2024 Neuroscience Clinical-Translational Research Symposium

Program & Abstract Book

NEUROSCIENCE
RESEARCH PRIORITY AREA

2024 NEUROSCIENCE CLINICAL-TRANSLATIONAL RESEARCH SYMPOSIUM

THURSDAY, OCTOBER 24 AGENDA

Healthy Kentucky Research Building

3:00-3:30pm: Check-In/Registration

3:30-5:00pm: **Research Data Blitz (5 minutes, 1 slide)**

Moderated by: Bjoern Bauer, PhD

Khine Zin Aung, MD, PhD: Exploring Clues from Causal Aspects (Mendelian Randomization): Systemic Review on the Inverse Relationship between AD/ADRD and Cancers.

Madison Blanton: Microglia-derived Peripheral Monocytes as a Window into the Brain: A Beneficial Tool for Understanding the Impact of AUD on CNS.

Ravichandra Davargaon, PhD: Amylin Vasculopathy Impairs Cerebral Aβ Efflux through Altering Cerebral Vasodilation.

Stefani Deschner: Pilot Study: Gut Function Following Experimental Cervical Spinal Cord Injury.

Diane Iradukunda, MS: Closed-Loop Thermoregulatory Manipulation of Mouse Sleep Architecture.

Rahul Joseph, MD: Low Cord Blood Progesterone Predicts Worse Neurocognitive Outcomes in Preterm Infants.

Albert Junior Nyarko: Sex Differences in the Impact of PTSD, Depression, and Sleep Apnea on Subjective Cognitive Decline Among Older Black Americans.

Sara Palacio: Extracellular Vesicles Derived from Glioblastoma After Radiation Promote Microglia-Mediated Neurotoxicity.

Meet Patel: Cdhr1a Connects the Outer Segment to Calyceal Processes via Interaction with pcdh15b, Implications for Pathogenesis of Cone Rod Dystrophy.

Cassandra Wolsh, PhD: Serum Biomarkers in Surgically Treated Degenerative Cervical Myelopathy Patients.

Anjana Subramoniam, MS: Effect of Transcutaneous Vagal Nerve Stimulation (tVNS) on Sleep Architecture in Healthy, Young Adults.

Jonathan Vincent: Differential Impact of Closed-Head Injury on CA1 and Dentate Gyrus Neuronal Functions in Mice.

5:00-6:00pm: **Neuroscience Networking & Social Hour, including Data Blitz Poster Session**

Beer, Wine, and Light Snacks provided

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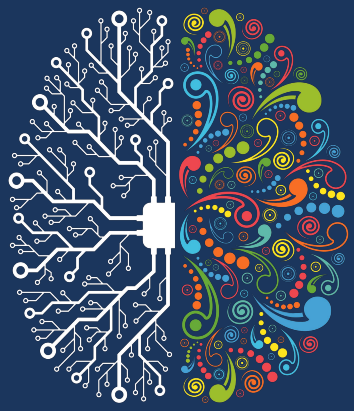
2024 NEUROSCIENCE CLINICAL-TRANSLATIONAL RESEARCH SYMPOSIUM



FRIDAY, OCTOBER 25

9:00 am

Healthy Kentucky Research Building

Room 150

Keynote Speaker

Katherine Hartmann, MD, PhD

Director, Center for Clinical & Translational Science
Associate Vice President, Clinical & Translational Science
Associate Dean, Research Development & Synergy
Professor, Obstetrics & Gynecology

"Avoiding Loss in Translation"

Dr. Katherine Hartmann is an epidemiologist and health services researcher with expertise in large community-recruited cohorts, behavioral interventions and clinical trials. Her primary research focuses on finding answers to questions that matter to women and their care providers including adverse pregnancy outcomes, uterine fibroids, pelvic floor disorders, and risks for cardiovascular disease. Her work crosses many policy-relevant areas including health outcomes, evidence-based practice, systematic reviews, cost analysis and informed medical decision-making.

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FRIDAY, OCTOBER 25 AGENDA

Healthy Kentucky Research Building (Lobby & Room 150)

8:00 - 9:00	**Check-In / Registration - HKRB Lobby**
9:00 - 9:10	**Welcome and Introduction: Larry Goldstein, MD & Linda Van Eldik, PhD**
9:10 - 10:00	**Keynote Presentation: *Katherine Hartmann, MD, PhD***
10:10 - 11:10	**Neurotrauma & Neurorehabilitation** **Moderated by: Kathryn Saatman, PhD**
	Gabriela Aparicio, PhD: Immunochemical and Transcriptomics Evidence of Schwann Cell Reprogramming in an In vitro Model of Human Peripheral Nerve Degeneration. **Velmurugan Gopal Viswanathan, DVM, PhD:** Neuron Specific LRP1 Knockout (NLKO) Mice are Protected Against Traumatic Brain Injury (TBI) Induced Mitochondrial Dysfunction. **Sarah Garcia Pava:** Gradations in Isometric Finger Extension Captured by Event-Related Changes in the EEG Mu-Beta Sensorimotor Rhythm. **Reena Kumari, PhD:** The Therapeutic Efficacy of a Well-defined Macrophage Depletion Technique Resulting in Improved Recovery after Spinal Cord Injury.
11:20 - 12:20	**Neurodegeneration** **Moderated by: Shannon Macauley, PhD**
	Xian Wu, PhD: Genetic Association Analyses of Longitudinal Cognitive Changes Related to Alzheimer's Disease in Diverse Populations. **Ting-Hsuan Lu, PhD:** Insulin Resistance and Alzheimer's Disease: Targeting Astrocyte Insulin Receptors to Address Gait Disturbances. **Zhihui Zhu, PhD:** The Sphingosine-1-phosphate Receptor 1 Antagonist Ponesimod Reduces TLR4-induced Neuroinflammation and Increases Aβ Clearance. **Akhil Pallerla:** The Role of APOE Genotype in Responses to Anti-amyloid Immunotherapy for Alzheimer's Disease.

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FRIDAY, OCTOBER 25 AGENDA

12:30 - 1:00	**Lunch** (assortment of boxed lunches will be available)
1:00 - 2:00	**Poster Session - authors at their posters**
2:05 - 3:05	**Epilepsy / Sleep** **Moderated by: Marilyn Duncan, PhD**
	Jordan Clay, MD: Ictal SPECT vs. Postictal MRI for Seizure Onset Zone Localization in Patients with Refractory Epilepsy. **Haleigh Whitlock, MS:** Sex and Genotype based Alterations in Sleep and Neuroinflammation in an Alzheimer's Disease Mouse Model. **Logan Eslinger, MD:** Improving Patient Experience in the Epilepsy Monitoring Unit: A Randomized Controlled Trial of Virtual Reality Technology. **Maxwell Lavin, MS:** Complementing EEG with Piezoelectric Motion Signals for Improved Seizure Detection and Characterization in Mouse Epilepsy Models.
3:15 - 4:15	**Stroke & Neurovascular Injury** **Moderated by: Keith Pennypacker, PhD**
	Hend Mansoor, PhD: Sex Differences in Prescription Patterns and Medication Adherence to Guideline-directed Medical Therapy Among Patients with Ischemic Stroke. **Hilaree Frazier, PhD:** A Mild Cortical Infarct Increases Neuroinflammation, Elevates Plasma Biomarkers, and Alters Hippocampal Synaptic Function in Male 5xFAD Mice. **Neha Anil:** Expression of Dementia Biomarkers in Appalachian and Non-Appalachian ELVO Patients during Thrombectomy. **Annabel McAtee:** Impact of Sex and Age on Immune Cell Profiles in Skull Bone Marrow and Dura Before and After Ischemic Stroke.
4:15	**Closing Remarks**

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Abstract Index

Data Blitz Session

Platform Sessions

Neurotrauma & Neurorehabilitation

24 Immunochemical and Transcriptomics Evidence of Schwann Cell Reprogramming in an In vitro Model of Human Peripheral Nerve Degeneration.

25 Neuron Specific LRP1 Knockout (NLKO) Mice are Protected Against Traumatic Brain Injury (TBI) Induced Mitochondrial Dysfunction.

26 Gradations in Isometric Finger Extension Captured by Event-Related Changes in the EEG Mu-Beta Sensorimotor Rhythm.

27 The Therapeutic Efficacy of a Well-defined Macrophage Depletion Technique Resulting in Improved Recovery after Spinal Cord Injury.

Neurodegeneration

29 Genetic Association Analyses of Longitudinal Cognitive Changes Related to Alzheimer's Disease in Diverse Populations.

30 Insulin Resistance and Alzheimer's Disease: Targeting Astrocyte Insulin Receptors to Address Gait Disturbances.

31 The Sphingosine-1-phosphate Receptor 1 Antagonist Ponesimod Reduces TLR4-induced Neuroinflammation and Increases Aβ Clearance.

32 The Role of APOE Genotype in Responses to Anti-amyloid Immunotherapy for Alzheimer's Disease.

Epilepsy / Sleep

34 Ictal SPECT vs. Postictal MRI for Seizure Onset Zone Localization in Patients with Refractory Epilepsy.

35 Sex and Genotype-based Alterations in Sleep and Neuroinflammation in an Alzheimer's Disease Mouse Model.

36 Improving Patient Experience in the Epilepsy Monitoring Unit: A Randomized Controlled Trial of Virtual Reality Technology.

37 Complementing EEG with Piezoelectric Motion Signals for Improved Seizure Detection and Characterization in Mouse Epilepsy Models.

Stroke & Neurovascular Injury

39 Sex Differences in Prescription Patterns and Medication Adherence to Guideline-directed Medical Therapy Among Patients with Ischemic Stroke.

40 A Mild Cortical Infarct Increases Neuroinflammation, Elevates Plasma Biomarkers, and Alters Hippocampal Synaptic Function in Male 5xFAD Mice.

41 Expression of Dementia Biomarkers in Appalachian and Non-Appalachian ELVO Patients during Thrombectomy.

42 Impact of Sex and Age on Immune Cell Profiles in Skull Bone Marrow and Dura Before and After Ischemic Stroke.

Poster Session

Addiction

45 Family History Density of Substance Use: Influence on Participant Substance Use Onset and Duration.

46 Genomic Assessment of the Prefrontal Cortex in Differential Responsivity of Sucrose Preference and Fentanyl Escalation in Sprague-Dawley Rats.

47 Unique Role of Calcium-Activated Potassium (BK) Channels After Fentanyl Self-administration in the Rat.

48 Olfactomedins May Mediate Hormonally Driven Nicotine Consumption in Women via a Negative Feedback Loop.

Neurodegeneration

50 Effect of Protective Mutation on Dynamics and Structure of APOE4: a Molecular Dynamics Simulation Study.

51 TDP-43 Citrullination: Scratching the Structural Surface on a Novel Post-Translational Modification in AD and Related Dementia.

52 Dysregulation of Transposable Elements in Alzheimer's Disease: Insights from Single-Cell RNA Sequencing of the Middle Temporal Gyrus.

53 Separation of Astrocytes from Brain Tissues via Use of GLAST and Dynabeads.

54 ß-Hydroxybutyrate Improves Mitochondrial Function in an Alzheimer's Mouse Model.

55 Investigation of EEG Slow Wave Recovery Following Sleep Disruption in a Mouse Model of Alzheimer's Disease.

56 Altering ApoE in Mice Expressing Human Amylin in the Pancreas Exacerbates Brain Microvascular and Parenchymal Amyloid Pathology.

57 Glia-Selective Replacement of APOE4 with APOE2: Response to Age and Inflammation.

58 Astrovascular Coupling in the Somatosensory Cortex of an Animal Model of Amyloidosis at Rest and During Ambulation.

59 Amyloid-Beta and Gait Dysregulation in Older 5xFAD Mice.

Neurophysiology

61 Effects of Claustrum Psychedelic Microinjections on Neuronal Excitability and Cognitive Flexibility after Cocaine Self-Administration.

62 The Regulatory Role of Asprosin in Hypertension via Oxytocin-Ptprd Signaling.

63 The Relevance of Exploring Siah-1 Targets in Retinal Development.

64 1-Deoxysphingolipids cause neurotoxicity. Can we stop them?

65 Astrocyte Ca2+ in the Dorsal Striatum Suppresses Neuronal Activity to Oppose Cue-Induced Reinstatement of Cocaine Seeking.

66 TRPA1 Deficiency Results in a Reduction of Cochlear Microphonic Amplitude After Noise Exposure.

67 PercevalHR Responses of Mixed Primary Hippocampal Cultures Exposed to Insulin are Sensitive to Extracellular Glucose Concentrations.

Neurotrauma

69 Modeling Post-Traumatic Epilepsy After Repeated Blast-Induced Traumatic Brain Injury.

70 Pathophysiological Alterations in Rats with Sepsis Following Spinal Cord Injury.

71 Temporal Dynamics of B Cell Infiltration after Contusive Traumatic Brain Injury in Mice.

72 Inducible Expression of Constitutively Active AKT Using Retrogradely Transported AAVs Improves Motor Function After Severe Spinal Cord Injury.

73 Spinal Cord Injury (SCI) Leads to an Intraspinal Inflammatory Response Including Infiltrating Blood Leucocytes.

74 Severe Hypoglycemia Causes Neuronal Degeneration and Neuroinflammation.

Other Neurologic Conditions

76 Optimizing Glyoxal as an Alternative Tissue Fixative to PFA.

77 Neurocognitive Processing Speed Enhancement from Gamma Entrainment.

78 Elucidating the Molecular Events Involved in Optic Fissure Fusion in Zebrafish Eye.

79 Perinatal Opioid Exposure and Maternal HCV Infection Compromise Microglia Development and Function.

80 Asprosin, an Analgesic Orexigenic Hormone.

81 Does Time of Administration Matter? Investigating The Effects of Exenatide, a GLP-1 Receptor Agonist, on Clock Gene Expression in the Diabetic Brain.

Stroke/Neurovascular

83 Proteomic and Demographic Comparisons of Patients Suffering from a Recurrent Ischemic Stroke.

84 The Role of Complement Component 5a Receptor 1 (CD88) Antagonist PMX205 i n Post- Stroke Depression: Clinical Findings and Therapeutic Implications.

85 Leukemia Inhibitory Factor (LIF) Improves Mitochondrial Bioenergetics after Ischemic S troke.

86 Validation of a Wearable Sensor-Based Device for Objectively Tracking Hand Function.

87 Plasma Extracellular Vesicle-Associated Markers of Endothelial Cell Activation in Cere brovascular Disease.

88 Developing Simple and Sensitive Behavioral and Vision Tests for a Mouse Model with Mild Stroke.

89 Utilizing Metabolic-Associated Protein Differences in Total and Astrocyte Enriched Ex tracellular Vesicles to Differentiate Cerebrovascular Disease.

Addendum

91 Vitamin B6 Homeostasis in Plants and Humans.

Data Blitz Session

KHINE ZIN AUNG, MD, PhD [1] • Su Su Zin, MD [2] • Xian Wu, PhD [3] • Erin Abner, PhD [4] • Peter Nelson, MD, PhD [5] • David Fardo, PhD [6] • Shama Karanth , PhD [7] • Yuriko Katsumata, PhD [8]
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Exploring clues from causal aspects (Mendelian Randomization): Systemic Review on the inverse relationship between AD/ADRD and cancers

Post Doc

According to prior published work including our own, Alzheimer's disease (AD)-type dementia is relatively less likely to develop in survivors of cancers, indicating an inverse association between the two clinical syndromes. However, this inverse association was predominantly reported from observational studies that are known to be vulnerable to potential biases and confounders and thus must be interpreted cautiously. In this study, we performed (1) a literature review and (2) two-sample Mendelian randomization (MR) to help address the causal question between AD/ADRD and cancers.

First, we conducted a literature search using PubMed, Embase, and Web of Science with an open time frame (freeze on 2024 August 1 st), and a total of 315 studies were detected and 13 papers were selected for this review after double-blinded data screening by two reviewers. Second, we conducted two-sample MR using the National Alzheimer's Coordinating Center (NACC) neuropathology (NP) data linked to whole genome sequencing (WGS) data from Alzheimer Disease Sequencing Project (ADSP), and cancer summary statistics from large genome-wide association studies (GWAS).

Results: The 13 MR studies highlighted that incidence of AD was inversely associated with neoplasms and in particular with specific cancer subtypes (such as colon, breast, endometrial, oral, bowel, lung, and prostate carcinomas, and leukemia). Fewer consistent associations were observed for the opposite direction (i.e., cancer was exposure) while glioma was associated with increased risk of AD. For Parkinson's Disease (PD), there were fewer consistent inverse associations, with some evidence of inverse relationships with specific cancers, such as high-grade serous ovarian cancer, but no significant associations with many other cancers using MR methods. Our preliminary two-sample MR analysis of neuropathology outcomes focused solely on leukemia. We observed an inverse association between leukemia and Braak NFT stages (0-2 vs. 3-6). However, neuritic plaques, Lewy bodies, and TDP-43 pathology were not significantly associated with leukemia.

Discussion: Inverse variance weighted two-sample MR analysis on leukemia supported a significant inverse association with Braak stage while robust MR approach including MR-Egger, weighted median, and mode did not support this significance, suggesting that we would need more sample size to validate this inverse association between leukemia and Braak stage. Another preliminary result of our group also reported that biological pathway-specific cancer polygenic risk scores were relatively likely to have a lower risk of AD/dementia-related phenotypes. Sample sizes, population-specific genetics, and selection of MR methods are still developing areas of this topics highlighting the need for further research using large sample sizes into the complex molecular links between neurodegenerative diseases and cancers.

