resembling sea-level altitude. Hypoxia treatment is one way to model high altitude conditions and it has been shown to be safe in humans at various severities and lengths of time. Voluntary exercise is also a safe and effective way to improve health outcomes in a variety of measures. For stroke patients, higher levels of pre-stroke physical activity are protective and lead to milder deficits (Krarup et. al 2008). A pilot study in humans showed that Alzheimer's patients who received H-L training had an upregulation of beneficial growth factors in their serum and CSF (Palmer et. al, 2010). With this in mind, we aim to apply H-L in a model of chronic spinal cord injury (SCI). In rodent models, increasing growth factors supports plasticity and functional recovery after an SCI (Mantilla et. al, 2013), and repetitive intermittent hypoxia has been shown to increase growth factor expression in both respiratory and non-respiratory motor neuron pools (Satriomoto et. al, 2016). In a previous study using rats with thoracic SCI, the group with unlimited wheel access showed greater improvement than the group with limited wheel access, suggesting a dose-dependent manner (Engesser-Cesar et. al, 2007). However, it is important to note that forced overuse or initiating exercise too early can exacerbate functional deficits after SCI (Krajacic et. al, 2009), and SCI patients often present with polytrauma that can limit early initiation or aggressive implementation of therapies. With all this in mind, we designed a study to investigate H-L training in a rodent model of chronic cervical SCI. The majority of SCI occurs at the cervical level, which often causes respiratory impairment and various degrees of tetra- or paraplegia, and the vast majority of human SCI patients are in the chronic phase. H-L training has been shown to be safe and effective in an elderly human population, so H-L therapy has the potential to be highly translatable to the human SCI population. We will use phases of moderate repetitive hypoxia exposure coupled with phases of unlimited wheel access. Since the rodent sleep/wake cycle is opposite of humans, the hypoxia will be administered for 4 hours during the day and then the animals will have unlimited access to monitored exercise wheels overnight. We believe the regular hypoxic exposures will have a synergistic effect with the benefits of exercise, promoting long-lasting improvements in motor and respiratory function. We plan to measure growth factor expression in the serum, anxiety, and motor function at several timepoints throughout the long-term experiment, and will also measure sensory function and respiratory function at the endpoint.

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High-Low training to induce long-term motor and respiratory plasticity after SCI

Student

Background: The technique of "living High, training Low" (H-L) is employed by athletes to improve exercise performance. It involves repeatedly performing typical lifestyle activities while mimicking high altitude conditions (i.e, hypoxia), coupled with an exercise regimen performed at sea-level conditions. Exercise can be protective before and after CNS injury, with higher pre-stroke physical activity correlating with lower stroke severity and better long-term outcome following first stroke (Krarup, 2008). Implementing an exercise regimen after spinal cord injury (SCI) can improve recovery of locomotion (Engesser-Cesar, 2007). Application of hypoxia promotes functional recovery in respiratory and non-respiratory motor neurons (Satriotomo, 2016) and induces respiratory plasticity following SCI (Lovett-Barr, 2012). The goal of this project is to investigate potentially synergistic effects of combining intervals of hypoxic exposure with voluntary exercise in a rat model of SCI.

Methods: In this preliminary investigation, 14 F Sprague-Dawley rats underwent baseline assessment 2 weeks prior to cervical SCI. They were separated into 2 cohorts of n=6 and n=8 based on age difference of 2 weeks. The 2 cohorts received a left C2 Hemisection at age=4mo. 9 survived past acute phase of injury and began assessments 5 weeks post-injury (wpi), with assays repeated every 4 weeks onward until the terminal EMG at 14wpi. Rat pairs were randomly assigned by cage to treatment of exercise/hypoxia or sedentary/normoxia. At 6-7wpi, n=6 rats assigned to exercise/hypoxia group began H-L training. Briefly, H-L involves placing rats into individual exercise cages with voluntary access to monitored running wheels overnight, and administering 11% O2 gas treatment for 4 hours in the afternoon, both for 5 days a week. The sedentary/normoxia group (n=3) serves as a control. They are housed individually in standard cages overnight and receive 21% oxygen gas treatment concurrent with hypoxia treatment for H-L rats. The assays performed at baseline, 5wpi, 9wpi and 13wpi are the Activity Box/Open Field for anxiety and rearing behavior, the Catwalk for locomotor activity, whole body plethsymography for respiratory measures, and blood draws for protein levels from isolated serum. At the 13wpi timepoint we will also perform the Hargreaves for thermal allodynia and the Locomotor Forelimb Scale for detailed assessment of motor deficits. At 14wpi, we will perform a terminal EMG to measure respiratory motor output. The spleen will be collected fresh in order to isolate and bank splenocytes for later analysis, while CNS tissue will be perfused with PFA to perform histology and immunohistochemistry.

Results: The first series of H-L training is in progress. After 7-14 days of treatment H-L seems well-tolerated in rats. Results expected Dec 2021. A second series is planned for Jan 2022.

Conclusions: If effective in the rodent model, H-L training could be highly translational to SCI patients.

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Considering Sex as A Biological Variable in Spinal Cord Injury

Student

Complement has been found to play a significant role in the inflammatory response after injury. This protein has the ability to fight infection by initiating a membrane attack complex, inducing phagocytosis, and attracting other inflammatory cells such as macrophages and neutrophils. It has recently been found that males produce more C1qa, a complement component, in monocyte-derived macrophages (MDM). Therefore, we wanted to further explore this by determining if this was specific to the monocyte-derived macrophages or if this finding was consistent throughout tissue post spinal cord injury. In this, we also looked at various components of the complement cascade to assess their role in the inflammation process to add additional evidence regarding injury effects and sex-specific effects. We found that there is only an injury effect in the increase of C1q, C4 and C5 proteins, and confirmed that there is a cell-type-specific increase of C1q in males.

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Time Course of Mitochondrial Bioenergetic Impairment in Mice After Severe Controlled Cortical Impact: Is Sex a Driving Force?

Student

Due to the growing number of women joining active military duty as well as women suffering from intimate partner violence, the need for female representation in traumatic brain injury (TBI) research has never been higher. Mitochondrial dysfunction plays a key role after TBI since it is the main energy source of the cell, as well as a cell death modulator. This dysfunction after injury has been well characterized in male animals, but has yet to be fully studied in female models resulting in a severe knowledge gap. The present study assessed mitochondrial bioenergetics in synaptic and non-synaptic fractions isolated from the cortical injury epicenter and ipsilateral hippocampus at various time points after severe controlled cortical impact (CCI; 1.0mm depth deformation) in both male and female mice *to test the hypothesis that female mitochondria are less impaired after CCI and have an altered time course of impairment from male mitochondria.* The goals of these experiments were to (1.) characterize the time course of mitochondrial dysfunction after CCI, (2.) determine the time of maximum bioenergetic impairment after CCI, and (3.) identify a time frame of impairment in which mitochondrial-based therapeutics can be administered to improve bioenergetic outcomes in female mice. Synaptic and non-synaptic mitochondrial fractions were isolated utilizing a novel and highly sensitive technique called fractionated mitochondrial magnetic separation (FMMS) 3, 12, 24, and 48-hours post-CCI or sham surgery; mitochondrial bioenergetic measurements were evaluated immediately after isolation. The results revealed male and female mitochondria had similar respiratory capacity in sham and CCI groups across all time points, tissues, and mitochondrial fractions. The time course showed synaptic mitochondrial bioenergetic impairment began at 12-hours, peaked at 24-hours, and persisted up to 48-hours post-CCI in the injured female mice compared to female sham. Together, these results indicate male and female mouse mitochondria have similar respiratory capacity and time course of dysfunction after severe CCI.

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Cervical spinal cord injury induces distinct changes in gut microbiome composition over time in rats

Student

Nearly 60% of all spinal cord injuries (SCI) occur at the cervical level. These high-level injuries can lead to quadriplegia, autonomic dysregulation, and interruption of the descending respiratory pathways required for breathing. As respiratory motor function is required for life, it is critical to restore breathing as soon as possible after cervical SCI. Indeed, therapeutic techniques in animal studies have been successful at restoring breathing function after SCI; however, these interventions appear to be more effective at chronic than acute timepoints post-injury. One potential cause for this observation is the impact the injury has on the gastrointestinal (GI) tract and gut microbiome, which have previously been shown to have detrimental effects on recovery outcomes, including lower limb motor function and emotional affect as indicated by increased anxiety-like behavior. However, the impact the gut microbiome has on the recovering spinal cord at different timepoints, levels, and injury severities still needs to be fully realized. We aimed to build upon these previous findings and investigate the impact of cervical SCI on the gut microbiome over time and up to chronic timepoints. We hypothesized that cervical SCI leads to transient changes in the gut microbiome, which are most severe acutely after injury impeding functional recovery of breathing, but resolve over time and thus eventually allow for more profound recovery. To test our hypotheses, we performed left C2 hemisections on adult female rats, collected fecal samples from injured, sham, and naïve animals, and assessed microbiome composition at various timepoints pre- and post-injury. Preliminary results suggest that following cervical SCI (up to 12 weeks post injury), robust differences in gut microbiome are apparent compared to non-injured animals. Future studies will classify bacterial identities and assess the impact of the post-injury gut microbiome on respiratory motor function and plasticity, as well as inter-institutional differences.