Funding: McCullers Scholar 2024, **P01AG078116 and P30AG072946**

Madison Blanton* [1] • Hami Hemati*, PhD [2] • Heather True, PharmD [1] • Rupak Khadka [3] • Kathleen Grant, PhD [3] • Ilhem Messaoudi, PhD [2]
Pharmaceutical Sciences University of Kentucky [1] • Microbiology, Immunology and Molecular Genetics University of Kentucky [2] • Division of Neuroscience Oregon National Primate Research Center, Oregon Health and Science University [3]

Microglia-derived peripheral monocytes as a window into the brain: a beneficial tool for understanding the impact of AUD on CNS

Student

Alcohol consumption dysregulates the central nervous system (CNS)-resident cells, including microglia, leading to escalated use, tolerance, and dependence. Microglia are the tissue-resident macrophages of the brain, playing a key role in immune surveillance, neuronal repair, synapse pruning, and myelination. Despite their importance in research, microglia sampling from *in-vivo* models, outside of terminal sample collection, is unfeasible. Therefore, we optimized an *in-vitro* approach to differentiate peripheral blood mononuclear cells (PBMC) from non-human primates (NHP) to microglia-like cells (iMG; induced microglia). PBMC were obtained from rhesus macaques who were either ethanol naïve or after 12 months of voluntary ethanol self-administration and subjected to a 14-day differentiation protocol. The phenotypic, morphological, and functional assessment confirmed that iMG expressed canonical microglia markers, including TMEM119, P2RY12, and TREM2, and were highly phagocytic. Further, RNA-seq data revealed that the transcriptome of iMG was more closely related to that of primary microglia than that of peripheral blood monocytes. The iMG derived after chronic alcohol consumption (CAC) produced lower levels of inflammatory mediators coinciding with increased expression of genes associated with negative regulation of responses to stimuli. Contrastingly, CAC was associated with enhanced phagocytosis a possible result of increased *MARCO* expression. Taken together, these data conclude that *in-vitro* differentiation of peripheral monocytes can serve as a less-invasive mechanism to survey the interplay between CAC and microglia in non-human primates, while also demonstrating that CAC rewires the functional and transcriptional profiles of microglia. (Supported by R01AA028735-04, U01AA013510-20, R24AA019431-14, P51OD011092, F31AA031600-01A1).

Ravichandra S Davargaon, PhD [1] • Noah S Leibold, MS [1] • Nirmal Verma, PhD [1] • David K. Powel, PhD [2] • Florin Despa, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Magnetic Resonance Imaging and Spectroscopy Center University of Kentucky [2]

Amylin vasculopathy impairs cerebral Aβ efflux through altering cerebral vasodilation

Fellow

Background

Impaired interstitial fluid drainage in the brain is indicated by the presence of perivascular **β**-amyloid (A**β**) deposits and is attributed to alterations in contractility and relaxation of vascular smooth muscle cells (SMCs). The brain microvasculature in Alzheimer disease (AD) accumulates amyloid-forming amylin secreted from the pancreas. Here, we tested the hypothesis that cerebrovascular amylin deposits perturbs cerebral A**β** efflux by impairing cerebral vasodilation.

Methods

Using transgenic rats expressing amyloid-forming human amylin in the pancreas (HIP rats) (aged 16-months) and wild-type (WT) littermates that express non-amyloidogenic rat amylin, we conducted comparative analyses of cerebral blood flow (CBF), pressure myography in isolated pial arteries and vascular SMC oxidative stress experiments.

Results

Longitudinal brain MRI measurements revealed consistent structural alterations that progressed more rapidly with aging in HIP vs. WT rats, leading to 14.9% reduction in CBF in HIP rats. Plasma nitrite and nitrate, stable nitric oxide (NO) end products, were increased in HIP vs. WT by 84.7% and 24.87%, respectively. Pressure myography experiments using pial arteries showed that both WT and HIP arteries developed arterial tone (e.g. pressure-induced constriction); however, arteries from HIP rats show significant elevations (56.9-142.3%) in arterial tone compared to WT rats at physiologically-relevant intravascular pressures (e.g. 60-100 mmHg). Consistent with these results, vascular SMCs from HIP rats showed elevated (12.6% increase) lipid peroxidation, which was replicated in SMCs incubated with exogenous human amylin (29.6%). Increased lipid peroxidation contributes to oxidative stress in the vascular wall and reduces NO bioavailability, altering vasodilatory function. Both arginase activity and expression (of Arginase 1 and 2) were increased in brain microvascular lysates from HIP rats compared to those from WT by 17.6%, 63.9%, and 57.8%, respectively, suggesting arginase-NO dysregulation. A possible impact of increased blood amylin concentration on cerebrovascular arginase-NO regulation was further tested in brain microvascular lysates from rats intravenously injected with amyloid-forming human amylin (55.0% reduction in arginase activity).

Conclusion

Our results indicate perivascular A**β** deposits in the setting of AD are potentially linked to amylin vasculopathy and altered spontaneous contraction/relaxation of cerebrovascular SMCs. Future experiments will focus on delineating molecular markers of amylin-induced alterations of SMC contractile phenotype.

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Spinal Cord and Brain Injury Research Center University of Kentucky [1] • undefined University of Hawai'i [2]

Pilot Study: Gut Function Following Experimental Cervical Spinal Cord Injury

Student

Spinal cord injury (SCI) has been linked to diminished gut function, with patients citing a return to normal bowel function as a high priority. Prior studies using a left C2 hemisection (LC2Hx) model have shown microscopic pathological changes that are reversed with probiotic (pbx) administration. This study aimed to identify macroscopic functional changes following a LC2Hx, and if probiotic administration can improve functional recovery. We completed two experiments, each with six female Springer-Dawley rats, over a two week timeline. We split the first cohort into naive and LC2Hx groups and the second cohort into LC2Hx gavaged with saline and LC2Hx gavaged with pbx groups. The second cohort received daily gavage. Fecal pellets were collected over 14 days, weighed, dried for 24 hours, then re-weighed to measure the fecal water content. Rats were also housed individually for 24 hours to measure the total fecal output. Once euthanized, the colons were weighed and measured to calculate the colon weight-to-length ratio – a macroscopic marker correlated with colon histopathologic scores. In the first cohort, the LC2Hx rats produced fewer fecal pellets and had lower fecal water content at 2 weeks post-injury compared to the naive rats. Furthermore, the colon weight-to-length ratio was higher in the LC2Hx group. There was no difference in fecal water content between the pbx and saline groups in the second cohort, but the pbx group returned to pre-injury levels of pellet production at 2 weeks, while the saline group remained low. The colon weight-to-length ratio was significantly lower in the pbx group than the saline group. Both the decreased pellet production and fecal water content in the LC2Hx groups of both cohorts are consistent with decreased gut motility and subsequent constipation post-SCI. Gut inflammation increased following SCI, as evidenced by the increased colon weight-to-length ratio in the LC2Hx groups. Prior studies have shown an increased Firmicutes/Bacteroidetes ratio - an indicator of gut dysbiosis, namely an imbalance of pathogenic to commensal bacteria - which could explain this finding of increased colon inflammation. The return to normal fecal pellet production and a decrease in colon weight-to-length ratio in the pbx group suggests treating this gut dysbiosis with probiotics mitigates gut inflammation, leading to improved gut function. This study shows evidence of gut dysfunction in a cervical model, with promising results of probiotics as a possible treatment. Studies are ongoing to verify these results.

Diane Iradukunda, MS [1] • Jun Wang,PhD [1] • Dillon Huffman, PhD [2] • Sridhar Sunderam, PhD [1]
Biomedical Engineering University of Kentucky [1] • undefined Signal Solutions LLC [2]

Closed-Loop Thermoregulatory Manipulation of Mouse Sleep Architecture

Student

Sleep is a physiological process essential for overall well-being. It is well known that temperature and other environmental factors can significantly impact sleep. The prevalence of sleep disorders emphasizes the critical need for innovative and creative research in this area. Manipulating ambient temperature can alter sleep patterns in mammals. However, experimental protocols rarely manipulate temperature in response to changes in vigilance state. Here, we investigate the impact of sleep-selective thermoneutral exposure on mouse sleep architecture. By manipulating temperature in closed-loop (CL) only during sleep, we aim to understand the relationship between temperature and sleep regulation, potentially informing interventions for sleep disorders.

All animal procedures were performed with IACUC approval at the University of Kentucky. Wild-type mice aged 7-9 months (7 females, 3 males) underwent temperature manipulation using a custom thermostatic chamber equipped with infrared ceramic heating lamps. A piezoelectric motion sensor placed on the cage floor was used to detect wakefulness and sleep by analyzing the root-mean-squared values of the piezo signal to control the heating lamps. Each mouse was surgically implanted with a head-mounted preamplifier for continuous EEG and EMG recording. After two weeks of recovery, the mice acclimated to the experimental cage over two days of baseline recording. During the experiment over the next three days, cage temperature was elevated from ambient (22°C) to thermoneutral (30°C) if sleep was detected for over a minute, with brief arousals disregarded. Wakefulness longer than a minute reset the cage temperature to 22°C. This CL protocol targeted daytime sleep over three days, with sleep patterns assessed by analyzing mean bout duration and percent time spent in each vigilance state. A yoked control (YC) group experienced thermoneutral temperature elevation in sync with CL-treated mice but irrespective of sleep state, while a SHAM control group remained at 22°C throughout.

Results showed that selectively increasing cage temperature at sleep onset significantly increased total sleep ($p=0.004$) and NREM sleep ($p=0.01$) and non-significantly decreased REM sleep compared to SHAM controls. Both total sleep mean bout duration ($p=0.004$) and NREM mean bout duration ($p=0.004$) significantly increased, suggesting enhanced homeostatic sleep recovery and more stable sleep architecture. Sleep fragmentation also decreased, potentially promoting more efficient vigilance cycling. However, differences between CL and YC were not statistically significant, potentially due to limitations in the CL system's ability to rapidly adjust temperature in response to sleep or wake transitions.

This study demonstrates the feasibility of modulating sleep architecture in mice through a CL thermoregulatory approach, with potential implications for treating sleep-related disorders.

Rahul Joseph, MD [1] • Luke Jones [2] • Hong Huang, MD, PhD [3] • Thomas Curry, PhD [4] • Aric Schadler , PhD [5] • Brandon Schanbacher, MS [6] • John O'Brien, MD [7] • Peter Giannone, MD [1] • John Bauer, PhD [6]

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Low cord blood progesterone predicts worse neurocognitive outcomes in preterm infants.

Fellow

Background: Progesterone (P4) modulates placental function, maternal immune response, and fetal development, rising 5- to 10-fold in pregnant mothers in the 3rd trimester. Progesterone supplementation in pregnancies affected by short cervical length reduces risk of preterm birth. Our prior study investigated cord blood P4 levels in mostly term infants (mean 655 ng/mL). No prior studies have investigated the association between umbilical cord blood P4 and postnatal outcomes in preterm infants.

Aim: Determine perinatal level of progesterone in preterm infants and investigate its relation to neurodevelopmental outcomes.
Methods: This study was a secondary analysis of the INDEED trial. We enrolled 216 infants with gestational age <31 0/7 weeks (mean 27 0/7) born at the University of Kentucky. We collected blood samples from cord blood as well as newborns at NICU admission and 24 hours of life. We analyzed samples using a CLIA certified progesterone assay through our Maternal-Fetal Medicine department. Samples were then correlated with individual infant scores on follow up neurodevelopmental assessments, including 3 month TIMP, 6 month EMPP, MCHAT and CBCL T-score at 24 months, and Bayley Scales of Infant Development III at 12, 24, and 36 months. Infants were stratified into two groups for analysis: those with cord blood P4 levels >1 standard deviation (SD) lower than our previously identified term infant mean (<287.8 ng/mL, considered low cord blood P4) and those with cord blood P4 <1 SD below the term infant mean and higher (>287.8 ng/mL).

Results: Cord blood samples were collected on 66.7% (144) of total subjects enrolled. Of those, 6.9% (10) were under the limit of detection of our P4 assay. Cord blood levels significantly increased with gestational age (GA) (p=0.0034), but serum levels decreased 10-fold by 24 hours of life (estimated blood half-life of <6h in neonates). Cord blood P4 levels were >1 SD lower than the term infant mean in 52% (75) of subjects (<287.8 ng/mL). Lower GA was associated with significantly worsened measures of neurodevelopment including head circumference at 28 days of life (p<0.0001), lower 3 month TIMP z-score (p=0.0269), higher 6 month EMPP score (p<0.0001), and lower Bayley III scores at 12 and 24 months in domains of cognition, language, and motor development. In preterm infants with low cord blood P4 levels, 24 month Bayley III social-emotional, cognitive, and general adaptive composite scores were significantly lower than in preterm infants with higher levels (>287.8 ng/mL).

Conclusions: Birth GA was strongly associated with both umbilical cord blood P4 levels and neurodevelopmental outcomes. Additionally, low cord blood P4 levels were predictive of worse neurocognitive outcomes in preterm infants in this study. Further investigation of the impact of progesterone on early life brain development, and potential replacement therapeutic strategies, are warranted.

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Sex Differences in the Impact of PTSD, Depression, and Sleep Apnea on Subjective Cognitive Decline Among Older Black Americans

Student

Chronic conditions are increasingly recognized as substantial risk factors for Alzheimer's disease and related dementia (ADRD), with emerging evidence indicating sex-specific vulnerabilities. While females are disproportionately affected by ADRD, there is a paucity of research examining sex differences in the impact of chronic illnesses on cognitive decline, particularly among Black Americans who are more likely to suffer from chronic diseases that are known precursors of ADRD. We investigated sex differences in the association between PTSD, depression, sleep apnea, and subjective cognitive decline (SCD) among older Black American males and females. We analyzed data from 231 participants (168 females; M_{age} = 75.26, SD_{age} = 7.49; mean years of education = 15.48) who are part of the University of Kentucky ADRC cohort. Our analysis revealed, that overall, PTSD was the strongest and only significant predictor of SCD (*b* = .342, *p* = .002). Further analysis by sex showed that for males, both PTSD (*b* = 0.415, *p* = 0.007) and sleep apnea (*b* = 0.213, *p* = 0.009) were significant predictors of SCD. For females, PTSD was the only statistically significant predictor (*b*= 0.387, *p* = 0.019). These findings emphasize the complexity of factors contributing to cognitive decline and highlight the need to account for sex differences in research on aging and brain health. The sex-specific findings, particularly the significance of sleep apnea in males, indicate that tailored interventions may be necessary when addressing brain health in aging Black Americans.

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Extracellular Vesicles Derived from Glioblastoma After Radiation Promote Microglia-Mediated Neurotoxicity

Student

Little is known about the underlying mechanisms of glioblastoma (GBM) and/or therapy-derived cognitive impairment. Our data indicates that GBM patients exhibit higher numbers of extracellular vesicles (EVs) compared to non-cancer patients and levels of EVs release are increased after radiation therapy. Importantly, these radiation-derived EVs (named Redox EVs), contain high levels of highly reactive 4-hydroxynonenal (4HNE), which participates in multiple pathological processes. Additionally, GBM patients experience cognitive changes both before and after their treatments. Given that EVs can function as messengers between cells, we seek to elucidate if GBM-derived Redox EVs trigger molecular mechanisms within glial cells that induce neurotoxicity. As a first step, we evaluated if microglia cells (HMC3) would uptake Redox EVs. EVs were collected from LN18-RFP, a GBM cell line transfected to express RFP in the plasma membrane, specifically in phosphatidylserine. After adding the EVs to microglia cells, confocal images showed that EVs are taken up within minutes of exposure and they spread evenly throughout the cells. To determine if Redox EVs

cause microglial activation, we treated HMC3 cells with Redox EVs and monitored their morphology as well as cytokines and H_2O_2 levels in the medium. Results showed that Redox EVs from LN18 cells caused 1) changes from ramified to ameboid morphology, 2) a significant increase in some cytokines and 3) a significant increment in H_2O_2 production as early as 3h and continued to increase at 24h. These data suggest that Redox EVs activate microglial cells that in turn release ROS and cytokines. To probe whether H_2O_2 is toxic to neuronal cells, Redox EVs were added to co-culturing chambers containing HMC3 cells and neuron cells (HCN2) for 48h. Cell viability of HCN2 cells was significantly reduced after co-culturing with Redox EVs-activated HMC3 cells. Importantly, the viability of HCN2 cells was rescued by pre-treating them with catalase. Next, we tested the effect of GBM-derived Redox EVs *in vivo*. We developed intracranial injections in mice as a delivery method for GL261-derived Redox EVs and subsequently those mice were tested with Novel Object Recognition and their brain was collected for IHC. Mice injected with Redox EVs showed altered memory and DNA damage in their cerebral tissue. Finally, we developed a clinically relevant orthotopic GBM model to study GBM- and GBM treatment-derived cognitive decline. Overall data shows that 1. GBM-derived RedoxEVs are taken up by microglia and induce morphological and secretory changes; 2.H_2O_2 released from microglia treated with EV could be a key for RedoxEV-mediated neuronal death and 3.GBM-derived RedoxEVs alter cognitive behavior in mice and cause DNA damage in the brain.

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Cdhr1a connects the outer segment to calyceal processes via interaction with pcdh15b, implications for pathogenesis of cone rod dystrophy

Student

Purpose : Mutations in CDHR1, a photoreceptor specific cadherin lead to cone rod dystrophy. Mice and xenopus Cdhr1 mutant models exhibit abnormal outer segment (OS) morphology and photoreceptor (PRC) degeneration. However, the function of CDHR1 underlying pathogenesis remains unknown. By using a cone dominant zebrafish model, we explored how loss of Cdhr1a function leads to cone rod dystrophy and also discovered that Cdhr1a interacts with Pcdh15b thus physically linking the outer segment with calyceal processes (CP).

Methods : To establish a zebrafish model of cone rod dystrophy we generated a CRISPR knock-out line for Cdhr1a (Cdhr1aD173) and verified it to be null using Immunofluorescence (IF). Cdhr1a KO maternal zygotes at 5 days post fertilization (dpf), 15dpf, 30dpf, 90dpf, and 180dpf were analyzed using confocal microscopy. Prph2 staining was used to demarcate OS morphology while fluorescent peanut and wheat germ agglutinins were used to distinguish between cones and rods. To examine subcellular localization, IF imaging for Cdhr1a and Pcdh15 was visualized using structured illumination microscopy (SIM). Finally, biochemical interactions between Cdhr1a and Pcdh15b were tested by coimmunoprecipitation and cell aggregation assay using HEK293T and K562 cells.

Results : Cdhr1a mutants exhibit minor gross cone OS morphology defects starting at 15dpf and severe OS disruption by 3 months while rod OS defects appeared at 3 months. Using SIM we observed juxtaposition of Cdhr1a at the leading edge of OS with Pcdh15b localizing to the CP of inner segments in zebrafish, human, macaque, xenopus, mouse, and gerbil PRCs. Additionally, coimmunoprecipitation in HEK293T cells confirmed cadherin-based interactions between Cdhr1a and Pcdh15b while co-culturing Cdhr1a and Pcdh15b transfected K562 cells led to cell aggregation indicating functional extracellular interactions. Finally, using SIM, we correlated the disruption of CP attachments to cone OS degeneration in the Cdhr1aD173 line.