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Dysregulation of inflammatory pathways in miR-223 knockout mouse brain following traumatic injury

Staff

Traumatic brain injury (TBI) elicits a cascade of secondary physiological and biochemical events that contribute to further injury. Neuroinflammation is recognized as a critical secondary event following TBI. While the acute inflammatory response is essential for removal of cellular debris and promoting cellular repair, dysregulation of this response leads to persist pro-inflammatory signaling and chronic inflammation impacting long-term neurological function. MicroRNAs (miRNAs) are a class of regulatory non-coding RNAs that play a key role in mediating gene regulation post-transcriptionally in almost all biochemical processes including inflammation. We previously showed that several inflammatory responsive miRNAs including miR-223-3p are dysregulated following TBI. Here we provide further evidence showing that miR-223-3p plays a key role in mediating several proteins that are key modulators of inflammatory signaling. Specifically, miR-223-3p directly targets upstream modulators and transcription factors associated with NF-κB and NLRP3 inflammasome. Deficiency of miR-223-3p resulted in elevated expression levels of several of these pro-inflammatory markers in the uninjured brain. These data suggest that miR-223-3p is a key regulator involving several inflammatory pathways, and modulation of miR-223-3p may serve as a novel strategy in limiting excessive neuroinflammation following TBI.

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Effects of Acute Ethanol Intoxication on Spinal Cord Injury Outcomes

Student

One in five individuals with a traumatic spinal cord injury (SCI) have a blood alcohol content above the legal limit at the time of their injury. Individuals with an elevated blood alcohol content at the time of admission are at an increased risk for having a longer hospital stay and developing pneumonia, urinary tract infections, and skin ulcers. All of which are associated with poor functional recovery. Preclinical studies which aimed to simulate this clinical scenario in rodent and feline models of SCI have suggested that acute intoxication leads to worse recovery and more severe hemorrhage into the spinal cord parenchyma. However, these experiments were not designed to understand the underlying mechanism of either of these observations. Through the combination of our established mouse models of acute ethanol intoxication and experimental SCI we aim to understand the impact of a high blood ethanol content on locomotor recovery after injury. We employed several behavioral assessments to asses locomotor recovery in order to tease out any differences in outcome. We found that the acute intoxication did no significantly alter locomotor recovery. However, In the future we plan to investigate the effect of both acute and chronic alcohol exposure on locomotor recovery, hemorrhage, as well as its impact on lesion area after SCI.

Other

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Using an injury-paradigm to upregulate key transcripts and proteins to enhance the repair mechanisms of a human investigational cell therapy

Faculty

We recently showed that the human gene expression profile in peripheral nerve is dramatically changed following a transection injury to the sural nerve (Welleford et al. 2020). As part of a clinical trial, we have been deploying regenerative autologous peripheral nerve graft tissue (termed APNG) to the substantia nigra in patients with Parkinson's disease (PD) to provide trophic support to sick and dying cells affected in PD. We performed a new, cellular level analysis of transcription along with a proteomics analyses of APNG to identify key transcripts and proteins. Participants with PD provided written consent to collection and analysis of tissue samples. As part of a clinical trial, the sural nerve was identified, transected, and closed. Two weeks later, the incision was reopened to identify the regenerating nerve and about 1 cm of the proximal end of the distal stump of the nerve was excised. Individual fascicles were isolated and samples were snap frozen. Samples were quantified using single nuclei RNA-Seq (Singulomics, NY) and a high throughput multiplexing suspension array system (Luminex, TX USA). Not all samples yielded results for individual proteins. Neurotrophic factors including brain derived neurotrophic factor (BDNF) (mean ± SD, 8.0 ± 7.7 pg/ml), glial-cell line derived factor (GDNF, 53.0 ± 36.1 pg/ml) and nerve growth factor (NGF, 42.5 ± 51.3 pg/ml) were present along with the putative anti-inflammatory, nuclear factor erythroid 2-related factor 2 (NRF2: 61.2 ± 22.0 pg/ml). Presence of growth and cell survival factors in APNG may provide a means of supporting sick cells in PD and other neurodegenerative disorders.

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Acute adipose afferent reflex stimulation increases neuronal activation in obese male mice exposed to early life stress

Staff

Visceral adiposity has been implicated in increased sympathetic activation during obesity-induced hypertension. The experimental stimulation of afferent excitatory signals from adipose tissue contributes to the increased sympathetic activation associated with obesity-induced hypertension as part of a sympathoexcitatory mechanism called adipose afferent reflex (AAR). Previous studies from our lab showed that male mice exposed to maternal separation and early weaning (MSEW), a mouse model of early life stress, display increased mean arterial pressure (MAP) when fed a high-fat diet (HF). These mice display exacerbated blood pressure responses to the acute stimulation of the AAR in epididymal white adipose tissue (eWAT) compared to controls. Thus, the aim of this study was to determine the neuronal activation in brain areas that receive afferent signals from eWAT and regulate blood pressure: organum vasculosum of the lamina terminalis (OVLT), subfornical organ (SFO), non-endocrine neurons in the posterior paraventricular nucleus of the hypothalamus (PVN), magnocellular neurons of PVN (PaLM), supraoptic nucleus (SON), dorsal raphe nucleus (DRN), rostroventrolateral medulla (RVLM), nucleus of the solitary tract (NTS), and area postrema (AP). C57BL/6J mice litters were separated from the dam from postnatal days 2 to 16 and weaned early on day 17. Undisturbed controls (C) were weaned on day 21. After weaning, male C and MSEW mice were fed low-fat diet (LF) or HF for 16 weeks (10% and 60% Kcal from fat, respectively). Next, epididymal WAT (eWAT) was microinfused with vehicle (VEH; 20 µL ethanol, 10 µL tween 80/mL normal saline) or 1.5 pmol/µL of capsaicin (CAP; 8 µL capsaicin solution over a period of 2 minutes in 4 different sites, bilaterally) for quantification of neuronal activation. VEH or CAP infusions in eWAT were performed in five replicates every 20 minutes and were perfused 10 minutes after the last infusion to determine Fos immunoreactivity (1:4000, RPCA-c-FOS; EnCor Biotechnology, FL), used as a marker of neuronal activation. In control and MSEW mice fed a LF, the AAR stimulation with vehicle or capsaicin did not change the number of Fos positive cells in the brain areas analyzed in this study between control and MSEW males. On the contrary, in control and MSEW mice fed a HF, CAP infusions in eWAT significantly increased the number of Fos positive cells in OVLT, posterior PVN, DRN, and RVLM in obese MSEW mice compared with vehicle infusions and capsaicin infusion in controls. The neuronal activation in SFO, PaLM, SON, NTS, and AP was similar between groups in response to VEH or CAP infusions. These results, along with the increase in MAP after acute CAP stimulation, suggest that the AAR could play an important role in the exacerbated blood pressure in obese MSEW males by increasing neuronal activation in brain areas that receive sensory signals from fat and regulate sympathetic outflow such as the OVLT, posterior PVN, DRN, and RVLM.

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Gaming Makes You Smarter? Exploring the Effects of Virtual Reality

Student

In the media, video games are often portrayed in a negative way, but research has demonstrated empirical evidence to highlight the positivity video games can have on cognition. This is shown in our study where participants completed game training via immersive virtual reality (VR) through a head-mounted display. The goal was to measure the affects that gaming has on cognition. To begin, 24 healthy participants were randomly assigned to either a non-intervention control or VR group (n=12). Before the intervention, a baseline evaluation was completed using a tablet based neurocognitive assessment. Over the course of 3-weeks participants completed 8-hours (3, 1-hour session/week) of game training. Twenty-hours hours following the final training session, participants completed a post-intervention evaluation using the same assessments used during baseline. Comparing the baseline and post-intervention assessment performances revealed significant improvements in memory, attention and processing speed in participants that completed VR training ($p<.05$). However, participants in the control condition demonstrated a significant improvement in processing speed, suggesting there is a practice effect for this measure. Therefore, the processing outcomes should be interpreted with caution. In the future, an optimal training duration should be explored to optimize outcomes.

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Why do I feel exhausted?: Exploring factors that may contribute to fatigue levels during the COVID Pandemic

Faculty

The COVID-19 pandemic has created an unprecedented disruption worldwide. With the first wave hitting the United States in early 2020 and the subsequent waves that have followed, many people across the US find themselves questioning when are we going to return to "normal?" In the midst of these uncertain times, we were interested in investigating how people were coping with the ever-changing climate of the COVID-19 pandemic. Participants completed an anonymous web-based survey that included the Profile of Mood Scale (POMS) and Connor-Davidson Resilience Scale questionnaires between March 2020 and February 2021. Four-hundred and thirty-six participants (82.3% female), aged 18-70 years old (M = 41.78, SD = 15.11) completed the survey. A linear regression analysis was conducted to determine if age, race, gender, employment status, having recently changed jobs due to COVID-19, being currently employed or going to school, living alone or with others, and resiliency (Connor-Davidson Resilience Scale), would predict fatigue measure on the POMS (0-28). Controlling for all other variables in the model, men scored 1.60 points lower on the POMS fatigue scores than women. Furthermore, every 10-years of age increase reduced a participant's POMS fatigue score by 0.6 points. Additionally, every 30-days spent social distancing increased POMS fatigue score by 1.31-points. Finally, every 1-point increase in resilience scores reduced the POMS fatigue score by 0.22-points. These results suggest that women reported higher fatigue levels than men, regardless of age, race, employment status, and living situations. Additionally, younger people reported higher fatigue levels, compared to older participants, and the duration of time social distancing, negatively affected fatigue levels. Finally, higher resilience levels protected against fatigue ratings.