Conclusions : Phenotypes observed in the Cdhr1aD173 phenocopy cone-rod dystrophy observed in patients. Our discovery of the interaction between Cdhr1a and Pcdh15b as being required for OS-CP interactions suggests a novel mechanism for pathogenesis of cone rod dystrophy.

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Serum Biomarkers in Surgically Treated Degenerative Cervical Myelopathy Patients

Post Doc

Degenerative cervical myelopathy (DCM) is the largest cause of spinal cord injury in adults. Decompression surgery is the standard treatment but surgery does not always result in a patient improvement. The goal is to identify serum based biomarkers that associate with the quality of functional recovery following surgery to appropriately recommend future patients as candidates for surgery treatment.Here, we have screened for markers of spinal damage in serum from DCM patients before receiving decompression surgery and 6 months after surgery. These markers include Glial fibrillary acidic protein (GFAP), Apolipoprotein E (ApoE), Neuron-Specific Enolase (NSE), Neurofilament Light Chain (Nf-L), Amyloid Beta Peptide (AB40, AB42) and others. Functional recovery is measured here by modified Japanese Orthopaedic Association scale (mJOA) score. We hypothesize that an increase in markers for neuronal damage before surgery will correlate to lower recovery rate and correlate to increased severity before surgery, as measured by mJOA. Utilizing a biomarker panel to predict functional recovery will help identify patients for surgical treatment. Eventually, these biomarkers may be utilized as a diagnostic tool to identify early signs of spinal cord damage in DCM as well as other spinal cord injuries. Serum based diagnostics will be more accessible to patients, allow earlier identification of disease so patients can get treatment as soon as possible, and aid physicians in recommending the correct treatment following spinal cord injury. Our goal is to eventually provide a non-invasive, accessible and cost-effective diagnostic tool that will facilitate earlier and more accurate prognosis of disease progression and treatment outcomes.

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Effect of Transcutaneous vagal nerve stimulation (tVNS) on sleep architecture in healthy, young adults.

Student

Sleep is a universal, multi-faceted behavior. Most of the previous sleep research has been focused on the neural processes occurring in the brain during sleep. However, complementary Autonomic Nervous System (ANS) fluctuations have been recorded across sleep stages (NREM and REM) (Cellini *et al.*, 2016; Whitehurst et al., 2016). Most of what we know is correlational, warranting the need to understand the causal effect of ANS on sleep. One key pathway through which the ANS modulates sleep is *via* the vagus nerve. Transcutaneous vagal nerve stimulation (tVNS) is a novel, non-invasive, technique that can be used during sleep to target the vagus nerve. Usage of tVNS has been mostly limited to studies conducted during the wake. Studies examining the impact of tVNS on sleep in healthy adults are scarce. The main aim of this project is to examine the impact of tVNS on sleep architecture.

The Institutional Review Board of the University of Kentucky approved all experimental procedures. 36 young (aged 18-65; 18M,18F), healthy participants engaged in a two-week, within-subject sham-controlled and counterbalanced study, spending two nights in the sleep lab. Each participant slept with polysomnography, and stimulation conditions were counterbalanced across the two visits. Polysomnography was monitored in real time and tVNS stimulation (active or sham) began once NREM Stage 2 onset was detected and continued for ninety minutes. Paired t-tests were used to compare the means of sleep architectural variables across the active and sham tVNS conditions within the same participants. Additionally, Pearson's correlation assessed the bivariate associations between the stimulation intensity and the sleep architectural variables.

We did not find any significant differences in the sleep architectural changes across the active and sham nights. Interestingly, participants with higher stimulation intensity exhibited reduced NREM Stage 3 minutes ($r=-0.324$; $p=0.05$), and increased NREM Stage 2 percentage ($r=-0.377$; $p<0.05$). Additionally, participants with higher stimulation intensity also showed increased wake minutes ($r=-0.367$; $p<0.05$) in the second quartile. The results showcase the nuanced effect of tVNS on sleep architecture. Future directions include assessing EEG spectral components during tVNS active and sham nights.

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Differential Impact of Closed-Head Injury on CA1 and Dentate Gyrus Neuronal Functions in Mice

Student

Objective: This study was designed to evaluate hippocampal neuronal functions, specifically targeting basal synaptic strength, presynaptic excitability, and population spike threshold at 1 week, 3 weeks, and 6 weeks after a closed-head injury (CHI) mouse model of traumatic brain injury (TBI).

Methods: 4 month old wild-type (C57BL/6) male mice underwent either a sham procedure or CHI to model TBI. We assessed neuronal function within the CA1 and dentate gyrus (DG) regions of the hippocampus at 1 week, 3 weeks, and 6 weeks post-injury using extracellular field potential recordings. Our evaluations focused on measuring basal synaptic strength, presynaptic excitability, and population spike threshold via input-output curves.

Results: Preliminary findings indicate that CHI mice exhibited alterations in hippocampal neuronal functions compared to sham controls at 6 weeks post-injury. In the CA1, CHI mice demonstrated a decrease in basal synaptic strength concurrent with a reduced population spike threshold, while presynaptic excitability was unaffected. In the DG, basal synaptic strength and presynaptic excitability remained unchanged, but population spike threshold was reduced.

Conclusion: The differential effects observed between the CA1 and DG highlight the nuanced vulnerability of hippocampal circuits to injury, suggesting that TBI induces a multifaceted disruption of synaptic homeostasis. These findings not only deepen our understanding of the pathophysiological consequences of TBI but also emphasize the critical need for targeted therapeutic strategies that address the specific neuronal dysfunctions associated with different hippocampal regions.

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Platform Sessions

Neurotrauma & Neurorehabilitation

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Immunochemical and transcriptomics evidence of Schwann cell reprogramming in an in vitro model of human peripheral nerve degeneration

Post Doc

Schwann cells (SCs) are activated upon nerve injury to acquire a repair phenotype able to promote axon regrowth. However, scarce information is available on the SC repair phenotype in humans. The goal of this project was to use immunostaining, microscopy, and spatial transcriptomics analysis to investigate injury-related responses in cells from human nerves degenerated in vitro.

We optimized methods for the culturing of isolated fascicles and whole nerve segments (floating explants) to recapitulate key events associated with the process of Wallerian degeneration such as SC activation. Microscopy analysis was done to characterize the cellular composition of the human nerves as well as the injury response.

A comparison of intact (uncultured) and cultured (degenerated) nerves stained with Ki67 antibodies provided evidence of cell proliferation in a subset of endoneurial cells, possibly SCs, in degenerated nerve segments without signs of apoptotic cell death. However, myelin and axon degeneration were both incomplete even after 2 weeks of culturing in vitro, as determined by measuring the expression of myelin protein zero and neurofilament, respectively. Spatial transcriptomics analysis of intact and degenerated tissues revealed dramatic changes in the gene expression profiles of all cell clusters particularly those consisting of or including SCs. Curiously, cellular reprogramming was not restricted to SC-containing clusters. Cells from epineurial and perineurial areas also reprogrammed their transcriptome after culturing in vitro.

Overall, we found that in vitro degeneration partially recapitulates human SC responses to injury, including myelin degradation and conversion into repair cells. This model may prove useful to investigate the complex molecular and cellular responses of human peripheral nerve cells after trauma or degenerative disease.

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Neuron specific LRP1 knockout (NLKO) mice are protected against traumatic brain injury (TBI) induced mitochondrial dysfunction.

Staff

Millions of individuals experience persistent cognitive deficits following traumatic brain injury (TBI), yet no approved treatment exists to date. Emerging evidence suggests that strategies aimed to reducing mitochondrial reactive oxygen species (ROS) production and maintaining mitochondrial bioenergetics post-TBI are neuroprotective. Our previous studies with Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) knockout (LKO) cells, demonstrated resilience to oxidative stress-induced mitochondrial dysfunction. Based on these findings, we developed two mouse models to investigate whether neuron-specific LKO (NLKO) could confer neuroprotection in a controlled cortical injury (CCI) model.

Neuron-specific LRP1 knockout (NLKO) mice were generated by crossing Camk2a-Cre with LRP1 $^{fl/fl}$ (LRP1$^{fl/fl}$::Camk2a-Cre$^{+/-}$). Successful NLKO generation was confirmed through immunostaining of brain sections for LRP1, as well as Western blot analysis. To evaluate the neuroprotective effect in NLKO, mitochondrial bioenergetics were assessed in control (LRP1 $^{fl/fl}$) and NLKO mouse brains by measuring the oxygen consumption rates (OCR) using the Seahorse XF analyzer, 24 hours post-injury. Mitochondrial dysfunction was significantly evident in the control-CCI group, whereas NLKO mice showed protection against mitochondrial damage compared to their respective sham group. Consistent with these functional assessments, Western blot analysis of brain lysates revealed a significant decrease in mitochondrial complex I protein expression in the control group but not in the NLKO group following injury. Additionally, mitochondrial complex II protein expression was elevated in NLKO mice compared to the control group even without injury, while no changes were observed in complexes III, IV, or mitochondrial transcription factor A (TFAM).

To further investigate mitochondrial dynamics and their correlation with function, we generated Cre-inducible, neuron-specific mitochondrial reporter Dendra2-green (mtD2g) mice in the NLKO background (NLKO-NmtD2; LRP1$^{fl/fl}$::mtD2$^{fl/-}$::Cre$^{+/-}$), with wild-type NmtD2 (mtD2$^{fl/fl}$::CaMK2a-Cre$^{+/-}$) serving as a control. Mitochondrial dynamics analysis from sham and CCI brain sections indicated that mitochondria in the NLKO-NmtD2 brain were healthier (characterized by a long and slender morphology) compared to NmtD2 control mitochondria (short and bald morphology) after injury. Furthermore, total mitochondrial volume was significantly reduced in NmtD2 compared to NLKO-NmtD2 mice post-injury. Overall, NLKO mice demonstrated protection against TBI, likely due in part to their ability to maintain mitochondrial homeostasis following injury.

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Gradations in Isometric Finger Extension Captured by Event-Related Changes in the EEG Mu-Beta Sensorimotor Rhythm

Student

Keywords: EEG, event-related desynchronization, brain-computer interface, graded motor effort

Brain-computer interfaces (BCIs) offer a revolutionary approach to assist individuals with disabilities from neuromuscular injuries or neurodegenerative diseases by directly translating brain signals into commands for external devices. BCIs commonly rely on non-invasive electroencephalography (EEG) to determine user intent. But the limited ability of EEG to differentiate between graded levels of effort beyond simple binary commands (e.g., hand movement vs. rest), poses a significant challenge. To address this limitation, we investigated EEG features indicative of graded motor effort associated with a finger extension task, which is critical for individuals with hand impairment.

With prior IRB approval and informed consent, 12 right-hand dominant subjects without neurological or physical impairments were asked to extend their fingers to a certain target level (no-go/rest, low, medium, or high) when prompted by a visual cue. Each session comprised 12 runs of multiple trials, alternating between the two hands. Each target was presented 4 times in random order within each run to prevent subjects from anticipating the task. Data from 32 EEG electrodes and bipolar EMG from both extensor carpi radialis muscles were recorded at 256 Hz. Event-related desynchronization (ERD) of the mu-beta (8-30 Hz) EEG power during each movement trial relative to the median of all pre-trial periods was computed at each scalp location. Then, the norm of the resulting ERD vector was calculated as a scalar measure of the ERD's strength for each target extension level. A test was performed to assess whether the ERD strength increased monotonically with the target extension level in each subject.

The mu-beta ERD strength increased monotonically from no-go to high finger extension in 7/12 participants on the left hand, a proportion greater than chance ($p=0.0143$), and in 6/12 participants on the right hand ($p=0.054$). These trends underscore the potential for deriving graded volitional signals indicative of fine motor control, linked to a progressive increase in cortical recruitment correlated with extensor activity. However, they also emphasize the necessity for personalized BCI therapies to accommodate individual differences.

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The therapeutic efficacy of a well-defined macrophage depletion technique resulting in improved recovery after spinal cord injury: Independent replica

Staff

Abstract: Spinal cord injury (SCI) triggers an intraspinal inflammatory response that contributes to secondary injury and neurodegeneration. Here, we attempted to replicate and independently verify the therapeutic potential of hematogenous macrophage (MØ) depletion for SCI. Specifically, we selectively depleted peripheral MØ using clodronate liposomes in a rat model of SCI. 10–12-week-old female Wistar rats received T9 contusion SCI (175 Kdyn) to model clinical SCI. Rats received intravenous injections of vehicle or liposome-encapsulated clodronate (2 mL of 7 mg/mL anionic) at 1, 3- and 6-days post-injury (dpi). We used standardized behavioral (Basso, Beattie, and Bresnahan locomotor test, horizontal ladder walk test, Catwalk XT) and neuropathological analyses for up to 8 weeks post SCI in 4 independent cohorts. Clodronate treatment significantly reduced intraspinal macrophage ($CD68^+$, IBA 1^+ and $CD11b^+$ cells) infiltration at 7dpi. Clodronate treatment significantly improved locomotor function in treated animals. Concordantly, we observed significant increase in tissue sparing in the spinal cords of clodronate-treated animals.

Next, we explored if extended clodronate dosing (1, 3, 6, 9, 13-dpi) efficiently depletes intraspinal macrophages. We observed a significant decrease in intraspinal $CD68^+$ macrophages but no differences were seen for other macrophages markers (IBA 1^+ and $CD11b^+$ cells) in clodronate treated animals when compared to control animals at 14 dpi. Dosing animals with clodronate for a second week post-SCI did not worsen outcomes, though, plasma levels of endotoxin were significantly higher in clodronate treated animals compared to control at 14 days post SCI. This difference in plasma endotoxin level at 14 dpi subsided at 42 dpi. We observed no significant differences in locomotor recovery between clodronate and vehicle treated groups at 14dpi, similar to our previous observation when treatment was delivered for only 1-week post SCI. Based on these preliminary results, we will study whether increased dosing results in improved locomotor recovery at chronic timepoints after injury and will examine sex as a biological variable.

Our observations implicate the crucial role of hematogenous MØ in secondary injury progression post-SCI. Furthermore, our results are consistence with previous observations made by an independent laboratory several decades before. Thus, our independent replication validates macrophage depletion as an adjunct therapy post-SCI.

Key Words: neuroinflammation; macrophages (MØ); regeneration; spinal cord injury (SCI); liposomes; immunosuppression.

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Neurodegeneration

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Genetic Association Analyses of Longitudinal Cognitive Changes Related to Alzheimer's Disease in Diverse Populations

Fellow

Background

Late-onset Alzheimer's disease (LOAD) is highly heritable. Recent research suggested that population-specific LOAD genetic risks may exist. The Mini-Mental State Examination (MMSE; a measure of global cognitive function) has been commonly used to monitor AD-related cognitive changes. MMSE raw scores have a strong ceiling effect (upper limit = 30 points). In a previous study, we demonstrated that the Tobit model utilizing ceiling information for estimation is a superior approach compared to the linear model. In this study, we aim to employ Tobit modeling to investigate associations of single nucleotide variants (SNVs) with MMSE scores to identify population-specific genetic risks.

Method

The phenotype data were drawn from the National Alzheimer's Coordinating Center (NACC) Uniform Data Set (UDS) September 2022 data freeze. The genotype data were obtained from the Alzheimer's Disease Genetics Consortium (ADGC). Alongside self-reported race/ethnicity, we applied the principal component analysis (PCA) and uniform manifold approximation and projection (UMAP) to identify genetic-ancestry (GA) groups (Fig. 1). Within each GA group, we performed association analyses of 82 AD-related SNVs identified by a genome-wide association study (Bellenguez et al., 2022), on MMSE using mixed-effect Tobit modeling, adjusting for age at baseline, sex, education, and 2-4 PC scores. We utilized Bonferroni correction to set $P = 6.10 \times 10^{-4}$ for multiple testing.

Result

Three GA groups were included in analyses: non-Hispanic White (NHW, n = 15,112), African American (AA, n = 2,392), and Hispanic (n = 1,159). Genetic variants were associated with MMSE, as shown in Table 1. At baseline, in NHW, genes *CR1, SORT1, PRKD3,*

BIN1, INPPSD, MME, UNC5CL, CD2AP, TMEM106B, SPDYE, USP6NL, MS4A4A, EED, SLC24A4, SPPL2A, MAF, PLCG2, and ABCA7 were associated with initial cognitive performance (ICP). In contrast, only *BIN1* showed an association with ICP in AA, while *LILRB2* was associated with ICP in Hispanics. Further, *CR1* in NHW, *NCK2* in AA, and *EED* in Hispanics were associated with changes in MMSE scores over time.

Conclusion

Our findings underscore the importance of considering population-specific genetic variants when studying AD-related longitudinal cognitive changes. Novel genetic risks may vary across GA groups and be further revealed in future studies.

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Insulin Resistance and Alzheimer's Disease: Targeting Astrocyte Insulin Receptors to Address Gait Disturbances

Post Doc

Alzheimer’s disease, a leading cause of dementia, leads to memory loss, confusion and mobility issues. Currently an estimated 5 to 7 million older Americans are affected, with this number potentially rising to 13.8 million by 2060, highlighting the urgent need for better treatments. Besides cognitive decline, Alzheimer’s patients often suffer from central insulin resistance. Insulin is vital for brain function, regulating energy use and impair the process of clearance of beta-amyloid, a protein that forms harmful plaques in Alzheimer’s patients. Astrocytes, a type of glial cell in the brain, contain insulin receptors that help manage glucose levels. Disrupted insulin signaling in these cells impairs glucose metabolism and may contribute to Alzheimer’s progression. Here we overexpressed a truncated human insulin receptor beta-subunit (hIR-beta) with constitutive activity in somatosensory astrocytes in the 5XFAD model (mice model of Alzheimer’s disease) independent to the extracellular insulin. Using advanced two-photon microscopes combined with GCaMP Ca^{2+} sensors, we examined network performance in somatosensory cortex in the aging mice. We investigated the neurovascular coupling, insulin-astrocytic network at rest and during ambulation. Overexpression of hIR-beta in astrocytes improved network connectivity and neurovascular coupling. This improvement could help recover impaired brain function and slow disease progression. This approach appears valuable for addressing dysregulated gait and may also benefit other brain regions, potentially improving overall cognitive function.

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The sphingosine-1-phosphate receptor 1 antagonist Ponesimod reduces TLR4-induced neuroinflammation and increases Aβ clearance

Staff

ABSTRACT

Background: Previously, we showed that the sphingosine-1-phosphate (S1P) transporter spinster 2 (Spns2) mediates activation of microglia in response to amyloid peptide A**β**. Here, we investigated if Ponesimod, a functional antagonist specific for the S1P receptor 1 (S1PR1), could prevent A**β**-induced activation of microglia and Alzheimer's disease (AD) pathology. **Methods:** We used primary cultures of mixed glia and pure microglia as well as the 5XFAD mouse model to determine the effect of A**β** and Ponesimod on glial activation, A**β** phagocytosis, cytokine levels and activation of proinflammatory cell signaling pathways, and AD pathology and cognitive performance.

Results: In primary cell cultures of astrocytes and microglia, oligomeric A**β**42 increased the levels of TLR4 and S1PR1 and induced the formation of a complex between the two receptors as shown by proximity ligation assays (PLAs) and co-immunoprecipitation experiments. Ponesimod prevented the A**β**-induced increase of TLR4 and S1PR1 as well as reduced the number of PLA signals and the amount of (co-) immunoprecipitated TLR4 and S1PR1. A**β**42 activated the pro-inflammatory signaling pathways Stat1 and p38 MAPK, which was prevented by Ponesimod, while Stat6 was activated by Ponesimod. Consistent with Stat6 activation, Ponesimod increased phagocytosis of A**β**42 in microglia in vitro. In comparison, FTY720, a functional antagonist of several S1P receptors, did not enhance phagocytosis of A**β**42. In 5XFAD mice, Ponesimod decreased TNF-**α** and CXCL10, two proinflammatory cytokines activating TLR4 and Stat1, while it increased the level of IL-33, an anti-inflammatory cytokine that activates Stat6 and induces A**β** phagocytosis in microglia. Consistent with reduced neuroinflammation and increased phagocytosis, Ponesimod decreased the number of IBA-1 (+) microglia and GFAP (+) astrocytes, and the size and number of amyloid plaques, while it improved spatial memory measured by a Y-maze test.

Conclusion: Targeting S1PR1 with Ponesimod is a promising therapeutic approach to reprogram microglia, reduce neuroinflammation, and increase A**β** clearance in AD

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The role of APOE genotype in responses to anti-amyloid immunotherapy for Alzheimer's Disease

Student

The traditional approval of Lecanemab (LEQEMBI, Biogen) by the FDA was a landmark in the treatment of Alzheimer's disease (AD), currently the 7th leading cause of death and the leading cause of dementia in the United States. Lecanemab, an anti-amyloid**β** (A**β**) monoclonal antibody, is the first disease modifying therapy for AD, slowing symptomatic progression by 6 months over the treatment course. While these drugs are beneficial in reducing dementia progression, a key adverse effect is of these drugs are amyloid related imaging abnormalities (ARIA). ARIA are findings of fluid effusion, edema, and microhemorrhages on MRI described in each major anti-amyloid antibody trial, and have been associated with severe symptoms and even death. While the cause of ARIA remains unknown, there is a strong link between ARIA and carriage of the E4 variant of Apolipoprotein E (*APOE*) . E4 increases the risk of AD up to 15-fold and the rate of ARIA is 2-3 times higher in this patient population. With ARIA more specifically affecting the highest risk individuals for AD, it is important to understand how E4 increases the risk for ARIA. However, a lack of preclinical models hinders this discovery; to date the majority of studies done on anti-amyloid therapies have been conducted in preclinical models of amyloidosis that express only murine *APOE,* which is significantly different from human *APOE*. For this reason, we here propose to utilize mice expressing humanized *APOE* (EFAD) in an attempt to develop a reliable and translatable model system of E4-associated ARIA. In an initial feasibility study, 11 E4FAD mice were given an acute treatment course with either chimeric aducanumab(chAdu), or a saline control. These mice underwent MRI scanning, and post mortem-brain tissue was collected for immunohistochemistry. The results showed a non-significant trend toward a decrease in total amyloid count normalized to area between chimeric aducanumab treated animals compared to saline controls. Additionally, while total counts of activated microglia and brain resident macrophages, measured with CD68, did not show significant differences between the two groups, we did observe an increase in CD68+ cell colocalization to amyloid plaques and with vascular deposits of Ab. These results may indicate a potential increase in phagocytic activity of microglia and perivascular macrophages with aducanumab treatment. Prussian Blue staining of spaced sections in these mice has also demonstrated microbleed occurrence in the E4FAD mice. This feasibility study is part of a larger ongoing study in which EFAD mice homozygous for E2, E3, and E4 are receiving 12 weeks of chAdu, IgG isotype control, or saline injection. As a part of this study, mice are undergoing MRI scans, and brains, livers, and splenic immune cells are being harvested for use in downstream immunohistochemistry, metablomics, transcriptomics, and flow cytometric analyses.

Epilepsy / Sleep

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Ictal SPECT vs. Postictal MRI for Seizure Onset Zone Localization in Patients with Refractory Epilepsy

Faculty

Purpose: 35% of patients with epilepsy are refractory to anti-seizure medications and could benefit from epilepsy surgery if the seizure onset zone (SOZ) can be localized. The presurgical evaluation of epilepsy aims to identify the SOZ, and determine if there is need for further investigation, such as invasive stereotactic EEG (sEEG) monitoring. This presurgical evaluation includes analysis of clinical semiology, ictal EEG, MRI, and PET but some complex patients require further workup with ictal SPECT. Ictal SPECT produces images of brain perfusion representing the time of radiotracer injection, and focal hyperperfusion can assist in localizing the SOZ. However, this study requires rapid injection within seconds of seizure onset. Failure of ictal SPECT often occurs due to not injecting (e.g., seizures occurring outside the window of radiotracer availability) or delayed injections. Postictal MRI using arterial spin labeling (ASL) is a novel proposal for SOZ localization. In the minutes after a seizure, postictal ASL can quantify perfusion changes that may provide a SOZ biomarker. The purpose of this study is to compare a novel postictal ASL workflow versus established ictal SPECT, thus exploring the clinical value of postictal ASL.

Materials & Methods: Patients admitted for an ictal SPECT study were recruited. After consent, MR-conditional EEG electrodes were applied. Standard clinical protocol was followed for ictal SPECT, generating images subtracted from a baseline scan. MRI, consisting of MPRAGE and PCASL, occurred at two time points: a baseline scan on the day of admission and postictally within 20-90 minutes after a seizure. Postictal ASL images were processed using AFNI and FSL to generate calibrated, co-registered maps, subtracted from baseline, and thresholded to display a maximum of 1% hypoperfusion and hyperperfusion. SOZ was determined by expert consensus, and if available, sEEG results.

Results: 15 patients were recruited, of which 11 had seizures, producing 23 total seizures. Ictal SPECT was achieved in 8/23 (35%) seizures and postictal ASL in 10/23 (43%). Ictal SPECT was concordant with the correct SOZ in 3/8 injected seizures (38%), while postictal ASL was concordant in 6/10 (60%). Postictal ASL captured more seizures and proved higher concordance than ictal SPECT.

Conclusion: In our cohort, postictal ASL outperformed ictal SPECT in both feasibility and accuracy for SOZ localization in refractory epilepsy. These early results suggest ASL could add value if integrated into presurgical evaluation of refractory epilepsy.

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Sex and Genotype based Alterations in Sleep and Neuroinflammation in an Alzheimer's Disease Mouse Model

Staff

Alzheimer's disease (AD) is a neurodegenerative disorder affecting nearly 7 million Americans, two thirds of whom are women. Early in disease progression, years before the onset of cognition deficits, AD patients often exhibit disrupted sleep patterns, including nighttime awakenings, daytime napping, and sundowning— agitation and hyperactivity in the early evening. Increased fragmentation of the daily sleep-wake rhythm is associated with increased risk of developing AD. The complex relationships between sleep, circadian rhythms, sex, and AD in aging populations are not well understood. Using an Alzheimer's mouse model with three humanizing knock-in mutations, referred to as SAA, we tracked the progression of both sleep and circadian rhythms from 2 to 19 months using a piezoelectric sleep recording system to monitor sleep for 1 week at 2-3 month intervals. At all ages, both genotypes (n=31,32 each) slept approximately twice as much during the light phase as in the dark phase. From 15-19 months of age, total sleep in the SAA mice was lower ($p<.001$) compared to WT controls, specifically driven by deficits in sleep during the light phase, the major rest phase. Females of all ages and both genotypes slept less than males, primarily during the dark phase. Because sleep is an important process for clearance of amyloid-beta, less sleep by females may contribute to the higher levels of amyloid-beta characteristic of this sex. Sex based differences were also observed in several measures of the circadian rhythm. At all ages, female mice showed higher mean activity levels and higher rhythm amplitudes, with the latter suggesting greater robustness of their circadian rhythms. In addition, we used a programmable sweeping bar cage system to induce sleep fragmentation in a separate cohort of 8-month old SAA mice to investigate the impact of daily sleep fragmentation on AD-like pathology and neuroinflammation. Male but not female SAA mice kept awake for four 1-hour intervals during light phase, for 5 days a week for 4 weeks, exhibited a significant increase in expression of multiple markers of neuroinflammation (e.g., IL-1β, IL-33, CCL2, CXCL2, CXCL10) compared to undisturbed SAA controls. However, undisturbed female SAA mice had an increased level of neuroinflammation, equal to that of sleep fragmented males. Neuroinflammation occurred specifically in the neocortex, but not the hippocampus, mimicking the early stages of AD development in humans. These findings indicate that daily sleep fragmentation can contribute to AD progression through stimulation of neuroinflammation. Female sex may increase the risk of AD through elevated neuroinflammation as well as reduced sleep. These findings in a mouse AD model help to elucidate the roles of female sex and sleep disruption in the risk and progression of AD.

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Improving Patient Experience in the Epilepsy Monitoring Unit: A Randomized Controlled Trial of Virtual Reality Technology

Other

The epilepsy monitoring unit (EMU) is commonly used for the elective admission of patients to the hospital for continuous video electroencephalogram (cvEEG) monitoring. Patients typically have a minimum of an overnight stay, but stays are often extended over several days to localize seizure focus or characterize events concerning for seizures. A common issue during EMU admission is premature patient-directed discharge, especially if no seizures have been captured after several days. This can lead to decreased patient satisfaction, as reflected in post-discharge surveys, and patients may feel that the admission was purposeless. Additionally, premature discharge makes it more difficult for epileptologists to develop effective treatment plans. Therefore, there is a need to improve the patient experience during potentially uneventful hospital admissions.

Several studies and systematic reviews have assessed the effectiveness of virtual reality (VR) technology in enhancing hospital admission satisfaction, including reducing overall and procedural anxiety, aiding in pain control, and facilitating cognitive and motor rehabilitation. We propose that VR technology could be used in the EMU to reduce anxiety associated with seizures and improve overall patient satisfaction.

The proposed study is a randomized controlled trial in which patients admitted to the EMU will be randomly assigned to one of two groups: a control group with standard admission and discharge protocols, and an intervention group provided with VR headsets in addition to standard protocols. Both groups will complete a standardized questionnaire upon discharge that includes both quantitative and qualitative measures of overall satisfaction with their admission. We hypothesize that the VR group will demonstrate significantly higher satisfaction compared to the control group.

As a secondary outcome, we will monitor whether the use of VR headsets affects the incidence of epileptic discharges and seizures using standardized EEG review methods. Given that some forms of epilepsy can exhibit photoparoxysmal responses, we will monitor for any potential VR-induced seizures. However, evidence of VR technology inducing seizures in photosensitive epilepsy is limited, highlighting the need for further research as technology advances. Recruitment for this study is estimated to start in late 2024.

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Complementing EEG with Piezoelectric Motion Signals for Improved Seizure Detection and Characterization in Mouse Epilepsy Models

Student

Electroencephalography (EEG) is an invaluable tool in preclinical epilepsy research. However, it does not by itself convey explicit information about seizure-related motor behavior, which is commonly used to grade seizure severity. Non-invasive motion measurements can help but are likewise limited in the information conveyed about epileptiform activity in the brain. All procedures discussed were approved by the Institutional Animal Care and Use Committee of the University of Kentucky. Here we examine EEG measurements in combination with piezoelectric ('piezo') motion signals from floor sensors to correlate patterns of cortical activity with overt behavior. Mice (n=6; 1-2 months old) were treated with pilocarpine to induce acute *status epilepticus* and then monitored for up to four weeks for signs of spontaneously recurring tonic-clonic seizures using surgically implanted EEG hardware (Pinnacle Tech.) and piezoelectric pressure sensors on the cage floor (Signal Solutions, LLC). A preliminary sampling of candidate events (n = 383) initially detected through piezo and labeled as either seizure (n = 221) or non-seizure (n = 162) via manual video review were used as the ground truth to train a three-stage Long Short-Term Memory neural network (LSTM) system. The first stage utilized a line length ratio applied to the EEG data to try and detect labeled events. This system detected a total of 2235 events, of which 371 correlated to ground truth events, showing this stage is overly sensitive to candidate events. From the 371 EEG detected events we extracted sixty seconds of EEG and piezo data centered on detection time and extracted four features (Teager Energy, Variance, Shannon Entropy, and Intensity Range) from both EEG and piezo. The second stage was our first LSTM which examined the features extracted from the EEG to classify events as either seizure or non-seizure. We were able to produce an average specificity and sensitivity of 84.3% ± 3% and 83.5% ± 3%, respectively. Finally, all seizure events were used in the third stage to train and test a second LSTM that utilized the piezo features to classify physical behavior based on the Racine Scale, a scale from one to five ranging from mild to extreme movement and loss of balance. When used on non-training/testing data only events classified as seizures by the EEG LSTM will be fed to the piezo LSTM, however, to properly train and test the model we chose to use all seizure events. We are currently limited in the number of labels for this model, so we can only show the ability to classify S5 seizures, which produced an average specificity and sensitivity of 63.3% ± 7% and 76.8% ± 8%, respectively. We hope that this work will increase the value of motion measurements as sources of objective quantitative descriptions of seizure severity in preclinical epilepsy models as well as improve the range of data extractable from EEG. This work was supported by National Institutes of Health Grant No. NS107148.

Stroke & Neurovascular Injury

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Sex differences in prescription patterns and medication adherence to guideline-directed medical therapy among patients with ischemic stroke

Faculty

Background: Ischemic stroke is a leading cause of death and disability. Society guidelines recommend pharmacotherapies for secondary stroke prevention. However, the role of sex differences in prescription and adherence to guideline-directed medical therapies (GDMT) after ischemic stroke remains understudied. The aim of this study was to examine sex differences in prescription and adherence to GDMT at 1-year after ischemic stroke in a cohort of commercially insured patients.

Methods: Using the Truven Health MarketScan® database from 2016-2020, we identified patients admitted with ischemic stroke. GDMT was defined as any statin, antihypertensive, and anticoagulant prescription within 30-days after discharge. Medication adherence was estimated using the proportion of days covered (PDC) at 1-year. PDC <0.80 was used to define non-adherence. A multivariable model adjusting for covariates was performed to identify the factors associated with non-adherence at 1-year. This analysis was restricted to new users of GDMT.

Results: Among 155220 patients admitted with acute ischemic stroke during the study period, 15,919 met the inclusion criteria. The mean age was 55.7 years, and 7,701 (48.3%) were women. Women were less likely prescribed statins (58.0% vs 71.8%), and antihypertensives (27.7% vs 41.8%). In this subset of patients with atrial flutter/fibrillation, women were also less likely prescribed anticoagulants (41.2% vs 45.0%). Women were more likely to be non-adherent (i.e., PDC <0.80) to statins (47.3% vs 41.6%, P<0.0001), antihypertensives (33.3% vs 32.2%, P=0.005), and the combination of both (49.6% vs 45.0%, P=0.003). On multivariable analysis, women were likely to be non-adherent to GDMT at 1-year (odds ratio 1.23, 95% confidence interval 1.08-1.41).

Conclusions: In this real-world analysis of commercially insured patients with ischemic stroke, women were less likely initiated on GDMT within 30 days after discharge. Women were more likely to be non-adherent to statins and antihypertensive agents at 1-year. Future efforts and novel interventions are needed to understand the reasons and minimize these disparities.

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A mild cortical infarct increases neuroinflammation, elevates plasma biomarkers, and alters hippocampal synaptic function in male 5xFAD mice

Faculty

Vascular dysfunction is one of the most common comorbidities reported in Alzheimer's disease patients and is thought to exacerbate neuroinflammation and cognitive decline. Previously, we found that diet-induced cerebral small vessel disease (hyperhomocysteinemia) in amyloidogenic mice was associated with worsened cognitive performance and impaired synaptic plasticity. However, this model is complicated by concurrent peripheral dysfunction, which limits its ability to report on CNS-specific alterations. For the present study, we therefore characterized the effects of mild cortical infarcts in 5xFAD mice, as this represents a milder, more CNS-relevant model of vascular injury. Male WT and 5xFAD mice (8-9 months old) received a 60 min tandem CCA/distal MCA occlusion localized to the anterior region of a single hemisphere. Seven days later, mice underwent behavioral testing (spontaneous open field activity, rotarod, and frailty index), followed by euthanasia between 13- and 30-days post-infarct for preparation of cortical tissue samples and acute brain slices for electrophysiology. Plasma samples were also analyzed for assessment of relevant Alzheimer's disease biomarkers. As expected, 5xFAD mice had elevated plasma NFL and GFAP levels. Extracellular field recordings in hippocampal area CA1 revealed alterations in several synaptic parameters, with 5xFAD animals having smaller EPSPs and reduced Late LTP compared to WT. Additionally, injured hemispheres from both groups had smaller fiber volley responses compared to non-injured, suggesting the cortical infarct may have led to distal effects on adjacent hippocampal neurons. These differences also correlated with increased neuroinflammation and enhanced infiltration of CD3+ peripheral cells in injured cortex. Overall, this work indicates that mild vascular injuries in the cortex of male 5xFAD mice can alter synaptic function in distal hippocampal regions and induce peripheral immune responses such as lymphocyte infiltration into the CNS.