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A Machine Learning Approach for the Prediction of Retinopathy of Prematurity (ROP) in Preterm Infants

Student

Background: Retinopathy of prematurity (ROP) is a leading cause of visual impairment in preterm infants and is associated with abnormalities of brain structure and metabolism. Predicting ROP plays a vital role in early diagnosis as well as preventing vision loss and brain injury in preterm infants. The objective of this study is to employ a machine learning algorithm with influencing factors (e.g., gestational age, birth weight, small for gestational age) as inputs to predict ROP.

Methods: Data were collected from 230 preterm infants (23 0/7 to 34 6/7 weeks gestation) at the Kentucky Children's Hospital, including 200 infants without ROP and 30 infants with ROP. A logistic regression-based model for predictive analysis was used to predict ROP. Model training was performed using seven independent variables including gestational age, birth weight, small for gestational age, gender, prenatal steroids, cesarean section, and multiple gestations. All analyses were performed using Python program and a data analysis tool of Pandas. The model performance was examined using metrics including the sensitivity, specificity, area under the receiver operating characteristic curve (ROC), and harmonic mean of the model's precision (F-score).

Results: Our logistic regression model predicts the ROP with the sensitivity of 0.74, specificity of 0.83, area under ROC of 0.86, and F-score of 0.52. Among seven independent variables, gestational age is the most significant factor for ROP prediction, which meets the clinical expectation.

Conclusions: With the promising logistic regression model established in this pilot study, we are now adding other influencing factors such as intermittent hypoxemia (IH) for better prediction and management of ROP as well as to investigate if exposure to IH would cause abnormalities of brain structure and function.

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Neurobank: Building a Unique Resource for Neuroscience Research

Staff

Intro: The University of Kentucky NeuroBank commenced operations in 2020. Under the VPR-funded Neuroscience Research Priority Initiative, the aims of Neurobank are to support neurologic researchers in better understanding the disease process by providing access to relevant human biospecimens and to accelerate translational and innovative research. The overarching end-goal is to facilitate diagnosis and treatment options for patients with neurologic diseases and disorders.

Background: Neurobank initially developed a workflow to incorporate patient collections from the clinic in Kentucky Neuroscience Institute and the Chandler Medical Center Neurology Service. To expand sample diversity, a Spanish language version of the consent was created. To expand CSF samples, Neurobank consented patients undergoing lumbar punctures both in clinic and in the hospital. Neurobank then evolved to encompass collections in conjunction with neurosurgery, pathology and trauma surgery, in order to include participants undergoing brain surgery and also support research in spinal cord injury and traumatic brain injury. In response to a demand for samples from epilepsy patients, Neurobank worked closely with the epileptologists and nurses on the Epilepsy Monitoring Unit to develop a collection of samples from patients with EEG-confirmed seizure in the acute time period. In the process, samples from patients with psychogenic non-epileptic spells were also collected.

Methods: Neurobank depends heavily on resident, clinician, and staff communication and collaboration. Neurobank staff identify potential participants and then request permission from the care team before approaching the patient. If the patient consents, Neurobank staff then manage the collection and processing of samples.

Results: Neurobank has approached 381 patients at the University of Kentucky, 351 patients provided their consent to either extra draws for research or leftover sample collection. As of now, only English and Spanish speaking patients are eligible for consent to biobanking. The overall rate of consent for the Biobank is 92.13%. Collection efforts are focused on blood, cerebrospinal fluid (CSF), and tissue. Currently, Neurobank has: 316 blood samples, 132 CSF samples, and 21 tissue samples. The average collection to processing times for biospecimen are: 54 minutes for blood and 44 minutes for CSF. The average times for collection to freezing (-80°C) for biospecimen are: 85 minutes for blood and 63 minutes for CSF. (For comparison purposes, per website description, Harvard's Biomarkers Study Biobank processes within 4 hours of collection).

Conclusion: Neurobank has evolved to incorporate innovative and researcher-responsive methods into collection of human samples to optimally facilitate translational research for neuroscience investigators. Researchers can request sample information and availability by filling out an inquiry form.

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A Fast Algorithm Towards Real-Time Laser Speckle Contrast Imaging

Fellow

Background: Laser speckle contrast imaging (LSCI) technology illuminates laser light on tissue surface to produce interference speckle patterns. A CCD/CMOS camera captures the spatial/temporal speckle pattern fluctuations resulting from motions of red blood cells, which corresponds to regions with the higher blood flow including, arteries and veins. With recent advancements in computational technologies, developing a real-time flow-mapping algorithm has gained significant attention. This study developed a parallel computing technology with more efficient functions in MATLAB.

Methods: We built a MATLAB-based algorithm that takes advantage of its Parallel Computing Toolbox along with Image Processing Toolbox functions. Traditionally, two nested for-loops are utilized for calculating LSCI statistical variance on an N × N pixel window. In addition, a third one iterates the window through every pixel of the image. We eliminated these for loops. Instead, we reduced every window's dimension from N × N to a column of length N × N, which MATLAB handles most efficiently. The codes were executed on a desktop with Intel(R) Core (TM) i9-10900K CPU @ 3.70GHz configuration. [YG1] [SD2] [SD3] With the help of MATLAB's parfor control flow statement, we were able to utilize ten "MATLAB workers" for this task corresponding to using ten CPU cores simultaneously. For this study, three hundred of 1040 × 1440pixel images were collected by a customized LSCI device. The subject was a mouse head with an intact skull. We measured the efficacy of our new algorithm for mapping cerebral blood flow by measuring its analysis time versus the one from the MATLAB-based traditional script.

Results: The traditional script took several hours to reconstruct the flow map for various window sizes. Our new algorithm registered 12.68, 51.80, and 103.77 seconds for the pixel windows of 3 × 3, 5 × 5, and 7 × 7, respectively. The flow image qualities from all pixel windows were comparable.

Conclusions: The analysis proved the possibility of obtaining a close to real-time flow-mapping speed of ~24 fps (i.e., 300/12.68) for the 3 × 3 pixel window. Running this code on a more powerful computer or a cluster may facilitate even faster flow-mapping on par with high-speed cameras.

METHODS OF DETERMINING MULTISENSORY SIGNAL REDUCTION OF ALCOHOL IMPAIRMENT

Student

It is well known that alcohol slows the ability to react to stimuli. Since reaction time is a key behavioral impairment underlying alcohol's broad disruptive effects, studies in cognitive psychology have now identified circumstances in which the presentation of multiple stimuli can facilitate performance under alcohol. Laboratory studies have shown that individuals respond more quickly when information is presented as multisensory, redundant stimuli (e.g., aurally and visually), rather than as a single stimulus presented to either modality alone. This phenomenon is referred to as the "redundant signal effect" (RSE; Todd, 1912). Under the influence of alcohol, multisensory stimuli across two modalities (e.g., visual and auditory) have been found to ameliorate alcohol's impairing effects. However, while research has well-validated performance improvement on RT tasks by the presentation of multisensory stimuli, the unit of analysis is typically the subject's mean RT. Therefore, little attention is paid to the distribution of RT and no study to date has examined how RSE ameliorates the slowing of RT across the distribution of RTs under alcohol. The purpose of this study was to replicate the finding that alcohol-induced impairment would be reduced by RSE and analyze this effect comprehensively through the use of standard (i.e., ANOVA and *t*-tests) and new (i.e., Cumulative Density Function) RT analysis. The present study examined a sample of 20 people in a lab who were administered a dose of alcohol and a placebo in counterbalanced order where reaction time was measured with a two-choice RT task. The results of the standard analyses show that alcohol slowed RT compared with placebo and responses were generally faster in the multisensory condition compared with the single signal conditions. Regarding the CDFs, consistent with RSE, multisensory signals generally ameliorated the slowing effects of alcohol on RT except for responses that were extremely slow or extremely fast. This understanding supports the use of CDFs as a methodological approach in analyzing RSE under alcohol by providing the details necessary to determine what specific RTs are impacted by alcohol and improved by multisensory signals.

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Neurodegeneration

A Potential Biomarker for Cerebral Amyloid Angiopathy: Cerebral Blood Flow Low-Frequency Oscillations Method using Diffuse Correlation Spectroscopy

Fellow

Background: Cerebral amyloid angiopathy (CAA) considers as a degenerative disease and cerebrovascular disease at the same time because it is one of the leading causes of cerebral microbleeds (CMBs) in older adults. Also, CAA contributes to neurodegeneration, cognitive impairment, substantial morbidity, and mortality. Non-invasive diagnosis of the CAA is rarely available, making the autopsy, parenchyma, and meningeal biopsy are the main methods for detecting CAA. However, CMBs can be seen visually based on magnetic resonance imaging (MRI), T2* weighted images. Accordingly, developing a specific and sensitive antemortem biomarker for CAA is crucial for pretreatment. However, currently available biomarkers for CAA lack sensitivity and fail to evaluate the cerebral hemodynamic influences of CAA responsible for CMBs and dementia. Spontaneous low-frequency oscillations (LFOs) method has been used to assess the cerebral autoregulation system (CA) by quantifying the relationships between the mean arterial pressure (MAP) and cerebral blood flow (CBF) as a gain transfer function (gain ~ 1/CA). The aim of this study is to use the near-infrared diffuse correlation spectroscopy (DCS) measurement as a noninvasive and low-cost technique with the LFO method for detecting CAA in the aging population.

Methods: Twenty-seven participants (77.88 ± 6.5 years, 14 female) were divided into two groups (CAA+ and CAA-) based on Boston Criteria. DCS instrument was used to continuously detect CBF from the anterior and posterior cortex in older adults, using a novel optical probe design. The data were collected before, during, and after four intervals (~2-min each) of 5% CO_2 inhalation alternating with room air. The LFO gains were computed and divided into three frequency intervals: metabolic activities (0.0095 -0.02Hz), neurogenic activities (0.02 - 0.07Hz), and myogenic activities (0.07-0.2Hz) to study the CA at each interval.

Results: The main significant changes between groups were found at the neurogenic activities compared to other intervals. The CAA+ group showed a weaker CA at the occipital lobe than the frontal, and the CAA- group showed a strong relationship between the frontal and occipital lobes. Also, the CAA- group showed the strongest correlation between lobes at the metabolic activities ($P < 0.001$, $R^2 = 0.8$ at Baseline and during CO_2 inhalation) compared to other intervals.

Conclusions: Based on our preliminary results, the LFO method with the DCS technique in relation to CA is a potential biomarker for investigating CAA in older adults. This result is promising to enable the future development of clinical translational therapeutic interventions for CAA. However, in future work, we still need to increase the sample size for a better understanding of the outcome.

Inhibition of p38 alpha MAPK signaling provides benefit in a mouse model of mixed dementia

Faculty

Background: The p38a MAPK signaling pathway is a well-established regulator of neuroinflammation that is aberrantly activated in Alzheimer's disease (AD). However, there is a lack of insight into the p38a MAPK signaling pathway involvement in non-AD comorbidities found in roughly 80% of probable AD patients. The most common of these co-morbidities is vascular pathology. Therefore, we explored the potential pharmacodynamic effects of MW150, a p38a MAPK isoform selective inhibitor currently in clinical trials, on neuroinflammation in a co-morbid environment with both AD and vascular pathology. To this end, we used a mouse mixed dementia (MD) model that combines amyloid pathology with vascular pathology induced by hyperhomocysteinemia (HHcy).

Method: The 5xFAD mouse model of amyloid pathology (MMRRC No. 34848-JAX) was used. 5-month-old animals were placed for 8 weeks on a B-vitamin deficient and methionine-supplemented diet (Envigo TD.97345) to induce HHcy and generate the MD model. In a preventative treatment paradigm (treatment before vascular pathology is present), the MD mice were given intraperitoneal injections of saline vehicle or varying doses of MW150. For baseline comparisons, WT littermates on a control diet and administered saline were included. At the end of the 8 weeks, mice underwent testing in the Morris Water Maze (MWM) at the Jackson Laboratory or were sacrificed in-house for pathological analyses, including measurement of pro-inflammatory cytokines and changes in various RNA transcript levels.

Results: In the MD mice, MW150 was able to rescue deficits in the MWM task, prevent proinflammatory cytokine IL-1b overproduction, and reduce several markers of microglial activation in a manner consistent with its known anti-inflammatory activity in other models.

Conclusions: These data indicate that early intervention with MW150 might be an efficacious strategy in the context of co-morbid amyloid and vascular pathologies. Work to test the efficacy of MW150 when administered in a therapeutic as opposed to preventative paradigm is currently ongoing.

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APOE genotype modifies microglial immunometabolism

Student

Background: Metabolic dysfunction and chronic neuroinflammation characterize Alzheimer's disease (AD), but it is unclear if these two facets of the disease are linked. The E4 allele of Apolipoprotein E *(APOE)* is the strongest genetic risk factor for late-onset AD. APOE4-expressing microglia exhibit a heightened pro-inflammatory response compared to APOE3. In addition, recent data from our lab and others show that E4 is associated with increased aerobic glycolysis. These two findings may be intrinsically linked through the concept of 'immunometabolism' - an emerging paradigm that implicates increased glycolytic activity as a core requirement during the pro-inflammatory activation of immune cells, whereas increased oxidative phosphorylation is required for anti-inflammatory activation. We propose that increased glycolytic activity and impaired mitochondrial function in E4 microglia predisposes E4 carriers to a pro-inflammatory and pro-neurodegenerative phenotype.

Method: Primary microglia were isolated from targeted replacement mice expressing human APOE2, APOE3 or APOE4. Cells were stimulated with pro-inflammatory (lipopolysaccharide; IFN**γ** + TNF**α**) or anti-inflammatory (IL10; IL-4 + IL-13) cytokine cocktails. Metabolic responses to these treatments were measured using the Agilent Seahorse platform and targeted metabolomics.

Result: The Seahorse glycolytic rate assay revealed increased glycolysis at baseline in E4 compared to E3 and E2 microglia, and this glycolytic activity was further increased following pro-inflammatory stimulation. Conversely, when treated with an anti-inflammatory stimulus, E4 microglia were shown to be deficient in mitochondrial respiration and oxidative phosphorylation compared to E3 and E2. Finally, targeted metabolomic profiling revealed increased levels of lactate, citrate, and succinate in E4 microglia, three metabolites known to accumulate in pro-inflammatory macrophages.

Conclusion: Our findings of increased glycolytic metabolism, decreased mitochondrial function, and an accumulation of lactate, citrate, and succinate all point to altered metabolic preference in E4 microglia, which may drive their increased pro-inflammatory response. When combined with the impaired mitochondrial function that we report here, these alterations in central carbon metabolism may prevent E4 microglia from promoting anti-inflammatory phenotypes, resulting in a failure to resolve inflammation or mount an effective tissue repair response. Thus, reprogramming immunometabolism in E4 microglia by inhibiting glycolysis and/or boosting oxidative phosphorylation may provide a novel therapeutic avenue for the treatment of AD.

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Expression and pharmacologic modulation of the Ca2+-permeable purinergic receptor P2X4 in hippocampal neurons and astrocytes

Fellow

It is well known that dysregulated Ca2+ in the brain contributes to deleterious changes, including decreased plasticity and altered excitability. Evidence suggests these changes may not be generalizable across phenotypes; yet most studies of Ca2+ dysregulation have been performed in the aged brain, with relatively few conducted in the context Alzheimer's disease (AD). Ionotropic purinergic receptors (P2XRs) are ATP-gated cation channels permeable to Ca2+ that have been implicated in several neurological disorders, including AD. Most of studies of P2X receptors have focused on P2X7; however, another subtype, P2X4, not only has a higher permeability to Ca2+, but is also known to be reduced in AD, making it an attractive target for AD-focused investigations. Here, we tested the impact of moxidectin (MOX), a P2X4 allosteric modulator, and 5-BDBD (BD), a P2X4 antagonist, on intracellular Ca2+ dynamics in neurons and astrocytes. We also measured P2X4 expression in the somatosensory cortex (S1) of Fisher 344 (F344) rats exposed to intranasal saline (INS) or insulin (INI). Intracellular Ca2+ measures were obtained in mixed, primary hippocampal cultures using FURA-2 imaging. Cells were first exposed to a static imaging solution containing DMSO, BD, or MOX for 2 min. Cells were then continuously perfused with this same solution for 4 min, followed by another 4 min of perfusion using a variation of the DMSO, BD, or MOX imaging solution that was supplemented with ATP and bradykinin to induce depolarization. Ca2+ transients were obtained by acquiring a fluorescent image every 10 seconds throughout the recording and used to derive ratiometric values. To assess the impact of age and insulin on P2X4 expression in the brain, Western immunoblots (WBs) were performed on homogenized S1 tissue from young and aged F344 rats obtained 30 or 120 min after acute exposure to INS or INI. WBs were quantified in ImageJ and band intensities were normalized to total protein load (Ponceau S staining). FURA-2 imaging results highlighted a significant impact of P2X4 ligands on intracellular Ca2+ transients compared to DMSO. WBs revealed a trend for an effect of INI on P2X4 expression 120 min after acute exposure. A significant interaction term was also detected, with the young INI group having reduced expression compared to young INS, while the aged INI-treated animals had increased expression compared to the aged INS group. No significant effect of INI was detected in either age group at the 30 min timepoint. Together, our findings suggest P2X4 ligands can alter intracellular Ca2+ in the hippocampus and that brain insulin signaling may be able to mediate these receptors in an age-dependent manner. We are currently assessing the impact of MOX and BD on S1 Ca2+ network dynamics in 5xFAD mice using *in vivo* 2-photon imaging. Future investigations of P2X4 expression using human tissue samples are also being planned in order to address the translatability of this work to the clinic.

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β-amyloid accumulation in the brain is decreased by knocking-down human amylin in mice with conditional expression of human amylin in the pancreas

Other

Rationale: Amylin dyshomeostasis is associated with mixed amylin- **β**-amyloid (A**β**) plaques in the brains of patients with Alzheimer's disease (AD).

Objective: To test whether lowering human amylin in the circulation decreases cerebral A**β** plaque formation in a conditional human amylin knockdown mouse model of AD.

Methods: A human amylin expressing mouse model was generated by targeted replacement of rodent amylin by floxed human amylin gene ($HuAmy^{f/f}$). Further, by crossing with pancreas-specific Ins1-Cre-ERT^2 (Cre) and (APP/PS1) mice, conditional human amylin knockdown mice ($Cre/HuAmy^{f/f}$) and conditional human amylin knockdown-APP/PS1 mice ($Cre/HuAmy^{f/f}$/APP/PS1) were made. Wild type (WT), amylin knock-out (AKO), $HuAmy^{f/f}$, $Cre/HuAmy^{f/f}$, APP/PS1, and $Cre/HuAmy^{f/f}$/APP/PS1 mice (n=10/group) were fed with high fat diet (HFD) for 2 or 4 months from 3 months of age to enhance amylin secretion in the circulation. Amylin expression in pancreas was downregulated conditionally in $Cre/HuAmy^{f/f}$ and $Cre/HuAmy^{f/f}$/APP/PS1 mice by treating with tamoxifen (or vehicle). Amylin-A**β** interaction, and metabolic and cognitive functions were measured terminally.