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Title: Expression of Dementia Biomarkers in Appalachian and Non-Appalachian ELVO Patients during Thrombectomy

Student

Background/Context: Vascular Cognitive Impairment and Dementia affects 25-30% of stroke patients and includes cognitive impairments caused by vascular injury, such as post-stroke dementia. There is currently no reliable method to identify those at risk of dementia after a stroke. Several biomarkers, including ADRD (Alzheimer's disease and related dementias) biomarkers (Ab, tau, NfL, and GFAP) and angiogenic factors (VEGF, Flt-1, Tie-2, PIGF, and FGF) have been associated with the development of dementia.

Populations in Appalachia experience a higher incidence of stroke and related mortality compared to other groups. Given the elevated stroke rates in Appalachian communities, this study aims to investigate potential proteomic differences between patients from Appalachian and non-Appalachian counties. The primary goal of the study is to characterize the expression of post-stroke cognitive dementia biomarkers and to explore differences in the proteomic profiles of Appalachian and non-Appalachian populations.

Methods/Approach: The Blood and Clot Thrombectomy Registry Collaborative (BACTRAC) protocol introduces a novel method for analyzing stroke by collecting intracranial blood samples from patients undergoing mechanical thrombectomy. During the procedure the thrombus and blood samples from areas distal and proximal to the thrombus are collected and undergo proteomic analysis. The control data was obtained from arterial blood collected during diagnostic angiograms from patients with cerebrovascular disease.

Propensity score models were used to perform a one-to-one match between stroke and control patients on age, sex, BMI, hypertension, and hyperlipidemia resulting in groups that were balanced on these measured prognostic characteristics. A Wilcoxon rank sum test was then used to assess differences in the 12 ADRD biomarkers.

Results: Compared to the controls, stroke patients had significantly higher levels of GFAP. The control patients had significantly higher levels of AB40, AB42, and VEGFA. In the Appalachian patient population, the control patients also had significantly higher levels of AB40, AB42, and VEGFA. Additionally, the Appalachian stroke patients had higher GFAP. In the non-Appalachian population only GFAP was significantly different between stroke and control groups, with it being elevated in the stroke group.

Conclusions: There was a notable difference in the levels of certain ADRD biomarkers between stroke patients and control patients. Specifically, in Appalachian populations, stroke patients showed significant differences in multiple ADRD biomarkers (AB40, AB42, and GFAP) compared to controls, a pattern not seen in non-Appalachian stroke patients, where only GFAP levels increased. This difference in ADRD biomarkers observed in Appalachian stroke patients could be attributed to a combination of socioeconomic and environmental factors unique to the Appalachian region.

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Impact of Sex and Age on Immune Cell Profiles in Skull Bone Marrow and Dura Before and After Ischemic Stroke

Student

Background: Stroke is a leading cause of death and disability, and elderly females have both the highest rate of stroke and the worst stroke outcomes. Recent studies show that the meninges play a key role in brain immunity, and immune cell populations in this location may shift to pro-inflammatory states with age. Acutely after stroke, there is an upregulation of hematopoiesis in the skull bone marrow (BM) adjacent to the infarct. However, no studies to date have examined immune activity in the skull and dura at subacute and chronic time points after stroke - periods that may contribute to long term cognitive decline, and how cell populations change with sex and age. Therefore, the objective of this study is to characterize immune cell populations in the skull BM and dura in aging and after stroke.

Methods: Adult (7-15-months, n=6/group) male and female C57BL/6 mice were used to establish methods, while a preliminary cohort of aged (>18 months, n=4/group) male and female C57BL/6 mice underwent a 30- minute transient middle cerebral artery occlusion (tMCAo) or sham surgery. Mice were sacrificed and femur BM, skull BM, and dura were collected. Samples were processed, stained for 8 general leukocyte markers (CD45, CD19, TcRβ, CD4, CD8, CD11b, NK1.1, Ly6G), and analyzed with flow cytometry. Data were gated in FlowJo and analyzed with 2-way and 3-way ANOVA with multiple comparisons (α=0.05) in GraphPad Prism.

Results: In the dura of the adult uninjured cohort, there was a significantly higher proportion of B cells ($p<0.05$) and lower proportion of innate immune cells ($p<0.01$) in females compared to males. In this uninjured cohort, males had a significantly lower proportion of B cells in the femur BM ($p<0.01$), and a higher proportion of T cells in the skull BM ($p<0.01$) compared to females. In the dura of the aged stroke cohort, B cell representation decreased ($p<0.01$) and T cell representation increased ($p<0.001$) after stroke in females only. In males, there was a significant decrease in T cell representation ($p<0.01$) in the dura. In the skull BM, but not femur BM, there was a significant decrease in overall CD19+ cell number after stroke ($p<0.05$). Further analysis with larger sample sizes is ongoing to confirm these results.

Conclusions: This study aimed to characterize differences in immune cell populations in the dura, skull BM, and femur BM based on age, sex, and stroke injury. Ongoing studies are further characterizing cell phenotypes in each of these locations. Determining the origin of potential pro-inflammatory cell populations after stroke will allow us to develop targeted therapies to reduce harmful inflammation without affecting beneficial cell phenotypes.

Poster Session

Addiction

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Family History Density of Substance Use: Influence on Participant Substance Use Onset and Duration

Student

INTRODUCTION: This study leverages the National Epidemiological Survey on Alcohol and Related Conditions-III (NESARC-III) dataset to investigate how weighted family history density (WFHD) influences the intergenerational transmission of SUDs, focusing on age at first use and duration of use in both sexes. Additionally, we examined sex-specific correlations between family and participant SUDs, as well as concordance in substance preferences.

METHODS: We conducted a cross-sectional analysis using NESARC-III data (2012-2013) from 36,309 adults. WFHD was defined as drug or alcohol problems among first- and second-degree relatives. Linear regression models assessed the relationships between WFHD, age at onset, and duration of substance use, adjusting for sociodemographic and geographic factors. We also examined substance preference concordance and correlations between family members' and participants' SUDs, with the latter stratified by sex.

RESULTS: Each unit increase in WFHD was associated with a 0.54-year earlier onset [β = -0.54, Standard Error (SE) = 0.02], and 0.48-year longer duration of use (β = 0.48, SE = 0.03). WFHD significantly predicted substance use before age 18 and duration one's current age, 5 years, and 10 years in adjusted regression models. Significant substance preference concordance was found between family and participants. Sex-stratified analyses revealed that males had the strongest correlations with paternal substance use, while females showed similar correlations with both maternal and paternal substance use, with a notably stronger maternal influence compared to males.

DISCUSSION: WFHD is strongly linked with earlier initiation and prolonged duration of substance use. These findings highlight the importance of family history assessments in prevention and intervention strategies. Future research should use longitudinal studies to establish causal relationships and explore interactions between WFHD and other risk factors, such as environmental stressors, epigenetic changes, or genetic markers.

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Genomic Assessment of the Prefrontal Cortex in Differential Responsivity of Sucrose Preference and Fentanyl Escalation in Sprague-Dawley Rats

Student

Background: The "loss of control" phenomenon seen in opioid use disorder (OUD), known as escalation of intake, is well-established in preclinical rodent models. Antecedent behavioral characteristics, such as valuation of hedonic reinforcers prior to drug use, may impact the trajectory of fentanyl intake over time. Moreover, phenotyping escalation of fentanyl intake may reveal the underlying genetic markers associated with OUD. **Methods**: Male and female Sprague-Dawley rats (n=72) were trained in a sucrose reinforcement task using a progressive ratio schedule. Individual differences in responsivity to sucrose were hypothesized to predict escalation of fentanyl intake. Rats (n=63) underwent daily 1h acquisition sessions for i.v. fentanyl self-administration (2.5 µg/kg; FR1) for 7 days, then 21 6h escalation sessions, then tissue from prefrontal cortex was collected for RNA sequencing and qPCR. Latent growth curve and group-based trajectory modeling were used to create distinct behavioral categories for rats based on fentanyl escalation to determine the association between sucrose performance and fentanyl self-administration. **Results**: Sucrose breakpoints were not predictive of fentanyl acquisition or escalation. Our permutation analysis did not identify associations between behavior and gene expression when evaluated overall, or between our ascertained phenotypes. Weighted gene co-expression network analysis (WGCNA) and gene set enrichment analysis (GSEA) determined several genetic targets associated with escalated fentanyl intake including voltage-gated potassium channels, calcium channels, and excitatory signaling. Further, transcription factor analyses identified EZH2 and JARID2 as potential transcriptional regulators associated with escalated fentanyl intake. Genome wide association studies (GWAS) terms were also generated and positively associated with terms relating to SUD. **Discussion**: The gene networks associated with fentanyl escalation highlighted in the present study may identify druggable targets that can treat OUD. The identification of SNPs and transcription factors associated with the "addiction prone" high escalating animals promotes the importance of translational preclinical models, and through a precision medicine approach, our results may aid in the development of patient-centered treatment options for those with OUD.

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Unique Role of Calcium-Activated Potassium (BK) Channels After Fentanyl Self-administration in the rat

Student

The escalation of fentanyl use presents a continuing public health crisis in the US. Cellular responses to opioids in laboratory animals and humans involve potassium channels. However, which of the many potassium (K^+) channel groups may underlie cellular and behavioral plasticity associated with fentanyl self-administration remains unclear.

Our study focused on investigating whether escalation of fentanyl intake recruits a unique sub-population of K^+ channels to impact neuronal excitability in the infralimbic prefrontal cortex (IL) and the nucleus accumbens shell (NAc). Male and female rats underwent jugular catheterization and assigned to one of three groups: contingent fentanyl, yoked fentanyl, and yoked saline. Following 19 days of fentanyl or saline administration in long-access (4 hrs) daily sessions, animals were sacrificed for brain extraction and brain slice preparation. Using whole-cell patch-clamp techniques, we isolated activity of six distinct groups of K^+ channels in the IL and the NAc neurons by applying channel-specific antagonists.

Escalation of fentanyl seeking was observed in the contingent fentanyl group as expected from prior literature. Preliminary results indicate that rats exposed to either contingent or yoked fentanyl display increased amplitude of calcium-activated, BK, channel-mediated current in the IL, compared to yoked saline controls. No significant differences were detected for currents mediated by the calcium-activated SK channels, delayed rectifier K^+ channels, A-type K^+ channels, M-type K^+ channels, and two-pore domain K^+ channels. Together, these results suggest a unique association between calcium-activated K^+ channels and fentanyl exposure. However, escalation of fentanyl seeking behavior could not be linked to a unique K^+ channel activity profile. Ongoing experimentation aims to further investigate the association between BK channels and calcium signaling in IL and additionally explores a potential association between fentanyl seeking and activity of G-protein coupled inward rectifier K^+ channels.

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Olfactomedins may mediate hormonally driven nicotine consumption in women via a negative feedback loop

Student

Estrogen has been shown to be implicated in nicotine use disorder such that increased levels of estrogen are associated with a higher likelihood to develop nicotine use disorders and increased nicotine consumption. Estradiol (E2) treatments in ovariectomized (OVX) female rats resulted in the increase of only the beta isoform of the estrogen receptor (ER) in brain reward circuitry area, the nucleus accumbens core (NAcore). From existing datasets, we identified the olfactomedin (OLFM) genes as responsive to estrogen, expressed in the brain, and having a hormone function. Estrogen treated uterine cells showed an increase in expression of OLFM1 and OLFM2. Interestingly, nicotine suppressed the estrogen-induced increase in OLFM1. We confirmed that this mechanism is ER**β** and OLFM1 specific by performing a chromatin immunoprecipitation assay. We found that OLFM1 promoter was enriched by ER**β** and not ER**α**. We treated OVX female rats with E2, nicotine, and E2 and nicotine combined and examined gene expression in the brain reward areas. We found that Olfm1 and Olfm2 were significantly increased in the NAcore and the ventral tegmental area. Again, nicotine suppressed the E2-induced OLFM expression in these areas. Finally, we performed extensive PamGene analysis with neuroblastoma cell line treated with the same treatments. This showed us that the combination of estrogen and nicotine resulted in kinase activity distinct from that seen with estrogen or nicotine alone. Furthermore, we found that the ER**β** interactome was majorly altered by estrogen and nicotine combination, whereas the ER**α** interactome didn't have as much change. These findings lead us to suggest that the ER**β** activation of olfactomedins might provide a feedback loop for nicotine consumption and potentially open up a new pathway for therapeutic targeting.

Neurodegeneration

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Effect of Protective Mutation on Dynamics and Structure of APOE4: a Molecular Dynamics Simulation Study

Student

Apolipoprotein E (APOE) 4 has been recognized as a risk factor for Alzheimer's Disease (AD). People who possess APOE4 proteins are expected to have a much higher chance of getting AD than those who possess APOE2 and APOE3. Two rare mutations on APOE4, V236E and R251G, have been discovered to reduce the risk of people developing AD compared to those possessing wild-type APOE4. Despite this encouraging information, the knowledge remains limited about the working mechanisms behind the two protective mutations. The functions of a protein rely on its structure, dynamics, and solvation. This work aims to shed light on the plausible working mechanisms of the two protective mutations. We performed molecular dynamics simulations to investigate how the two protective mutations may induce variation in the dynamics and structure of an APOE4 protein. The wild-type APOE2, APOE3, and APOE4 are used as the reference. The investigation focuses on three functional regions of APOE protein: the lipidation region, the protein aggregation region, and the hinge region. The simulation results show that mutations in APOE4 alter its structural flexibility, making it more similar to APOE2 and APOE3. Specifically, the V236E and R251G mutations affect the lipid-binding, oligomerization, and hinge regions. These changes reduce flexibility in some areas while increasing it in others, ultimately narrowing structural disorder in key regions of APOE4.

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TDP-43 citrullination: scratching the structural surface on a novel post-translational modification in AD and related dementia

Student

Transactive response DNA-binding protein 43 kDa (TDP-43) is a nuclear DNA/RNA binding protein linked to the neuropathology of a spectrum of disease—notably by mislocalized cytoplasmic inclusions. These inclusions are classically seen as stress granules, which undergo liquid-liquid phase separation (LLPS) and hyperphosphorylation. Post-translational modifications, like phosphorylation or acetylation, are proposed to alter protein structure and function. Our laboratory has recently discovered a previously unknown but significant post-translational modification of TDP-43: citrullination by peptidyl arginine deiminase (PAD4), changing the positively charged arginine to neutral citrulline. This study aims to investigate the impact of PAD4 on TDP-43 pathology.

To investigate the effects of citR TDP-43 aggregate morphologies, full-length or low-complexity domain recombinant human (TDP-43/TDP-43^{LCD}, respectively) were subjected to an enzymatic reaction in vitro with recombinant human PAD4 to produce citrullinated proteins (citR TDP-43/citR TDP-43^{LCD}, respectively). Citrullination was confirmed by Western blot utilizing our novel, site-specific citR TDP-43 antibodies. TDP-43/TDP-43_{LCD} and citR TDP-43/citR TDP-43^{LCD} were incubated with yeast total RNA to determine differences within TDP-43 RNA binding motifs and C-terminus, which are known to have a direct effect on stress granule assembly and liquid-liquid phase separation (LLPS). Thioflavin T (ThT) and turbidity kinetics found a significant delay in citR TDP-43 interaction with RNA compared to unmodified TDP-43. Interestingly, citR TDP-43^{LCD} alone showed droplet-like formations under higher protein concentrations, suggesting self-crowding LLPS formation. To further investigate the structural changes of citRTDP-43, aggregated proteins were analyzed by transmission electron microscopy (TEM) tomography, showing a drastic size reduction from an amorphous aggregate state (full-length) or fibril (TDP-43^{LCD}), to granular/ condensate morphological profile.

We propose that citrullination serves as a molecular switch for LLPS and liquid-solid phase separation (LSPS) transitions, possibly through altered RNA-protein interactions, a known enhancer for normal stress granule assembly. These alternate accumulates can stray from the canonical LLPS aggregates found in stress granules, and may serve as a separate inclusion pathway for TDP-43 pathology.

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Dysregulation of Transposable Elements in Alzheimer's Disease: Insights from Single-Cell RNA Sequencing of the Middle Temporal Gyrus

Student

Background: Transposable elements(TEs) have increasingly been implicated in the pathogenesis of Alzheimer's disease(AD), where their dysregulation may contribute to neurodegenerative processes. While bulk RNA-seq studies have hinted at general TE dysregulation in AD, the specific impact at a higher resolution remains unclear. This study leverages single-cell RNA sequencing(scRNA-seq) from the Seattle ALzheimer's Disease Brain Atlas(SEA-AD) consortium to investigate differential TE expression in the middle temporal gyrus(MTG) of AD patients compared to healthy controls, a region heavily affected in AD.

Methods: Using SoloTE, a specialized tool for quantifying TE expression in single-cell RNA-seq samples, we generated expression matrices for both genes and TEs in these samples. Seurat was employed for sample-specific data processing, clustering, and filtering to ensure data quality. The resulting matrices were then aggregated at the TE subfamily level, enabling detailed comparisons of TE expression between AD cases and controls.

To further analyze this data, we performed a pseudo bulk differential expression analysis with DESeq2, allowing for robust comparisons of TE activity between AD and control groups. In addition, we performed separate differential expression analyses for genes and TEs, providing a granular view of molecular changes and highlighting significant TE subfamily upregulation and downregulation in AD patients.

Results: Our findings reveal significant TE dysregulation in AD, with subfamilies like LTR53B, MER112, *AluYa8*, L1PBb, and LTR15 showing strong upregulation. The upregulation of *Alu* elements, such as *AluYa8*, and L1 elements like L1PBb, is particularly noteworthy because *Alu* elements and L1s remain active in the human genome. These TEs may disrupt gene regulation and promote genomic instability by interfering with gene regulatory regions and mobilizing within the genome, potentially causing mutations and disrupting normal cellular functions. These results suggest that TE activity may contribute to cell-type-specific dysfunction in AD.

Conclusion: Further analysis is underway to expand this investigation to other brain regions affected by AD, potentially uncovering novel insights into the role of TEs in disease progression. By integrating single-cell approaches with TE subfamily-level analysis, this study provides a high-resolution view of the complete interplay between TEs, gene expression, and neurodegeneration in AD, offering new avenues for potential therapeutic interventions targeting TE dysregulation.

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Separation of Astrocytes from Brain Tissues via use of GLAST and Dynabeads

Staff

Background: We have recently published that overexpressing a constitutively active form of the insulin receptor beta subunit (IR-β) in hippocampal neurons ameliorates spatial memory performance in the F344 rat model of aging. Because astrocytes express insulin receptors and are central to cellular energy transfer and information processing in the brain, here we focus on the separation of astrocytes from tissue samples of the primary somatosensory cortex (S1) in the 5xFAD model.

Methods: Fresh plugs from the S1 cortex were removed from mice and placed into 0.125% trypsin for 8 to 15minutes then triturated for 7 minutes until quenched with 0.5% BSA in PBS (buffer). The tissue was resuspended with mouse GLAST antibody andbuffer then placed at 4°C. The solution was then exposed to Dynabeads to separate the astrocytes from the rest of the tissue. A magnet was used to separate the beads containing astrocytes from the rest of the liquid sample labeled astrocyte depleted (AD). A western blot buffer was added to the Dynabeads to release the astrocytes forming the astrocyte enriched sample (AE). Samples were run on a western blot gel or stored at -80°C. PVFD membranes were probed with antibodies to GFAP, Neun and β-Actin to determine the quality and quantity of separation.

Results: Initial results showed success at separation with a significant difference in the band intensity in the AE samples compared to the AD samples. Because further experiments appeared less reliable, we are investigating different digestion times, buffers and antibody concentrations maximize the signal and astrocyte isolation.

Conclusion: Magnetic cell separation is a promising method to confirm and quantify the specificity adeno-associated virus delivery system to particular cell types

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ß-Hydroxybutyrate Improves Mitochondrial Function in an Alzheimer's Mouse Model

Student

Background: Cerebral hypometabolism is a prevalent disease mechanism in Alzheimer's disease (AD), contributing to energy deficits in the brain. Beta-hydroxybutyrate (BHB), a ketone body, can provide an alternative energy source when glucose metabolism is impaired. Improved management of cerebral hypometabolism has reduced dementia rates, including in cases linked to traumatic brain injury (TBI). With over 6.7 million Americans affected by AD, effective therapies like BHB are needed. We hypothesize that BHB treatment will improve mitochondrial function in APP/PS1 KI mice, potentially offering a therapeutic approach to addressing energy deficits in AD.

Methods: Alzet mini-osmotic pumps (0.25 µL/hr) were used to deliver 0.83 mM/kg/day BHB dissolved in sterile saline subcutaneously. Control groups received pumps with saline. 17-20-month-old $APP^{NLh/NLh}$/$PS\text{-}1^{P264L/P264L}$ KI mice were implanted with either BHB or saline-filled pumps under sterile conditions. After 4 weeks, mice were euthanized for mitochondrial bioenergetics analysis.

Results: APP/PS1 KI mice exhibited significantly lower State III oxygen consumption rates (OCR) compared to WT mice ($p=0.008$). BHB treatment trended toward restoring State III OCR in the cortex ($p= 0.06$), with no significant effect in State V Complex I. A significant decrease in State V Complex II OCR was observed in the cortex of KI compared to WT ($p=0.025$). No genotype or treatment effects were seen in the hippocampus.

Conclusion: Our findings suggest that long-term BHB treatment partially restores mitochondrial function in the cortex of APP/PS1 KI mice, as indicated by improved OCR in State III respiration. However, no effects were observed in the hippocampus, and the mechanism of BHB warrants further investigation. The data support BHB as a potential therapeutic strategy to improve cerebral bioenergetics in AD, but further studies are needed to demonstrate its efficacy.

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Investigation of EEG slow wave recovery following sleep disruption in a mouse model of Alzheimer's disease

Student

Sleep is crucial for maintaining brain function and cognitive health. However, in Alzheimer's disease (AD), sleep is fragmented by repeated interruptions. These interruptions are not just symptomatic but also contribute to disease progression, correlating with increased amyloid beta (Aß) accumulation in the brain. While previous experiments have demonstrated that a reduction in slow-wave sleep is associated with an increase in the accumulation of Aß in the brain, whether consolidation of slow-wave activity (SWA) occurs in the periods following interrupted sleep remains unexplored.

To investigate the effects of a sleep fragmentation (SF) protocol that mimics the sleep disruption frequently observed in AD patients, we conducted an IACUC-approved study utilizing electroencephalography (EEG) and electromyography (EMG), in 8-month-old male APP/PS1 knock-in mice that are genetically predisposed to amyloid plaque formation. Mice underwent surgery for the implantation of EEG/EMG headmounts, secured to the skull by bone screws positioned to detect cortical field potentials. Following a two-week recovery period, we established baseline sleep patterns from a week of EEG recording. In the next four weeks, SF was performed by sensory stimulation, in a manner designed to mimic the pattern of disrupted sleep seen in AD patients. This protocol was carried out Monday through Friday, with one-hour-long blocks of sleep disruption at four different times of the 12-hour light phase separated by 90-minute undisturbed intervals. Following this intervention, EEG analysis was conducted. Particular attention was given to 0.5-2 Hz low delta power (LDP) in the EEG as a measure of SWA. Control mice were recorded alongside without sleep disruption.

Our preliminary EEG analysis of treated mice revealed surges in LDP during sleep following sleep disruption periods, compared to baseline levels. These results are suggestive of a homeostatic recovery of SWA during the expected sleep rebound. Notably, the surge in LDP diminishes as the day progresses, potentially due to the onset of the inactive dark phase in the diurnal cycle or due to adequate prior recovery of SWA. In contrast, no consistent trends in LDP were observed in control mice, further highlighting the large swings seen during the SF protocol. While further data collection is ongoing to investigate the significance of these observations, this study is expected to yield useful insight into whether SWA is consolidated between periods of sleep disruption in an experimental SF protocol and the correlation with pathology in a widely used murine model of AD.

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Altering ApoE in mice expressing human amylin in the pancreas exacerbates brain microvascular and parenchymal amyloid pathology

Student

Background:

The *APOE* **ε**4 allele is the most prominent genetic predisposition for sporadic Alzheimer's disease (AD). Amylin, a neuroendocrine hormone co-secreted with insulin from the pancreas, is increased in blood in AD and readily forms neurotoxic homo- and hetero-oligomers with **β**-amyloid in AD. Here, we investigated whether mice humanized for amylin and ApoE demonstrate ApoE isoform-specific alterations in cerebrovascular amylin deposition and **β**-amyloid homeostasis.

Methods:

Mice humanized for ApoE3 or ApoE4 and amylin (ApoE3HIP and ApoE4HIP) and amylin without ApoE expression (ApoE-KO-HIP) were tested for behavior deficits before brain microvessel isolation and amylin/**β**-amyloid quantification. GFAP-amylin colocalization in the brain was quantified using immunohistochemistry (IHC), double-immunofluorescence was used for amylin-ApoE colocalization, and immunoprecipitation experiments were conducted to confirm brain amylin-ApoE binding interactions.

Results:

ApoE4HIP mice demonstrated worsened behavioral deficits vs. E3HIP mice. IHC of ApoE4HIP and ApoE3HIP brains revealed increased deposits of GFAP and amylin in ApoE4HIP mice. Amylin in brain parenchyma was higher in ApoE4HIP vs. ApoE3HIP mice while ApoE-KO-HIP mice demonstrate reduced amylin in microvessels and parenchyma. **β**-amyloid 40 levels were elevated in ApoE4HIP brain and microvessels.

Conclusions:

Our data suggest ApoE may function as a transporter of amyloid-forming amylin in the brain with amylin binding ApoE4 stronger than ApoE3. The increased affinity of amylin for ApoE4 coincides with worsened brain amyloid pathology (in parenchyma and microvasculature), disrupted **β**-amyloid homeostasis, increased astrogliosis suggesting neurodegenerative insult, and functional impairments due to elevated amylin amyloid burden. These data may implicate the amylin-ApoE interaction as a mechanism underlying ApoE4-specific neuropathology.

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Glia-selective replacement of APOE4 with APOE2: response to age and inflammation

Student

Background: Apolipoprotein E (APOE) is the strongest genetic risk factor for late-onset Alzheimer's Disease (AD) and is inherited in three common allelic variants: E2, E3, E4. Compared with E3, E4 increases AD risk up to 15-fold while the E2 allele decreases AD risk by more than 50%. In the CNS, ApoE is primarily synthesized by astrocytes and microglia, making these two cell types promising targets for *APOE* gene therapy approaches. To study the potential effects of such a therapy, our lab has generated an inducible allelic "switch" model (APOE4s2) in which we can conditionally replace the E4 risk allele with the protective E2 allele in a cell-specific manner. To elucidate potential mechanisms by which astrocyte or microglia E2 expression may modulate AD risk independent of amyloid or tau pathology, we characterized the glial response of *APOE*astrocyte- or microglia-specific "switch" mice during aging and following an acute inflammatory challenge.

Methods: Aged APOE4s2 mice were administered tamoxifen to induce an *in vivo* transition from expression of E4 to E2 selectively in astrocytes (*Aldh1l1-CreERT*) or microglia (*Tmem119- CreERT2*) for one month. A separate cohort of astrocyte- or microglia-specific "switch" mice received LPS to induce an inflammatory response 24 hours prior to tissue collection. Immunohistochemical analysis of gliosis markers (GFAP, IBA1, P2ry12, CD68) and cytokine measurements were performed on brain tissue collected from the experimental groups listed above.

Results: Following LPS administration, cytokine levels in the plasma and brain of aged astrocyte- and microglia-specific "switch" mice reveal no difference compared to aged E4 expressing mice. Astrocyte-specific replacement of *APOE4* with *APOE2* resulted in increased astrocyte and microglia reactivity compared to E4 expressing mice following LPS administration. Replacing microglial *APOE4* with *APOE2* increased microglial, but not astrocytic, reactivity compared to E4 expressing mice following LPS administration.

Conclusion: The ability of glial cell-selective *APOE2* expression to regulate astrocytic and/or microglial reactivity in response to age and inflammation, even in the presence of continued E4 expression by other CNS cell types, may represent a targetable mechanism whereby *APOE* modulates AD risk.

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Astrovascular coupling in the somatosensory cortex of an animal model of amyloidosis at rest and during ambulation

Staff

Background: We shifted our attention from studying neuronal network to the studies of astrovascular coupling in the awake, free-ambulating 5xFAD animal model of amyloidosis. S1 neuronal and astrocytic activity is recruited during movement likely through feedback sensory engagement and, as we have previously shown, is sensitive to both sex and age where older females that perform better on ambulatory tasks, also display signs of enhanced neuronal activity with reduced neuronal synchronicity of the network.

Methods: Here, we present data from 17 younger (3 mo. old) and 30 older (9 mo. old) 5xFAD and wildtype mice. All mice received GCaMP8f, an imaging window and head plates over the S1 region. Four weeks later, imaging sessions were conducted on a two-photon microscope following retro-orbital rhodamine dextran delivery to visualize blood vessels. Both astrocytic and vasoreactivity were acquired simultaneously during periods of rest & ambulation.

Results: The astrocytic network parameters (Astro-Astro; both + & - correlations, connectivity, and activity) showed main effects of genotype during ambulation in older animals, while younger mice only showed main effects of sex along with alterations in the number of connections. No genotype effects were noted on the astrocytic vasoreactivity and astrocytic velocity correlations in both younger and older mice. The correlations between vasoreactivity and movement highlighted main effect of genotype with reduction in the older animals.

Conclusion: In this study, prior to overt Aβ deposition, some functional alterations were noted in the younger 5xFAD mice. The older 5xFAD mice exhibit reduced functional network connections, correlations strengths, as well as astrocytic density during ambulation, yet these network alterations do not appear to directly impact vasoreactivity or associations with ambulation. Only associations between vasoreactivity and ambulation were altered by age and genotype.

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Amyloid-Beta and Gait Dysregulation in Older 5xFAD Mice

Staff

Background: Gait dysregulation is a hallmark of Alzheimer's Disease (AD), particularly affecting the primary somatosensory cortex (S1). To investigate the relationship between amyloid-beta plaque accumulation and gait disturbances in older mice, the 5xFAD amyloid mouse model was used. In a previous study, our lab found no gait dysregulation in young 3-month-old male mice of this model. To further explore amyloidosis across different genotypes and sexes, immunofluorescence (IF) was performed on S1 and hippocampal sections to assess reactive astrocytes and plaque load in 5xFAD mice compared to wild type (WT).

Methods: Mice underwent craniotomy injections with a combination of AAV5-Gfa104-GCaMP8f and AAV5-Gfa104-Luciferase in the S1, four weeks prior to gait analysis on a custom-built three-plane visualization apparatus. Gait parameters were quantified using Matlab and ImageJ. Following gait analysis, brains were perfused and sectioned at 40 µm using a cryostat. Immunofluorescence staining was performed on S1 and hippocampal sections, probing for GFAP and amyloid-beta. Z-stack images were captured using a confocal microscope, and plaque areas were quantified after flattening the stacks.

Results: Coordinates from each mouse were used to calculate average stride length, stride length deviation, average speed, stride time deviation, paw precision index, deviation from center, number of steps per cm, and number of steps per second. Plaque quantification was based on a predetermined area of 250,000 µm², with measurements taken from three sections per region. Statistical significance was determined using 3-way and 2-way ANOVAs.

Conclusion: Significant genotype differences were observed in nearly every gait parameter (average stride length, stride length deviation, average speed, stride time deviation, paw precision index, number of steps per cm, and number of steps per second), with increased plaque load in 5xFAD mice compared to WT. The 5xFAD mice, particularly females, showed greater deviations from WT, aligning with clinical data.

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Neurophysiology

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Effects of Claustrum Psychedelic Microinjections on Neuronal Excitability and Cognitive Flexibility after Cocaine Self-Administration

Student

Psychedelic drugs have recently attracted widespread research and public interest. Early clinical outcomes probing the potential of psychedelics for the treatment of substance use are promising, but a large gap remains in understanding of mechanisms by which psychedelics may achieve therapeutic outcomes. It has recently been proposed that psychedelics improve cognitive flexibility to enhance adaptive behavioral strategies. Cognitive flexibility deficits are characteristic of substance use disorders, contributing to difficulties with abstinence and making relapse more likely to occur. We explored the hypothesis that psychedelic drugs may engage neurons in the claustrum (CLA), a subcortical nucleus with extensive cortical projections and a high density of serotonin receptors, to induce neuronal plasticity linked to cocaine-seeking behaviors.

Using qPCR and RNAscope, we demonstrate that 5-HT2A and 5-HT2C receptors subtypes on both glutamatergic and GABAergic neurons account for the bulk of serotonin receptor expression in the CLA, but that other subtypes (notably 5-HT1A) are also expressed at substantial levels. Whole-cell patch-clamp electrophysiology indicated that 5-HT inhibits intrinsic excitability and glutamatergic neurotransmission in CLA neurons projecting to the anterior cingulate cortex (CLA-ACC neurons). In contrast, we found that DOI, a potent psychedelic 5-HT2 receptor agonist, excites CLA-ACC glutamatergic signaling via the 5-HT2C, but not the 5-HT2A, receptors. At the behavioral level, we demonstrate that contingent cocaine self-administration, but not the non-contingent (i.p.) injections of cocaine, produced cognitive flexibility deficits in the set-shift task. Preliminary data indicate that microinjections of DOI into the CLA impact both cognitive flexibility performance and electrophysiological signatures of CLA-ACC neuron excitability during cocaine withdrawal. For example, we find reduced sensitivity of CLA-ACC neurons to membrane effects of a 5-HT1A antagonist in cocaine-experienced animals relative to yoked saline controls. Finally, we show that DOI administration unlocks spike-timing dependent long-term potentiation in CLA-ACC neurons that is absent under control conditions.

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The regulatory role of asprosin in hypertension via Oxytocin-Ptprd signalling

Fellow

Asprosin is a novel adipokine, identified through the study of genetic disease called neonatal progeroid syndrome (NPS). So far, two spatio-temporally distinct functions of asprosin have been discovered. Asprosin cell-autonomously induces hepatic glucose release and stimulates appetite via the activation of agouti-related protein (AgRP) neurons of the hypothalamus. Asprosin performs these two spatio-temporally distinct functions via two different receptors. Ptprd (Protein Tyrosine Phosphatase type **δ**), a membrane-bound phosphatase receptor mediates asprosin's orexigenic function, while a G-protein coupled receptor, Olfr734 (mouse ortholog of OR4M1),

In this ongoing study we are assessing the role of asprosin in regulation of blood pressure (BP). Our preliminary results show that asprosin deficient female mice (NPS) present with significantly lower BP, which can be completely rescued with intra-nasal treatment of recombinant asprosin. Further, at the mechanism level, our preliminary data shows that asprosin's hypertensive effects are mediated by Ptprd signaling in the oxytocin neurons. Both male and female mice, with genetic loss of *Ptprd* from oxytocin +neurons (*Oxy-cre*$^{+}$*;Ptprd*$^{flox/flox}$) had significantly lower MAP (mean arterial pressure) when compared to wild type littermate controls (*Oxy-cre*$^{+}$*;Ptprd*$^{+/+}$). This study identifies a novel function of asprosin and represents a new avenue for therapeutic development for treatment of hypertension.

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The Relevance of Exploring Siah-1 Targets in Retinal Development

Student

The ubiquitin proteasome system is a selective degradation pathway that plays a key role in cell proliferation, protein quality control, transcriptional regulation, and cellular homeostasis. Protein degradation occurs in two successive steps which includes: the covalent attachment of multiple ubiquitin to the target protein, and degradation of the polyubiquitin chain by the 26S proteasome complex. This pathway relies on the binding of the E3 ubiquitin ligase to a specific target protein. Seven In Absentia Homolog (Siah-1) is a member of the RING finger family of E3 ubiquitin ligases that is involved in: cell trafficking, DNA signaling, DNA repair, and specifically retinal development. Siah-1 targets substrates for degradation by recognizing the degron motif, PxAxVxP. Siah's ability to recognize substrates based on their degron motif governs both the specificity and targeting via the UPS system. Previous work in the Famulski lab has uncovered connections between Siah1 and retinal development. To expand on these findings, I scanned the zebrafish proteome for potential target proteins using the known degron motif sequence. One of the targets I identified was DDB1-Cul4 Associated Factor-17 (DCAF-17) which is a member of the DCAF-family gene that encodes receptor proteins for cullin-ring E3 ubiquitin ligases and provides target substrate specificity for protein ubiquitination. Cul4 has previously been shown to play a role in retinal development. I therefore hypothesize that the targeting of DCAF-17 by Siah-1 E3 ligase may be crucial for regulating Cul4 and therefore retinal development. To date, I have confirmed retinal expression of dcaf17 along with Siah1. Furthermore, using HEK293 cell culture I have determined that co-expression of dcaf17 and Siah1 leads to siah1-dependent targeting of dcaf17 for proteasomal degradation. My future directions include confirming the Siah1-dcaf17 interaction, testing direct targeting of dcaf17 via the degron motif, and lastly examining phenotypic effects of dcaf17 targeting by Siah1 on retinal development.