Results: In comparison to WT and AKO mice, $HuAmy^{f/f}$ mice show increased insulin resistance, blood glucose and glucose intolerance, and neurological deficits. Amylin knockdown in $Cre/HuAmy^{f/f}$ mice reverses metabolic and neurological deficits at the same levels as in WT and AKO mice. In $Cre/HuAmy^{f/f}$/APP/PS1 mice, amylin knockdown significantly decreased A**β** plaques and improved brain function.

Conclusions: Reducing amylin dyshomeostasis in human amylin expressing mice improves metabolic and cognitive function and slows down AD-like pathology progression. Further studies are needed to uncover molecular mechanisms by which anti-amylin therapies may reduce AD in the setting of type-2 diabetes.

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Diabetes-related Cerebral Amylin Vasculopathy Induces Brain Hypometabolism

Student

Background: Type-2 diabetes is a metabolic disorder that increases the risk for cerebrovascular diseases and dementia. We and others reported previously that this risk is associated with elevated blood levels of amylin (an amyloidogenic hormone synthesized and co-secreted with insulin), which promotes amylin amyloid deposition in the brain microvasculature (cerebral amylin vasculopathy). We also showed that inducing amylin dyshomeostasis in rats by pancreatic-specific expression of human amylin (amylin from rodents is non-amyloidogenic) leads to cerebral amylin vasculopathy and neurological deficits. Here we tested the hypothesis that cerebral amylin vasculopathy is a contributing factor to diabetes-associated brain hypometabolism. **Methods:** We conducted brain RNAseq analysis and proteomics in 16-month old rats expressing human amylin in the pancreas (HIP rats), amylin knockout (AKO) rats, and normal rats expressing wild-type (WT) rat amylin (n =10 males/group). Library and construction, and sequencing were performed using commercial service (Omega Bioservices). Briefly, sequence reads were aligned to the Rattus norvegicus genome (rn5) using STAR. Individual sample reads were quantified using HTseq and normalized via Relative Log Expression (RLE) using DESeq2 R Library. Isolated brain homogenates from WT and HIP (n =7/group) rats were used for western blot analysis for enzymes significantly changed in RNA seq analysis that is important in the glycolytic pathway. **Results:** In the brains of rats with amylin vasculopathy (HIP rats), RNA seq analysis shows a significantly altered pathway important for metabolism compared to WT littermates. The genes in these pathways were associated with glycolysis and revealed that amylin significantly decreases the expression of these proteins. Many of these pathways were inversely regulated in AKO vs. WT rats. **Conclusion:** Increased amylin levels in the circulation provokes cerebral amylin vasculopathy causing significant alteration of the brain gene expression. Cerebral amylin vasculopathy is a contributing factor to diabetes-related brain hypometabolism through the dysregulation of enzymes important in the glycolytic pathway.

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Using Crystallization and Kinetic Assays to Understand Insulin-degrading Enzyme-Phosphatidylinositol Interactions

Student

The Insulin-degrading enzyme (IDE) is a widely expressed protein responsible for breaking down insulin, the amyloid beta peptide (involved in the development of Alzheimer's Disease) and other cellular substrates. This research aims to identify how IDE gains access to its substrates, which are typically found in endosomes within a cell. Our hypothesis is that a small fraction of the IDE present in a cell's cytosol is drawn into the endosomes by binding to phosphatidylinositol phosphate (PIP) lipids located on the outer membranes of these subcellular compartments.

While we have not yet obtained conclusive results, much of our time in lab has been spent attempting to crystalize rat IDE produced in insect cells and bound with the polar portion of a PIP to better define the binding site on the enzyme. Computational modeling has identified the most likely sites of phosphatidylinositol binding, but successful crystallization of an IDE-phosphatidylinositol complex would support our hypothesis and guide future investigations of IDE's biochemical activity. Efforts are also underway to test the proposed binding site by determining if the PIP polar portion acts as an inhibitor of the enzyme.

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Sex differences in age-related false alarm and differential temporal and frontal responses during non-emotional working memory retrieval

Student

Inaccurate and prolonged working memory retrieval is hallmark of mild cognitive impairment (MCI) induced by Alzheimer's disease (AD) and related dementia pathologies. Increased false memory retrieval has been reported in normal cognitive aging (e.g. thinking a car in a parking lot is your car when it is not). It is unclear why there are more men in the US suffering from MCI (early stage of dementia), while AD disproportionally affect women. Here we test the hypothesis that sex differences in brain aging have differential effects on brain volumes and network associated working memory performance in preclinical cognitively intact older adults.

Forty-four (44) cognitively normal older adults (25 women; mean age 77), from the University of Kentucky ADC cohort, participated in the Bluegrass Working Memory Task inside the 3T Siemens magnetic resonance imaging (MRI) Scanner. The task is a 11-min modified delayed match-to-sample task (Jiang et al., 2016). A false alarm was defined as identifying a non-match visual object as a match target held in memory. MRI data was analyzed using Free Surfer. Spearman correlation and linear regression analyses were performed to test the hypothesis.

The mean false alarm rate in males was 3.74%, and 4.03% in females (no sex differences). Mean reaction time during false alarms (FA_RT) in men was 657 ms, and in women 792 ms (no sex differences, $p>0.05$). False alarm rate was negatively correlated with brain volumes in frontal and temporal regions. We observed significant correlations in FA_RT in all subjects in frontal and temporal regions. However, when subjects were separated by sex, FA_RT in males significantly correlated positively with frontal regions (e.g. rostral middle frontal gyrus and fontal pole) only while FA_RT in females correlated positively with temporal regions (e.g. transverse temporal gyrus).

We found that cognitively healthy older men and women made similarly rate of false alarm in working memory retrieval. However, the older men were significantly faster than women in false memory retrieval and involves differential cortical mechanisms. False alarm memory retrieval is associated with decreased frontal volumes in older men but decreased temporal volumes more often in older women.

Stroke & Vascular

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Amyloidogenic amylin accumulates in emergent large vessel occlusions

Staff

Emergent large vessel occlusions (ELVOs) result in severe ischemic strokes without appropriate treatment with thrombolysis and/or thrombectomy. Diabetes is a complicating factor and is associated with poorer functional outcomes, prolonged hospitalizations, higher readmission rates, and increased risk of recurrent stroke. Amylin is an amyloidogenic peptide co-secreted with insulin from the pancreatic beta cells and has been associated with vascular pathology. We collected thrombi and arterial systemic blood at the time of mechanical thrombectomy for ELVO. Arterial blood samples were also collected from cerebrovascular control patients having diagnostic angiograms. Red blood cells (RBCs) and plasma were separated by centrifugation. RBCs from all patients and thrombi from stroke patients were homogenized. Total protein concentration was calculated using the bicinchoninic acid assay (BCA) and the magnitude of amylin immunoreactivity was measured through a competitive enzyme-linked immunosorbent assay (ELISA). Inter-tissue amylin immunoreactivity ratios were calculated for each patient. Analysis of the immunoreactivity ratios revealed a significant increase in the uptake of amylin by RBCs in stroke patients. These results provide novel insights into the role of amylin in acute stroke in patients with diabetes. Future research in understanding how amyloid aggregates impact stroke risk is ongoing.

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SpeckleFlow: rapid spatio-temporal analysis of superficial cerebral blood flow dynamics in high resolution laser speckle contrast imaging

Student

Background: Laser speckle contrast imaging of the superficial cerebrovasculature provides simple measures of blood flow dynamics. Spatial-temporal characteristics of cerebral blood flow (CBF) in response to focal cerebral ischemia and cortical spreading depression have been previously investigated using laser speckle contrast imaging and have established excellent measurement validity (Dunn et al., 2001). However, the analysis of such imaging data contains many steps and takes much time, introducing error and bias. This lack of reliable, rapid analysis may discourage investigators from adopting this technology in their research and warrants an algorithm that expedites extraction, analysis, and processing from laser speckle contrast imaging.

Methods: We have created SpeckleFlow, a FIJI/ImageJ macro for rapid analysis of spatio-temporal dynamics in CBF using laser speckle contrast imaging output. SpeckleFlow can take time-stacked Tiff data, an output from simple, high resolution laser speckle contrast imaging, and provide local speckle contrast, a unique measure of CBF dynamics. Using a reference image, a region of interest (ROI) can be defined by the user and masked on speckle stacks. Additionally, the speckle stacks can be sectioned in blocks and analysed separately to measure the local speckle contrast for baseline, stimulation, and recovery periods by using frame number. Here, we demonstrate the utility of SpeckleFlow by analysing in vivo laser speckle contrast imaging stacks collected from the superficial barrel cortex of awake mice before, during, and after whisker stimulation.

Results: Compared to the standard approach of creating and organising multiple files, z-stack projects, and measurements one step at a time, SpeckleFlow accurately reported spatio-temporal characteristics of CBF, while reducing analysis time. SpeckleFlow measured changes in CBF dynamics of the superficial barrel cortex at baseline, during whisker stimulation, and during the post-stimulation recovery period. Local speckle contrast for these three periods were accurately and rapidly quantified, providing evidence that SpeckleFlow can be used in laser speckle contrast imaging applications.

Conclusion: SpeckleFlow is a strong, utile macro for rapid spatio-temporal analysis of superficial CBF dynamics in high resolution laser speckle contrast imaging.

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Characterization of Hospitalization Outcomes between Symptomatic and Asymptomatic Urinary Tract Infection in Acute Ischemic Stroke Patients

Other

Objective

Characterize demographics and hospitalization outcomes of symptomatic and asymptomatic UTI in stroke patients compared to a control group without infectious complication.