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1-Deoxysphingolipids cause neurotoxicity. Can we stop them?

Student

Taxane-induced peripheral neuropathy (TIPN) is a major side effect of taxane (paclitaxel and docetaxel) chemotherapy treatment affecting up to 60% of the patients. Previous work from our laboratory has shown that increased levels of atypical sphingolipids, the 1-deoxysphingolipids (deoxySLs), are associated with the incidence and severity of neuropathy in patients treated with paclitaxel. The deoxySLs are generated when the first enzyme of the sphingolipid biosynthetic pathway, serine-palmitoyltransferase, utilizes L-alanine. L-Alanine has very low affinity to serine-palmitoyltransferase compared to its higher affinity substrate, L-serine. L-Serine is the precursor of the canonical sphingolipids. DeoxySLs have slower degradation and accumulate when produced in excess, leading to neurotoxicity as in the case of TIPN. The neurotoxic mechanisms of the deoxySLs remain unclear, including the question whether structurally different individual deoxySls, such as positional isomers 4E and 14Z, show different levels of toxicity.

The neurotoxic effects of individual deoxySLs species were tested in two neuroblastoma cell lines, SMS-KCNR and differentiated Neuro-2a (N2a). We used increasing concentrations of the individual deoxySLs and at different time points assessed their neurotoxicity by evaluating the morphological changes of the treated cells. Our results showed that in differentiated N2a cells the individual deoxySLs species induced the formation of neurite swellings, neurite retraction, and degradation. In KCNR cells, the neurotoxic effects were manifested by retraction of the cell processes, resulting in rounding of the cells. Our results suggest that all individual deoxySLs species can cause neurite damage, however at different concentrations and/or exposure times, suggesting a transient fashion of the neurotoxic morphological changes.

In order to search for approaches to prevent deoxySLs accumulation, we tested if addition of the preferred L-serine substrate will decrease the production of deoxySLs in RSC96 cells treated with paclitaxel. Our results showed that the addition of L-serine resulted in decreased cellular levels of deoxySLs. Next, we tested in mice treated with paclitaxel the effects of a diet enriched in L-serine on the levels of deoxySLs and on their neuropathic behaviors. Our data showed a decrease in the levels of deoxySLs in plasma and in the DRG of the mice. Importantly, the enrichment of L-serine in the diet rescued neuropathic behaviors due to paclitaxel administration. These results provide a base to investigate further in patients if decreasing the levels of deoxySL can alleviate symptoms of the TIPN.

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Astrocyte Ca2+ in the dorsal striatum suppresses neuronal activity to oppose cue-induced reinstatement of cocaine seeking

Student

Cocaine use disorder remains a critical challenge to understand within the neuroscience community. Recent studies suggest that astrocytes may play an active role in facilitating cocaine seeking behavior, with astrocytic Ca^{2+} emerging as a key mechanism underlying such regulation. However, the specific influence of astrocytic Ca^{2+} on neuronal activity in brain areas associated with cocaine seeking and reinstatement have yet to be fully explored. To address this gap, we overexpressed the cytosolic Ca^{2+} extruder pump, hPMCA2, in the dorsal striatum astrocytes of rats trained to self-administer saline or cocaine along with the neuronal Ca^{2+} reporter, GCaMP6f, in the same brain area. While no notable differences were observed throughout saline self-administration, hPMCA2 animals exhibited increased cocaine self-administration compared to animals injected with the control tdTomato virus. No behavioral differences were seen between either group during extinction. However, while no significant differences were detected between saline hPMCA2 and saline tdTomato animals during cue-induced reinstatement, suppression of astrocytic Ca^{2+} led to increased cue-induced reinstatement in cocaine hPMCA2, relative to cocaine tdTomato animals. Subsequently, brain slices were collected for *ex vivo* calcium imaging. In slice imaging experiments, reduction of astrocyte Ca^{2+} increased neuronal event amplitudes, an indirect measure of cell excitability, in the cocaine-administering animals, but not the saline-administering ones. Additionally, suppression of astrocyte Ca^{2+} reduced the duration of neuronal Ca^{2+} transients in cocaine self-administering animals. Interestingly, these results were not associated with differences in neuronal response to exogenous cocaine and were not linked to differences in the overall extracellular Ca^{2+} levels. In a separate cohort of animals, we examined the effects of astrocyte Ca^{2+} on neuronal activity in awake behaving animals. Fiber photometry experiments, conducted throughout extinction and reinstatement sessions, were used to associate neuronal activity to discrete behavioral events. Our preliminary results indicate that dorsal striatal astrocytic Ca^{2+} may regulate the associations between lever press behavior and neuronal activity. On-going analyses investigate whether astrocyte Ca^{2+} regulation of neuronal excitability during discrete extinction and reinstatement of cocaine-seeking parallel the overall increase in neuronal excitability observed in the slice preparation.

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TRPA1 Deficiency Results in a Reduction of Cochlear Microphonic Amplitude After Noise Exposure

Student

TRPA1 channels are master sensors of tissue damage found throughout the body, including within the Organ of Corti. We recently showed that after acoustic trauma, TRPA1 activation in cochlear supporting cells regulates hearing sensitivity and is a component of a temporary threshold shift (TTS). Our ex vivo findings showed that TRPA1 activation in the Hensen's cells leads to prolonged calcium responses that propagate across the organ of Corti and cause long-lasting tissue displacements. In vivo, such shape changes in the non-sensory supporting cells would be expected to affect the geometry and/or stiffness of the cochlear partition thus affecting cochlear amplification. Consistent with this hypothesis, five days after noise exposure (which leads to the generation of TRPA1 agonists in the cochlear epithelium), wild-type mice exhibited smaller distortion-product otoacoustic emissions and higher auditory brainstem response (ABR) thresholds when compared to littermates deficient in TRPA1 (*Trpa1*$^{-/-}$). These results indicate that TRPA1 activity is a component of the noise-induced TTS. However, we still do not have direct evidence of TRPA1-mediated changes to the geometry and/or stiffness of the cochlear partition in adult mice. Here, we extracted cochlear microphonic (CM) data from ABR recordings to evaluate noise-induced changes in the operating point of the organ of Corti in the presence or absence of TRPA1. Our results show significant differences in the amplitude of the summating potential (SP) in click-evoked auditory brainstem responses between *Trpa1*$^{-/-}$ mice and wild-type littermates. However, five days after a 30-minute exposure to broadband noise at 100 dB SPL, when both wild-type mice and *Trpa1*$^{-/-}$ mice still exhibited elevated hearing thresholds, the SP differences were no longer observed due to an overall reduction in CM amplitudes in the *Trpa1*$^{-/-}$ mice which was not seen in wild-type littermates. In addition, mice exhibited a direct current (DC) shift in the CM elicited by an 8 kHz tone burst as the sound intensity increased, which was delayed in *Trpa1*$^{-/-}$ mice. Our results indicate that TRPA1 signaling during loud sound stimulation triggers a change in the operating point of outer hair cell mechanotransduction, which might serve as a protective mechanism to minimize noise induced tissue damage.

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PercevalHR Responses of Mixed Primary Hippocampal Cultures Exposed to Insulin are Sensitive to Extracellular Glucose Concentrations

Student

Brain homeostatic equilibrium is a well-maintained metabolic process. Loss of this homeostasis is linked to brain aging and is often detected as hypometabolism in Alzheimer's Disease (AD). Recently, brain insulin has been identified as an essential component in regulating cognitive function, particularly in the hippocampus, where it has been shown to ameliorate spatial memory recall deficits. Here, we investigated the energetic status of neurons and astrocytes in mixed primary hippocampal cultures using the ATP:ADP nanosensor PercevalHR. Embryonic rat hippocampi (E18) were extracted and maintained for 12-16 days *in vitro*. Cultures were exposed to a PercevalHR lentivirus (Human Ubiquitin C promoter) for determination of ATP:ADP. To correct PercevalHR's pH bias, some experiments were conducted concomitantly with the intracellular pH sensor pHrodo. To normalize glucose transporter function following ~12 days in high glucose concentration (30 mM), we returned the cells to a serum-free 5.5 mM glucose media ~24 h prior to imaging. After imaging an initial baseline, cells were treated with one of several compounds (0.5 mM, 5.5 mM, and 10 mM glucose; 50 mM KCl; 20 µM glutamate; 10 nM insulin). Glutamate and KCl exposures resulted in rapid decreases in ATP:ADP. Surprisingly, insulin exposures and glucose had relatively small effects on ATP:ADP. To validate our findings, ECAR and OCAR assays were performed and do indeed corroborate the lack of insulin response found during imaging. These data evaluate the bioenergetic status in two closely associated cell types that are known to share metabolic intermediates. Ongoing studies are investigating the ATP:ADP of astrocytes *in vivo* using 2P imaging.

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Neurotrauma

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Modeling post-traumatic epilepsy after repeated blast-induced traumatic brain injury

Student

Introduction: Blast-induced traumatic brain injury (bTBI) is a critical concern in military contexts, with ongoing biomedical efforts focused on elucidating and mitigating the neurological sequelae associated with bTBI. Some studies show a relationship between bTBI and post-traumatic epilepsy (PTE) but bTBI-induced PTE remains a relatively underexplored, yet critical, potential long-term consequence of bTBI. This study aims to investigate the interplay between bTBI and PTE, focusing on the potential identification of sleep disturbances as a biomarker for epileptogenesis and neurovascular damage.

Methods: Male and female Sprague-Dawley rats are subjected to repeated blast exposures (21 psi peak overpressure) at a 24-hour interval using a helium-driven shock tube (McMillan Blast Device) to induce severe bTBI. One cohort (N=8) was housed in piezoelectric cages at two months post-injury and animals were continuously monitored to capture sleep/wake cycle patterns and to assess the potential onset of seizure activity and progression of PTE. The piezoelectric cages are equipped with piezo-sensitive sensors and continuous video surveillance to measure sleep duration and detect seizure activity. Sleep is assessed over a 12:12-hour light/dark cycle. To investigate vascular pathology, we developed a protocol in preliminary studies for isolating large cerebral vessels and capillaries from rat brain. Additionally, we also stained for smooth muscle and pericyte markers to confirm purity of isolation of distinct vascular fractions.

Results: The blast pressure data were consistent across all cohorts for the initial severity level of 19.98±1.24 psi, as measured by AstroNova TMX18 and analyzed via AstroViewX64. Sleep data, analyzed using the integrated sleep statistics module within the piezo cage system, indicated that the percent sleep during the light and dark cycles the eight animals in the first cohort were 61.33 ±5.49 %and 42.71±5.38 %, respectively. Additionally, no seizure activity was detected between 2 months and 3 months following bTBI. The isolated large vessels and capillaries upon low magnification imaging showed distinct vascular fractions due to vessel size.

Conclusion: We successfully established a consistent bTBI model for the study of post-traumatic epilepsy (PTE) development. Although the initial experiment indicates no bTBI-induced alterations in sleep or seizure parameters, future directions involve extended monitoring, detailed recording, and analysis of pathophysiological marker expression. Disruptions in sleep cycles, along with alterations in both large vessel and capillary markers, serve as clear indicators of pathological changes after bTBI that could contribute to the development and progression of PTE.

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Pathophysiological Alterations in Rats with Sepsis following Spinal Cord Injury.

Fellow

Spinal cord injury (SCI) is results in motor paralysis. Post-injury complications result in compromised quality of life. Sepsis after SCI is one of the predominant secondary complications that results in impairment of spontaneous motor recovery and increased mortality in human patients. Interestingly, no clinically relevant experimental model is available to study complications in sepsis survivors after SCI. The current study is designed to develop a novel rodent model that mimics the long-term complications in sepsis survivors post-SCI.

All animals were randomly divided in 4 groups – Sham, Sepsis, SCI and SCI+Sepsis. Rats in SCI and SCI+Sepsis received T10 laminectomy and contusion (200kDyn) using Infinite Horizon impactor. Sepsis was induced by injecting cecal slurry (3ml) intraperitoneally immediately after SCI. All animals received fluid resuscitation and antibiotic 8 hours after SCI and/or sepsis induction, then twice daily for 5 days. The animals were monitored for survival and body weight as well as they undergo blood sampling and tissue collection including spinal cord, spleen and leg muscles at designated time post-SCI and/or -sepsis to assess for cytokines levels. For long-term study, animals are tested for hind-limb locomotor function using weekly BBB, horizontal ladder and in vivo skeletal muscle strength. Spinal cords were collected for quantitative histology to determine spared gray and white matter.

Significant blood bacteremia was observed in SCI + Sepsis group compared to sepsis alone at 6hr post-sepsis induction. No blood bacteria were found in Sham or SCI alone group. Lowest survival was observed in SCI+Sepsis (~39.3%) followed by Sepsis alone group (~over 55% whereas all animals survived in SCI and Sham groups. SCI+Sepsis resulted in most impaired hindlimb locomotor recovery compared to all three groups. Rats in SCI+Sepsis group were able to stand or walk without support (BBB~9) whereas rats in SCI group walked with occasional coordination (BBB~12) at 12-weeks post-SCI. Animals in Sham and Sepsis groups did not show any hindlimb locomotor deficits. In vivo muscle-strength test also showed significant muscle weakness in SCI+Sepsis versus SCI. At acute time points, Splenomegaly, reduction in weights of leg skeletal muscles and elevated blood cytokines in SCI+Sepsis group were observed. Ongoing studies are analyzing cytokines and histological changes in spinal cord.

In summary, this study is the first step towards understanding underlying mechanisms of sepsis post-injury and paving the way to elucidate therapeutic strategies for SCI.

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Temporal Dynamics of B Cell Infiltration after Contusive Traumatic Brain Injury in Mice

Student

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity for young adults. TBI survivors face persistent cognitive and neurobehavioral deficits. Repeated unsuccessful clinical trials targeting neuronal injury mechanisms have motivated investigations of other cell types in the complex secondary injury cascade following trauma. The roles of astrocytes and microglia in driving neuroinflammation are well established, as are contributions of systemic innate immune cells such as neutrophils and monocytes. Much less is understood about the adaptive immune response to TBI. B cells are a critical component in the secondary line of defense and injury progression, interacting with T cells, secreting cytokines, and producing antibodies. After TBI, various central nervous system proteins can act as antigens to activate B cells and stimulate antibody production. Although clinical studies describe systemic adaptive immunity engagement through peripheral autoantibody production, knowledge of the timing and regional extent of B cell diapedesis into the brain following TBI is limited, as previous studies in experimental TBI were largely restricted to a single timepoint. We hypothesize that contusion TBI triggers delayed B cell diapedesis into the cortex. Adult male mice received a lateral controlled cortical impact (CCI) TBI or sham injury and brains were collected at 1, 3, 7, 14 or 28 days (n = 6-8 CCI and 4 sham/time point). Series (1:10) of coronal brain sections were immunolabeled with the B cell antibody B220. Regional cell counts were performed, excluding B cells within hemorrhagic regions. Compared to sham mice, CCI-injured mice exhibited increased numbers of B220+ B cells within the injury epicenter of the cortex at 1 and 3 days, which peaked at 7 days before declining at 14 and 28 days. A significant increase in B cells was also observed at 7 days postinjury in the cortex adjacent to the contusion and in the cortex of the contralateral (non-impacted) hemisphere. Small numbers of B cells were observed in deeper brain regions as well, with increased cell numbers in the ipsilateral and contralateral hippocampi at 3 and 7 days postinjury, respectively, suggesting migration or delayed diapedesis. Though overall B cell numbers outside hemorrhage areas are low, the potential for production of autoantibodies by B cells could have implications in injury progression and neuroinflammation. Furthermore, B cell activation and inflammatory status could influence pathology. Future studies will characterize morphological and phenotypic characteristics of B cells within the injured brain to gain insight to their potential function and impact on injury pathology.

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Inducible expression of constitutively active AKT using retrogradely transported AAVs improves motor function after severe spinal cord injury.

Student

Repair of the damaged spinal cord remains an unsolved problem that limits function after spinal cord injury (SCI). Inducing growth and regeneration of damaged spinal axons can restore functions after SCI, however, to date very few regenerative treatments utilized in animal models maintain clinical viability due to the unspecific and off-target effects of pharmaceutical-based approaches. We have previously demonstrated that a single spinal injection of retrogradely transported AAVs (AAVrg) effeciently targets only spinal-projecting neurons of interest while also affecting near-all axons projecting from difuse regions of the brain. AAVrg's hold promise as a clinicaly viable approach to treat the injured spinal cord, but target identification and the permanence of transgene expression remain as barriers to translation. Our prior work knocked out the phosphatase and tensin homologue protein (PTEN; a potent inhibitor of AKT/mTOR) to treat SCI and observed a remarkable restoration of function. A permanent knockout of PTEN, however, is likely not viable as a clinical treatment. In this project we utilized AAVrg's to express a constitutively active AKT (myrAKT) to bypass PTEN inhibition, and used a neuron-specific doxycycline-inducible promoter system to gain temporal control of transgene expression. As a first approach, we utilized two AAVrg vectors to deliver the rtTA trans activator under a synapsin 1 promoter along with a separate vector expressing myrAKT under the Tetracycline Response Element. Mice were treated with 225 ppm doxycyline feed for 9-12 weeks after SCI and monitored for locomotor recovery. Female myrAKT-treated mice recovered significantly more locomotor functions compared to control treated mice. Similar to our observations after PTEN-knockout, we did not observe axon regeneration into or beyond the lesion, implicating spared axons as causal to functional improvements. We demonstrate the ability to eliminate doxycycline from rodent diets to turn off myrAKT expression, effectively gaining temporal control over regenerative signaling. Collectively, our work paves way for a clinically viable approach to deliver gene-therapies to treat SCI by combining AAVrg's, neuron-specific and inducible promoters, and the expression constitutively active proteins that regulate neuronal repair.