Background

Urinary Tract Infection (UTI) impairs recovery in stroke patients. Infectious Disease Society of America guidelines do not support treating asymptomatic UTI in the absence of objective markers of infection.

Method

This is a retrospective cohort study using the 2019 ischemic stroke database at the University of Kentucky. Cases were defined as patients with UTI confirmed on urine studies, and without other infection. Cases were subdivided into sUTI (concerning subjective history or objective vitals) and aUTI group (concerning lab or NIHSS change). Controls were defined as patients without evidence of infection, and matched to the case group by age and sex. Demographics (including stroke mechanism) were compared using Chi-squared analysis. Outcomes included length of stay (LOS), change in pre-admission to discharge modified Rankin Scale (delta mRS), delta NIHSS (discharge - admission NIHSS), and % change in NIHSS ((highest NIHSS -lowest NIHSS)/admission NIHSS)). Mean and standard deviation between the 3 groups were compared using ANOVA.

Results

A total of 91 cases were identified as having UTI (sUTI = 34; aUTI = 57), with nearly all receiving antimicrobials. Stroke mechanism based on TOAST classification is distinct for the aUTI cohort (predominately cardioembolic, p=0.047 vs. control). Both sUTI and aUTI had greater morbidity as defined by delta mRS compared to control (p=0 and 0.011 respectively). Only sUTI had longer LOS compared to control (p=0). No difference in delta or % change NIHSS was observed between the 3 groups.

Conclusion

Both sUTI and aUTI are associated with poor outcome in mRS, which may suggest impaired stroke recovery. Withholding antibiotic treatment in aUTI, as suggested by IDSA guidelines, may worsen outcomes. Further study is needed to determine the impact of non-treatment.

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The Impact of Exercise Prior to Stroke in Cathepsin B Knockout Mice

Student

Background: LG3 is a peptide derived from the C-terminus of perlecan, a proteoglycan in the vascular extracellular matrix, and plays a neuroprotective/neuroreparative role in the brain after ischemic stroke (PMID: 24323378, 21747167). Crucial to this derivation is cathepsin B, a ubiquitous cysteine myokine protease, which increases in plasma post exercise in both humans and mice. In addition, increased cathepsin B levels contributes to beneficial effects on memory and neurogenesis (PMID: 27345423). Cathepsin B knockout (ctsbKO) mice do not benefit from exercise due to the inability to derive LG3 (PMID: 27345423). **We hypothesize that the neuroprotective benefits of exercise before stroke is lost in ctsbKO mice.**

Methods: 3-9 mos.-old ctsbKO male (n=23) and female (n=24) mice were split into sedentary (Sed) and exercise (Ex) cohorts for 3 wks prior to transient middle cerebral artery occlusion (tMCAo), with inclusion criteria &<80% baseline cerebral blood flow (CBF via laser Doppler flowmetry) reduction, returning to <50% baseline CBF at reperfusion. Euthanasia occurred 3 days post-tMCAo. Ex cages contained a monitored running wheel to measure distance run. Submandibular blood was drawn weekly and spleen, blood, and brain collected for flow cytometry (spleen), histology (brain), and ELISA (plasma). Data were analyzed using FlowJo v10.7.2 Software and stats using GraphPad Prism v9.1.0.

Results: ctsbKO mice had significant exclusion based on CBF values with tMCAo in Sed vs Ex cohorts for both sexes (both p>0.05), including a high incidence of reperfusion and hemorrhagic transformation. There was no difference in post-stroke neurological deficit score (NDS) between cohorts. All female mice exhibited increases in activated B and CD4 T cells, and innate cells in the spleen. Ex increased monocyte and neutrophils while decreasing macrophages, again only in female mice. There was no correlation to pre-stroke exercise intensity for splenic populations, and male mice did not show differences in post-tMCAo splenic immune cell populations.

Conclusion: Sed ctsbKO mice have underlying pathology that precludes successful tMCAo induction similar to other strains previously published (e.g. PMID: 32051245). Exercise had no beneficial effect on the post-stroke NDS of ctsbKO mice, and no peripheral immune effect in male mice. Future studies will confirm infarct volume by histology, and plasma LG3 levels by ELISAs to determine loss of pre-stroke intervention benefit in the absence of cathepsin B.

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Laser Speckle Contrast Imaging of Cerebral Blood Flow Using Picosecond Pulsed Laser Illumination

Student

Background: The study of numerous physiological and pathological situations in the brain necessitates the measurement of blood flow and oxygen saturation in cerebral vasculature. For the brain to operate normally, it requires enough cerebral blood flow (CBF), and appropriate oxygen supply. During an ischemic stroke, vascular morphology is disrupted, resulting in irregular blood flow and decreased tissue oxygenation, compromising brain function. Conventional laser speckle contrast imaging (LSCI) technique illuminates continuous-wave (CW) laser light on tissue surface. A CCD/CMOS camera captures the temporal/spatial speckle pattern fluctuations, resulting from motions of red blood cells (i.e., blood flow). In this study, we tested a picosecond-pulsed laser as the LSCI source for 2D imaging of cerebral blood flow (CBF) in mice. This new system has the potential to be integrated with other imaging techniques using pulsed illumination such as photoacoustic imaging (PAI) and fluorescence angiography (FA).

Methods: A mouse model of transient global ischemia was used to test the integrated imaging system. A sterile surgical suture was wrapped around each common carotid artery (CCA) and a loose knot was tied on each suture without impeding blood flow to the brain to cause acute global ischemia. After a baseline measurement of ~8 minutes, the right CCA knot was tightened for ~8 minutes to occlude the right CCA, then the left knot was tightened for 2 minutes to cause transient global ischemia. The left and right knots were then released in order, allowing blood flow to the brain to be restored. We assembled an integrated LSCI system combining a CW laser (DL785-120-SO, CrystaLaser) at 785 nm and a picosecond pulsed laser (KATANA-08 HP, NKT photonics) at 775 nm. The glass and engineered diffusers were placed in front of the lasers for uniform illuminations. A 12-bit CMOS camera (BFS-U3-16S2M-CS, PointGray, pixels: 1440×1080, pixel size: 3.45μm) collected images from the mouse head with intact skull using varied exposure times from 1 to 5 milliseconds.

Results: The picosecond pulsed laser with the engineered diffuser and 5 ms exposure time captured more details of cerebral blood vessels compared to the CW laser with glass diffusers. The consecutive ligations of left and right common carotid arteries resulted in significant CBF reductions, which agreed with clinical expectations.

Conclusions: This research lays the ground for future work to develop multimodal imaging systems integrating LSCI, PAI, FA, and other time-resolved imaging systems with shared pulse illuminations.

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BACTRAC Protocol Can Be Utilized In Determining Proteomic Expression In Populations At Increased Risk For Emergent Large Vessel Occlusion (ELVO)

Student

Introduction: Despite the introduction of therapeutic advancements in managing ischemic stroke (IS) patients, IS continues to be a leading cause of morbidity and mortality. Endovascular treatment (EVT), a groundbreaking advancement, has become increasingly popular since it began to replace the mainstay of IS treatment, intravenous tissue plasminogen activator (IV- tPA), in 2015. Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol (clinicaltrials.gov NCT03153683) utilizes EVT to collect systemic and intracranial arterial blood at the time of stroke, which following proteomic analysis, results in molecular biomarkers that are recorded in BACTRAC-associated databases. We have utilized these biomarkers from subjects at increased risk for ELVO to determine di!erences compared to control subjects.

Methods: 61 subjects to date have underwent EVT for ELVO, where intracranial and systemic arterial blood specimens were collected during EVT via BACTRAC protocol. Proteomic expression analysis was performed at Olink Proteomics (Olink Proteomics, Boston, MA), where expression of 92 cardiometabolic and 92 inflammatory proteins was measured.Additional data, including age, sex, race, body mass index (BMI), diagnosed medical comorbidities, medications, smoking status, tPA administration, thrombus location, time from last known normal (LKN), infarct volume, edema volume, National Institutes of Health Stroke Scale (NIHSS), modified Ranklin Scale (mRS), and Thrombolysis in cerebral infarction (TICI) score were collected for each subject.

Results: Utilizing BACTRAC-associated databases, recorded clinical information has served to categorize each subject, and this categorization has been utilized to establish novel relationships between protein biomarkers in intracranial and systemic blood and populations at increased risk for emergent large vessel occlusion.

Conclusion: EVT for ELVO provides an opportunity to collect valuable proteomic datafrom usually inaccessible intracranial arterial blood, along with systemic arterial blood, which both typically go unused. This information can be used for establishing novel relationships between IS risk factors and development of an IS, which also can serve as therapeutic targets to hopefully decrease morbidity and mortality.

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Enlarged perivascular spaces in the centrum semiovale predict MoCA scores among cognitively normal older adults

Student

Cerebral small vessel disease (cSVD) is an important risk factor leading to the development of vascular contributions to cognitive impairment and dementia (VCID). cSVD is characterized by several in-vivo neuroimaging biomarkers including enlarged perivascular spaces (ePVS). PVS are fluid-filled spaces believed to play a role in the glymphatic system's removal of waste from the brain. Reduced clearance may cause backup and enlargement of the PVS and subsequent accumulation of toxic solutes characteristic of neurodegeneration, including A**β**. Evidence supports ePVS role in aging and dementia, but the relationship between ePVS and cognitive function remains unclear**.** Due to their presence in brain regions that support cognition, we hypothesized that quantitative, cross-sectional ePVS counts in cognitively normal older adults would predict baseline cognitive performance. We explored the relationship between ePVS and the Montreal Cognitive Assessment (MoCA) in 71 cognitively normal older adults ranging in age from 60-86. Participants were scanned on a 3T Siemens Prisma scanner with a 64-channel head coil. All ePVS counts were performed on T1 MPRAGE and T2 FLAIR images by an experienced rater blinded to participant demographics and under the direction of a trained neuroradiologist. In line with previously established guidelines, ePVS were defined as regions of hypointensity less than 3mm in diameter on T1 imaging and were distinguished from lacunar infarcts by the absence of T2 FLAIR hyperintensity. ePVS were individually and manually counted in a single, representative slice in the axial plane of the white matter centrum semiovale (CSePVS), basal ganglia, hippocampus, and midbrain. Linear regression analyses controlling for age, sex, intracranial volume, and education demonstrated a significant, negative relationship between total ePVS counts combined across the four regions of interest and MoCA score (β = -0.282, P = 0.022). The strongest relationship was found among CSePVS and MoCA (β = -0.337, P = 0.005). These findings suggest that ePVS burden, driven primarily by CSePVS, is associated with cognitive performance at baseline. Ultimately, our results support the continued investigation of ePVS as an early neuroimaging biomarker of cSVD-related cognitive dysfunction. In particular, additional work that addresses the longitudinal relationship between ePVS and MoCA is needed to strengthen the findings that ePVS predict cognitive dysfunction.

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A Wearable Optical Sensor for Continuous Monitoring of Cerebral Ischemia in Rodents and Piglets

Student

Background: Wearable technologies are needed for continuous monitoring and management of cerebral ischemia. Currently existing cerebral monitoring techniques are either too invasive, too large/heavy to carry, too shallow in detection depth, or too costly. This presentation reports an innovative, inexpensive, wearable, multi-scale diffuse speckle contrast flowmetry (DSCF) probe for transcranial imaging of cerebral blood flow (CBF) in rodents and a neonatal piglet.

Methods: 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 red blood cell motions in the deep brain (i.e., CBF). CBF variations during sequentially transient ligations of left and right common carotid arteries (CCA) were concurrently monitored by the DSCF and established diffuse correlation spectroscopy (DCS) with an integrated hybrid probe fixed on the animal head.

Results: Significant reductions in CBF during transient bilateral CCA were detected by the DSCF (-35±13% in two mice and -59% in a piglet), which meet clinical expectations. Results from the DSCF and DCS were consistent and significantly correlated.

Conclusions: Based on the promising results from this pilot study, we are currently developing a wireless, wearable DSCF probe for continuous cerebral monitoring in freely behaving subjects, including rodents, piglets, and human neonates. Ultimately, we expect to offer a unique, non-invasive, low-cost, fast, multiscale brain imaging tool for basic neuroscience research and clinical applications.

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Influence of Body Mass Index on Adenosine Deaminase and Infarct Volume in Mechanical Thrombectomy Subjects

Student

Background:

Emergent Large Vessel Occlusion (ELVO) strokes are devastating ischemic vascular events for which novel biomarkers and therapies are needed. The purpose of this study is to investigate the role of Body Mass Index (BMI) on protein expression and signaling at the time of ELVO intervention. Uncovering relationships between BMI, proteomic expression, and stroke outcome measures will aid in personalizing prognosis and future treatment of ELVO stroke.

Methods:

The Blood And Clot Thrombectomy Registry And Collaboration (BACTRAC) is a continually enrolling tissue bank (clinicaltrials.gov NCT03153683) from stroke patients undergoing mechanical thrombectomy (MT). N=61 human carotid plasma samples were analyzed for inflammatory and cardiometabolic protein expression by Olink Proteomics. Statistical analyses used t-tests, linear, logistic, and robust regressions, to assess the relationship between BMI, proteomic expression, and stroke related outcomes.

Results:

The 61 subjects studied were broken into three categories: Normal weight (BMI 18.5-24.9) which contained 19 subjects, Overweight (BMI 25-30) which contained 25 subjects, and Obese (BMI $\geq$30) which contained 17 subjects. Normal BMI group was a significantly older population (mean 76 years) when compared to Overweight (mean 66 years) and Obese (mean 61 years) with significance of p=0.041 and p=0.005, respectively. When compared to Normal weight and Overweight categories, the Obese category had significantly higher levels of adenosine deaminase (ADA) expression (p=0.01 and p=0.039, respectively). Elevated levels of ADA were found to have a significant positive correlation with both infarct volume and edema volume (p=0.013 and p=0.041, respectively), and were associated with a more severe stroke (NIHSS on discharge) and greater stroke-related disability (mRS on discharge) with significance of p=0.053 and p=0.032, respectively). When controlling for age and sex, increased infarct volumes were predicted by higher ADA levels in the Obese population (p=0.009), while increased ADA levels were not predictive of increased infarct volumes in the Normal weight category.

Conclusions:

When examined according to BMI, subjects undergoing mechanical thrombectomy for ELVO demonstrate significant differences in the expression of certain plasma proteins including ADA. The protein ADA is a deaminating enzyme that degrades adenosine, which has been shown to be neuroprotective in ischemia. Levels of ADA were found to be significantly higher in the Obese population when compared to Normal or Overweight groups. Increased levels of ADA in the Obese group were predictive of increased infarct volumes. Further testing will explore the relationship of BMI and ADA on cognitive function outcomes utilizing MoCA scores at both discharge as well as 90-day follow ups. These data provide novel biomarker candidates as well as treatment targets while increasing the personalization of stroke prognosis and treatment.

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Preliminary data tracking B cell diapedesis during functional recovery in post-stroke mice

Student

Background: Molday Ion Rhodamine B (MIRB) is a superparamagnetic iron oxide (SPIO) particle, which can be used in labeling & tracking cells in animals & humans via MRI (PMID 27175074). Using MRI, cell cultures, immunohistochemistry, & behavioral analysis, we will test the hypothesis that there is a correlation between functional recovery & B cell diapedesis in specific brain regions mediating recovery. Behavioral tests such as Catwalk, Open Field, & Marble Burying will be used to analyze long-term functional recovery in mice that receive MIRB-labeled B cells 3 days prior to euthanasia.

Methods: Young male (4-6 mos) C57Bl/J6 mice were given a 60-min tMCAo & recovered for 2 or 6 weeks. At 1-week pre-euthanasia, CD19+ B cells were isolated using magnetic beads (Stem Cell Technologies) and cultured at ~30x10^6 cells (1x10^6 cells/mL) per T75 flask. Cultures were stimulated with 5µg/mL LPS. At 24 h, 12.5 µg/mL MIRB was added. At 48 h, flasks were pooled, washed, resuspended in 1xPBS, and divided into 5x10^6 B cell aliquots for daily intraperitoneal (i.p., 300 µL) injections for 3 days prior to euthanasia. Motor recovery was measured via the Catwalk (Noldus), an automated way of measuring gait in mice (PMID 27816479). For our experiments, we focused on footfall patterns & the *Regularity Index (RI)*, which quantifies normal gait. Anxiety was measured using the Superflex open field box (Omnitech). Mice are allowed free roam for 5 minutes on a floor that tracks movement. The program assigns zones, which can be analyzed for anxiety-like behavior (PMID 30535687). Marble burying is another anxiety measurement (PMID 2017455). In 10 cages, 20 black glass marbles are evenly placed on top of ~3 inches of bedding. Mice were placed in the cages & left to roam for 30 minutes. Behaviors such as digging & marble burying can be observed, and visible marbles counted and subtracted from the total. Healthy mice were used as control cohorts. GraphPad Prism analyzed behavioral data using a one-way or two-way ANOVA.

Results: Mice (n=8; 2 wk cohort, n=14; 6 wk cohort, n=4 control) were tested 1 wk after tMCAo, with the 6-wk cohort also tested at 5-wks post-stroke. Catwalk RI showed significant deficits between control mice at both 1 & 5 wks post-tMCAo (both p=0.0001). Within the 6-wk post-stroke cohort RI, there was a significant difference between mice (p=0.0002), as well as for time post-injury (p<0.0001), including 2 mice with significant decreases at 5-weeks that had no lesion visible on post-mortem MRI. Open Field revealed no significance in total distance traveled between 1 & 5 wks but again there was a significant (p<0.0001) effect of time for behavioral testing, with more time spent in the inside zone (p<0.0001) at 5-wks post-tMCAo within mice, indicative of a decline in general anxiety. Marble Burying data revealed no significance between healthy controls & injured mice. Histology is ongoing to correlate B220+ B cells with MR images of potential B cell diapedesis.

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Noncontact Optical Assessment of Disrupted Cerebral Functional Connectivity in a Piglet Model of Transient Ischemic Stroke

Student

Perinatal ischemic stroke results from the lack of blood supply to brain tissue, possibly leading to cerebral ischemic/hypoxic stress, neurological disorder, and brain network impairment. Preterm infants with ischemic stroke are prone to alterations in cerebral blood flow (CBF) and associated spontaneous low-frequency oscillations (LFOs). Assessments of LFOs provide information for better understanding of neurovascular coupling and functional networking. Such information is useful for instant monitoring and management of ischemic stroke and associated complications. However, there are no established noninvasive imaging methods for continuous monitoring of CBF alterations at the bedside in neonatal intensive care units (NICUs). An innovative camera-based speckle contrast diffuse correlation tomography (scDCT) technology has been recently developed in our laboratory, which enables noncontact, noninvasive, and high-density 3D imaging of CBF distributions in cerebral cortex.

In the present study, the capability of scDCT technique for 3D imaging of CBF distributions in a neonatal piglet model of transient ischemic stroke was demonstrated. In our scDCT technology, a galvo mirror remotely delivered coherent point near infrared light to multiple source positions for deep tissue penetration and a sCMOS camera as high-density 2D detector array to measure spatial diffuse speckle contrasts on the tissue boundary in a selected region of interest on the head. A noncontact scDCT probe was setup above the piglet head, and 25 sources were used in the selected region of interest. Movement of red blood cells in the measured tissue volume generated diffuse laser speckle fluctuations on the tissue surface, which was captured by the sCMOS camera. Power spectral density analyses of LFOs and the network connections over the brain were assessed before and after the induction of acute ischemic stroke. The stroke resulted in a substantial decrease in CBF, attenuations in resting-state LFOs over the LFO frequency band (0.01–0.08 Hz), and functional connectivity disruptions in motor and somatosensory cortices.

This pilot study demonstrated the feasibility and safety of scDCT for noninvasive detection of resting-state LFO alteration and functional connectivity disruption after stroke. The scDCT technique has many unique advanced features over other competitive technologies, including fully noncontact hardware, FEM-based image reconstruction of objects with arbitrary geometries, and a low-cost portable instrument. Thousands of pixels provided by the sCMOS camera significantly improve the sampling density and temporal/spatial resolution. The 3D imaging method reduces the partial volume effect from the overlaying skull on the deep brain. We are currently testing this fully noncontact scDCT technology for 3D imaging of brain hemodynamics in the NICU, with the ultimate goal of instantly evaluating and managing brain injury/health to improve the clinical decision and outcome.

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Completing the NIHSS Score Prior to CT Scan to Reduce Time to Acute Intervention for Stroke: A Quality Improvement Project

Faculty

Objectives: Identify any potential delay for acute intervention in our stroke alert process due to the interruption of obtaining the NIHSS score during the transfer of the patient to the CT scanner and completion of imaging in order to improve patient outcome and prognosis.

Background: Ischemic stroke is a leading cause of morbidity and mortality in the United States. Two acute stroke interventions exist, including tissue plasminogen activator (tPA) and mechanical thrombectomy. Unfortunately, both are highly time sensitive and outcomes depend on rapid intervention. The typical patient will lose 1.9 million neurons per minute during acute stroke while awaiting treatment. Therefore it is in the best interest of patients and our health care system that patients have rapid times to intervention for acute stroke.

Methods: The FOCUS-PDSA model was used to determine how our stroke alert process could be improved. The stroke alert process previously involved initiation of the NIHSS score when a patient arrived to the emergency department, viewing imaging in real time, and following the patient back to their room to complete the NIHSS score before calling for acute intervention (i.e. tPA or thrombectomy). An opportunity for improvement was identified in which the NIHSS score could be completed outside the CT scanner and prior to imaging. By eliminating interruptions in the examination, we hoped treatment with tPA and thrombectomy could be initiated soon after reviewing imaging. A multidisciplinary team was developed including members who had extensive knowledge about the process. The change in protocol was made in July 2020. Data was analyzed for 6 months before and after the protocol change to determine whether the new protocol had improved times to tPA administration and thrombectomy.

Data & Results: Times to tPA administration before and after intervention were compared. Fifty-two patients received tPA prior to the protocol change and 36 received tPA after the protocol change. The average times to tPA administration increased after the protocol change from 53.25 minutes to 63.20 minutes. We further broke this data down into four categories and found that there were 12 (27.27%) in the >60 minute group pre-protocol change and 14 (40%) in the post-protocol change groups. When analyzing the other categories, there was a shift from the 46-60 minute group to the 31-45 minute group after the protocol change. Time to thrombectomy improved after the protocol change from an average of 52.46 minutes to 43.63 minutes.

Conclusions: Door-to-needle times did not change significantly after allowing the neurology resident to complete the NIHSS score prior to CT scan. However, time to thrombectomy did improve with this protocol change. A larger sample size is required to effectively address this clinical question. This should prompt additional analysis of the barriers to rapid tPA administration in appropriate patients and warrants further investigation.

The use of blood and CSF to identify unique populations in the rapid post mortem brain using flow cytometry

Student

Background:

The purpose of the following study is to identify leukocyte populations in the brain parenchyma using advanced flow cytometry techniques. We developed a flow cytometry panel that identifies leukocytes in human blood and CSF samples and are applying it to the rapid post-mortem brain (RPM). The following study branches off of our paper on identifying leukocyte populations in stroke patients (PMID: 34250820). The blood samples from stroke patients (BACTRAC), subarachnoid hemorrhage CSF samples, and cardiovascular control samples were used to compare leukocyte populations with RPM samples.

Methods:

The analysis includes intracranial stroke patient samples (n=8), systemic stroke patient samples (n=9), cardiovascular disease control samples (n=21), subarachnoid hemorrhage CSF samples (n=2), and rapid post mortem brain tissue samples (n=4). All samples obtained were stained with a general immunophenotyping panel containing a viability stain (Ghost Dye 780), CD45, CD3, CD4, CD8, CD19, CD11b, CD14, NK1.1, CD66b, CD11c, CXCR3, and CD138. All gating and analyses were performed in FlowJo V10. The samples were down sampled and concatenated into a single file, and analyzed using t-Distributed Stochastic Neighbor Embedding (tSNE). This is an unsupervised nonlinear dimensionality reduction algorithm that enables the visualization of flow cytometry data sets in a dimension-reduced data space. Manual gating based on linage markers were defined and overlaid on the tSNE plots for confirmation of specificity. Cell populations were identified using expression profiles.

Results:

The tSNE analysis showed that CSF T cell, Monocyte and Macrophage, are similar to BACTRAC systemic and intracranial populations. CSF samples had the highest number of neutrophils (24.25%) versus BACTRAC (3.81%) and cerebrovascular control (5.33%) groups. RPM had a large population of monocytes and macrophages (14.6%) that demonstrated a more distinct expression profile when compared to the other groups. The RPM group also showed a larger unidentified population of cells (42%) that was different from the other groups. Future directions include identifying novel cells found in the rapid post mortem brain and to increase our subject numbers in order to run a statistical analysis.

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Skin Capillary Amylin Deposition Resembles Brain Amylin Vasculopathy

Faculty

OBJECTIVE

The aim of this preclinical study is to test the hypothesis that skin capillary amylin deposition correlates with cerebral small vessel amylin deposition and is higher in rats with pancreatic overexpression of amyloidogenic (human) amylin polypeptide (HIP rats) compared to wild type (WT) rats that express non-amyloidogenic rat amylin.

BACKGROUND

Human amylin is a 37 amino-acid pancreatic peptide that forms neuro-toxic aggregates that deposits in the endothelium of brain and skin capillaries of diabetic patients causing cerebral small vessel vasculopathy and peripheral neuropathy, respectively. Endothelial amylin deposition causes cell membrane injury by lipid peroxidation and production of reactive aldehydes such as 4-hydroxynonenal (4-HNE). Amylin also deposits on erythrocyte membranes and impairs tissue oxygen delivery thereby activating hypoxia pathways mediated by hypoxia inducing factor (HIF).

METHODS

Immunohistochemistry (IHC) was performed for human amylin and collagen IV in brain and skin sections of HIP and WT rats using antibodies binding amylin as well as those binding HIF -1**α** and HIF-2**α**. Amylin-4HNE adduct was measured in the skin from HIP and WT rats.

RESULTS

Brain capillaries isolated from HIP rats had 1.7 times higher amylin content compared to WT rats using Western blot with anti-amylin antibody. The immunoreactivity signal of HIF-1**α** and HIF-2**α** in skin tissue from HIP rats was 4 times higher than WT rats. Amylin-4HNE adduct formation was 2.2 times higher in HIP rats compared to WT rats. In both HIP and WT rats, there was a phenotypic similarity between brain and skin capillary amylin when co-stained for human amylin and collagen IV.

CONCLUSION

Skin capillary amylin deposition resembles brain capillary amylin deposition and indicates worse cell membrane injury and chronic hypoxia in HIP rats compared to WT rats. This study also provides preliminary evidence that a skin biopsy might reflect the extent of brain amylin vasculopathy.

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Examination of Subventricular Zone Stem Cell in Neonatal Piglet

Faculty

Domestic piglets may be an excellent model for human neonatal brain development and perinatal neurologic diseases. The gross anatomical features, including gyral pattern and distribution of gray and white matter of the neonatal piglet brain, mimic that of human infants. Also, similar to humans, the major brain growth spurt in piglets extends from the late prenatal to the postnatal period, during which the ventricular zone stem cells (SC) and their distribution through the germinal matrix system will vanish, while the SC released from the subventricular zone (SVZ) and new migration path along the newly formed vasculature will be the major contributor to the early brain development. However, the distribution and structure of the SVZ SC population and their niches are still understudied. We harvested the naive piglet brains at age of 7days and 28days after birth, sliced the brain into coronal sections, and dissected out the SVZ zone and lateral ventricular wall as whole-mount pieces. Since the SC markers are highly conservative among species, we have successfully stained the SC/progenitor cells at the SVZ zone in both coronal sections and whole-mount SVZ pieces using a battery of commercial antibodies. Extremely complicated interactions between the SVZ SC/progenitor cells and other brain regions were observed, as well as well-organized niches in the SVZ. These niches showed typical well-organized, clustered SCs in the core, with an opening to the ventricle, and are surrounded by the support cells. Better examination of the morphology and structures of SVZ niches and SCs, as well as their interaction with other cells and brain regions, will help our understanding of the SVZ-SCs' turnover, proliferation, differentiation, migration, as well as their contribution to brain development and its repair after injury.