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Spinal cord injury (SCI) leads to an intraspinal inflammatory response including infiltrating blood leucocytes.

Fellow

Spinal cord injury (SCI) leads to an intraspinal inflammatory response including infiltrating blood leucocytes. Some of these subsets of immune cells (monocytes) contribute to ongoing tissue degeneration after SCI. Currently, there are no FDA-approved therapies for SCI. One promising therapy, clodronate liposomes (Formumax), depletes monocyte-derived intraspinal macrophages and several independent laboratories have reported therapeutic effects after lower thoracic SCI. The extent to which clodronate liposomes (CL) are effective after severe SCI or higher thoracic (T3) SCI is understudied. Here, we determined the effectiveness of CL after T3 SCI of multiple injury severities. Adult female Wistar rats were subjected to T3 spinal contusion with two different forces 300 kdyn (5s dwell time) and 400 kdyn (5s dwell time). For each severity, injured rats were randomly divided into two groups, one group received 2 ml Clodronate (7mg/ml) on days 1, 3, and 6 post-injury (once a day) through tail vein injections, and the control group received vehicle (2 ml saline). Spinal cords were isolated 7 days post-injury and histological assessment will be performed. Initial analyses reveal significant decreases in macrophage accumulation after T3 injury. Ongoing studies will determine if macrophage accumulation and the magnitude of CL-mediated depletion are injury severity-specific. Identifying the effectiveness of CL across multiple severities is clinically significant.

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Severe Hypoglycemia Causes Neuronal Degeneration and Neuroinflammation

Student

Background: Severe hypoglycemia is a serious complication for individuals with insulin-treated diabetes. When blood glucose is very low, the brain is deprived of its main fuel source and neuronal damage can result. The areas most affected by hypoglycemia-induced damage are the learning and memory areas of the brain, such as the hippocampus. This project aims to develop a novel rodent model of neuronal damage caused by insulin-induced severe hypoglycemia and determine mechanisms involved.

Methods: To induce brain damage, 10-week-old male Sprague-Dawley rats underwent a single episode of severe hypoglycemia (10-15mg/dL for 90 minutes) induced by a subcutaneous injection of Humulin R insulin (15U/mg/kg) and compared to a control cohort of saline injected rats. One week following the intervention, the animals underwent transcardiac perfusion and their brains extracted. Brain sections (40μm) of the hippocampus were obtained for immunohistochemistry staining using Iba-1/CD68, Cleaved Caspase 3 (CC3), and Fluoro-Jade C (FJC), to identify potential mechanisms by which neuronal damage from severe hypoglycemia occurs. Stains were analyzed using ImageJ software and total number of cells between control and severe hypoglycemic control groups were analyzed using a Student's *t* test.

Results: Rats that underwent insulin-induced severe hypoglycemia had four-fold more FJC+ cells (a neuronal degeneration marker) in comparison to control rats (SH=2510±848, CON=650±214, $p<0.001$). Similarly, the severe hypoglycemic group had a higher number of cells that expressed CC3, an apoptotic marker, in comparison to control animals (SH=9833±980, CON=7204±853, $p<0.001$). Iba-1/CD68 staining, that identifies microglial immunoactivity, was five-fold higher in the severe hypoglycemic group in comparison to controls (SH=576±191, CON=114±60, $p<0.0001$).

Conclusions: This novel rodent model of severe hypoglycemia causes brain cell death indicated by FJC and CC3 immunohistochemistry staining. Severe hypoglycemia also causes neuronal inflammation indicated by Iba-1/CD68 staining.

Other Neurologic Conditions

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Optimizing Glyoxal as an Alternative Tissue Fixative to PFA

Staff

Paraformaldehyde (PFA) is widely used as a tissue fixative for histological purposes. However, due to its high toxicity and carcinogenic effects, there have been efforts to find an alternative fixation solution. Glyoxal, a small dialdehyde, has recently shown promise for this position. Glyoxal is considerably less toxic than PFA and finds utility in a wide range of fields. It has been the focus of several papers in the last 10 years that praise its effectiveness as a tissue fixative due to its ease of use and high-quality staining results using immunohistochemistry. Our lab took on the task of optimizing glyoxal as a fixative for mouse brain tissue. Using concentrations ranging from 3 – 50% and a variety of antibodies targeting the CNS immune system, we evaluate the effectiveness of this potential alternative. Most papers cite that 3% glyoxal is sufficient for tissue fixation, however, our lab found that at least 7% is required, with closer to 50% yielding optimal structural results for tissue stability. We also tested the addition of ethanol to the solution to act as a catalyst in the fixation reaction and determined 10% ethanol to be the preferred concentration. Finally, in terms of solution, we determined that sodium acetate buffer with a pH of 4.0 provided the best diluent for the other components. We used a variety of antibodies to stain for microglia, astrocytes, leukocytes, and vasculature using immunohistochemistry and compared those results to the same stains on PFA-fixed tissue. We found striking differences in the presence and quality of staining compared to the two fixation methods.

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Neurocognitive Processing Speed Enhancement from Gamma Entrainment.

Student

Inducing neural oscillations (neural entrainment) has been established in the behavioral neurology literature and has been used to study how said patterns correspond to various cognitive functions. One of the more recent applications has been to use neural entrainment to stimulate cellular activities that often diminish as a function of neurodegeneration. Specifically, microglial cells are highly sensitive to a 40 Hz gamma signal and show subsequent increased proliferation after gamma entrainment. Therefore, inducing neural activity into a gamma oscillation pattern (i.e. causing cells in your brain to mimic an induced 40 Hz pattern) via both auditory and visual stimulation has garnered significant attention as a viable non-invasive, non-pharmaceutical intervention for individuals suffering from neurodegenerative conditions. Gamma-entrainment using sensory stimuli (GENUS) has mostly revolved around the improvements seen on memory-related tasks and almost solely for neurodegenerative populations. While the literature has primarily focused on neurodegenerative populations, gamma entrainment is a safe neural intervention that can be applied to any individual and there are limited reports of associated adverse consequences. As such, there are many tools using GENUS (e.g., headsets, audio-files, Youtube clips, etc.) that are commercially available and marketed as “cognitive enhancers” for everyday use. However, there has not been a thorough and systematic assessment yet of how and if GENUS leads to any improvements for neurotypical adults in general. This project piloted to establish a broader assessment of GENUS on behavioral and neurological function in neurotypicals beyond memory functioning. EEG-validated gamma activities were assessed in the auditory and visual cortices of neurotypical participants for sessions (n=7, Daily 1-hour daily GENUS, 8-consecutive days, BME Electronics: Gamma 40Hz Audio-Visual Sensory Stimulation; Day 9 GENUS + EEG, EGI 64-channel nets; impedance < 20kΩ), coupled with pre-post GENUS assessments of cognition using the NIH Toolbox V2 (Day 1 and Day 10). Repeated measures t-test pre-post gamma entrainment revealed significant differences in overall cognition ($p=0.02$) mainly driven by processing speed enhancement in this sample ($p=0.01$). This proof of concept revealed results consistent with the literature in clinical populations for neurotypicals’ speeded tasks, providing pilot evidence warranting larger scale experiments with additional domain-specific experiments, and assessment of applicability to other clinical samples beyond neurodegenerative conditions.

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Elucidating the molecular events involved in optic fissure fusion in zebrafish eye.

Student

During eye morphogenesis, the neuroepithelium from the developing forebrain evaginates to form two single layered optic vesicles. Subsequently, the distal end of each optic vesicle invaginates to form a double layered optic cup. A result of this folding is a ventral opening in the retinal tissue called the optic fissure (OF) that remains open at early stages and allows vasculature entry into the retina. A precise and timely closure of the OF is very important for retinal development. Failure of fusion leads to a congenital blinding disorder called coloboma. Previous studies have identified transcriptional regulator pax2 (pax2a in zebrafish), vax 1, and vax2 as critical for proper OF fusion. However, the molecular mechanisms driving OF fusion still largely remain unknown. Therefore, to understand this dynamic process in detail, we conducted a comprehensive analysis of the transcriptional changes associated with OF fusion. To do so we employed a combination of a transgenic zebrafish optic fissure reporter line, a detailed time course of samples, and scRNA sequence analysis. Using transgenic Tg[rx3:GFP] zebrafish embryos and FACS, we have generated a comprehensive OF fusion single cell transcriptome at 24, 26, 28, 30, 32, 34, 36 and 48 hpf. rx3:GFP expression is restricted to the OF and therefore specifies our analysis to only OF cells. On average, we have collected more than 4500 cells/timepoint covering up to 20,000 genes. To filter for OF fusing cells I had primarily focused on cells expressing pax2a. From this strategy I have already identified a few potential target genes that are likely to be involved in the OF fusion process. My analysis was done using Cloupe5 and Partek flow software for clustering while trajectory/pseudotime analysis of OF associated clusters was performed using Monocle3 and Partek flow software. To date, I have confirmed the expression of some novel target genes in the OF via in situ hybridization, namely *claudin19 (cldn19), atypical chemokine receptor 3b (ackr3b), chemokine (C-X-C motif) ligand 12a (cxcl12a),* and *sprouty homolog 4 (spry4). cldn19* is found in tight cellular junctions, *spry 4* is involved in notch signaling pathway which facilitates cell to cell communication, *ackr3b* and *cxcl12a* are predicted to act as chemokines and chemo attractants respectively, and thus indicating potential roles in epithelial fusion. Interestingly, they show significantly low expression at the OF in the pax2^{noi} coloboma line. We hypothesize that these genes play important roles in OF fusion, and are performing functional analysis using CRISPR. With this study, we hope to offer the first thorough transcriptome investigation of OF fusion and hopefully shed new light on the elusive etiology of coloboma.

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Perinatal opioid exposure and maternal HCV infection compromise microglia development and function

Student

Prenatal opioid exposure (POE) is associated with poor fetal neurodevelopment outcomes, including microstructural brain injury and poor Mental and Psychomotor Development Index scores. The mechanisms underlying these changes have yet to be well characterized. POE may impair fetal brain development directly via opioid accumulation in placental tissues, which could lead to impairment of the microglia. Microglia are critical regulators of CNS development through their ability to regulate neuronal survival and differentiation, axon outgrowth, and oligodendrocyte progenitor cell (OPCs) maturation and subsequent myelination. Thus, dysregulation in microglia development could have lifelong consequences for microglial function and neurodevelopment.

To unravel the mechanisms underlying how POE affects fetal neurodevelopment, we utilized a model of microglia-like cells derived from umbilical cord blood mononuclear cells (UCBMC), which mimic the morphology and function of microglia in vivo. Differentiation of microglia-like cells from UCBMC is confirmed by morphology and phenotyping (DAPI, TMEM119, PU.1, IRF8). We then assessed the functional landscape of microglia using multiplex Luminex, phagocytosis assays, and bulk RNA sequencing. Finally, we further stratified our findings by maternal HCV status at the time of labor and delivery, given the high prevalence of HCV infection in subjects who inject drugs.

Our results indicate that POE increases the activation of UCBMC-derived microglia, as shown by the increased production/expression of inflammatory mediators and genes. However, these microglia-like cells are functionally defective, exhibiting reduced phagocytosis and attenuated responses to LPS stimulation. Functional alteration potential was accompanied by transcriptional changes in genes associated with nervous system development, synaptic pruning, and innate immune responses. Overall, these findings suggest a significant impact of POE on microglia development and function, providing insight into the poor neurocognitive outcomes observed in newborns with POE.

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Asprosin, an analgesic orexigenic hormone

Student

Nearly forty percent of the United States adult population suffers from some form of pain, making it the most common reason for seeking clinical care. While persistent pain can be maladaptive, the acute pain response is an adaptive mechanism that protects the organism against dangerous stimuli. Rodent studies suggest that hunger selectively inhibits the behavioral responses to pain and that this analgesia is substantially mediated by a subpopulation of hypothalamic agouti-related protein- (AgRP) expressing neurons (the key hypothalamic cell type responsible for appetite stimulation). In 2016, Dr. Mishra's lab discovered asprosin, a fasting- induced hormone that is highly expressed in adipose tissue. Upon secretion, asprosin stimulates appetite and hepatic glucose release. Dr. Mishra identified Protein Tyrosine Phosphatase Receptor **δ** (Ptprd) as the central receptor that asprosin engages to activate AgRP neurons. Our current results suggest that asprosin, an orexigenic hormone, also alters the nociceptive response in mice, providing a potential endocrine mechanism for appetite induced analgesia. We have found that asprosin deficient mice feel more pain (hyperalgesia), asprosin overexpression causes analgesia in wild type mice, and 7BIA treatment decreases pain tolerance in wild type mice. This study thus far suggests an analgesic function of asprosin and its potential as a therapeutic for alleviating chronic pain.

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Does time of administration matter? Investigating the effects of exenatide, a GLP-1 receptor agonist, on clock gene expression in the diabetic brain.

Student

Clock genes create and regulate circadian rhythms through a series of interlinked transcription-translation feedback loops. These loops generate oscillations in gene expression and protein levels that repeat approximately every 24 hours. These genes play a crucial role in controlling various physiological and behavioral patterns such as sleep-wake cycles, hormone release, body temperature, cardiovascular function, and metabolism. There is a bidirectional relationship between Type 2 diabetes and circadian disruption and, by extension, worsened cardiometabolic outcomes and sleep disturbance.

We have previously demonstrated that *db/db* mice exhibit circadian rhythm disruption, manifesting as loss of food intake rhythm, nondipping or reverse dipping blood pressure, and blunted 24-h oscillation of sympathetic nervous system biomarkers. Moreover, we demonstrated that ZT0 (light/rest phase start) administration of exenatide can effectively restore food intake rhythm, reestablish normal blood pressure circadian rhythm and reduce hypertension, and reinstate normal norepinephrine excretion pattern. In contrast, ZT12 (dark/active phase start) administration of exenatide worsens food intake pattern, blunts blood pressure circadian rhythm or causes a reverse dipping pattern, and exacerbates misaligned norepinephrine excretion pattern *db/db* mice.

The current study measures changes in clock gene mRNA expression at ZT5 or ZT17 in the brain and muscle at baseline or after treatment with exenatide at ZT0 or ZT12 in *db/db* mice. The cortex of *db/db* mice had the most statistically significant effect, with ZT0 injection of exenatide, enhancing or restoring the day to night difference in bmal1, per1, rev-erb**α**, rorc. While these findings are promising, the implications by which ZT0 exenatide-induced changes to clock gene activity have on cardiometabolic outcomes and sleep disturbance, requires additional experiments.

Stroke/Neurovascular

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Proteomic and Demographic Comparisons of Patients Suffering from a Recurrent Ischemic Stroke

Student

Ischemic stroke is the third leading cause of combined death and disability world-wide. Due to improved acute interventions and diagnostic imaging, more patients are surviving ischemic stroke, but the rates of recurrence have remained relatively unchanged over the past couple decades. This population with high rates of recurrence highlights a need for secondary prevention of stroke recurrence, an area in which there have been limited advancements. This study aims to utilize the Blood And Clot Thrombectomy Registry And Collaboration (BACTRAC) tissue bank to identify proteomic and demographic differences in patients suffering from a recurrent ischemic stroke compared to a first stroke. Blood samples were collected during mechanical thrombectomy in patients suffering from large-vessel occlusion ischemic strokes. Plasma levels for 184 inflammatory and cardiometabolic proteins were measured in systemic blood and intracranial blood from the area of infarction. Differences in categorical variables were analyzed using Fisher's Exact Test. Differences in continuous variables were analyzed using Student's independent samples t-tests or Welch's t-tests when variances were unequal. Non-parametric Mann-Whitney U tests were performed for variables with a non-normal distribution. Proteins were divided into systemic and intracranial proteins, and independent samples t-tests were performed with a False Discovery Rate (FDR) of 5.0% for each group using the two-stage step-up method of Benjamini, Krieger, and Yekutieli. Significant variables identified by these methods were used in a multiple logistic regression to predict patients with a prior stroke, using $\alpha = .05$ for statistical significance. There were 20 patients in the prior stroke group and 121 patients in the first stroke group. The prior stroke group had a significantly higher percentage of females than the first stroke group (80.0% vs 50.4%, Odds Ratio (95% CI): 3.93 (1.30-11.26), p=0.016) and a significantly lower rate of HLD comorbidity (10.5% vs 35.5%, Odds Ratio (95% CI): 0.21 (0.05-0.84), p=0.034). Two proteins were significantly higher in those with a prior versus first stroke: systemic CCL14 and systemic FGF-19. The difference in CCL14 level means was 0.505 (0.134) (difference (SE); q = 0.022). The difference in FGF-19 level means was 0.863 (0.227) (q = 0.022). Multiple logistic regression performed with age, sex, HLD comorbidity, CCL14 levels, and FGF-19 levels found CCL14 and FGF-19 levels to be significant. Higher levels of CCL14 and FGF-19 were associated with a greater likelihood of the stroke being a recurrent stroke. Serum levels of CCL14 and FGF-19 obtained during thrombectomy are easily accessible and quantifiable biomarkers and are possible therapeutic targets for recurrent stroke prevention. These findings provide two new proteins that, with other demographic variables, could provide a predictive model to identify patients with a high probability of recurrent ischemic strokes.

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The Role of Complement Component 5a Receptor 1 (CD88) Antagonist PMX205 in Post-Stroke Depression: Clinical Findings and Therapeutic Implications

Staff

Stroke affects approximately 800,000 individuals in the United States and is frequently accompanied by long-term neuropsychological consequences, including post-stroke depression (PSD), which develops in about 33-50% of stroke patients. The complement system, a key component of the innate immune system, plays a crucial role in inflammation and its dysregulation or aberrant activation has been linked to various brain disorders. Using the BACTRAC tissue bank, we directly analyzed inflammatory pathways near infarcts from stroke patients. Our data reveal a significant increase in the mRNA expression of complement component 5a (C5a) in both systemic and intracranial blood collected during thrombectomy, compared to control subjects. Furthermore, C5 expression correlates positively with the severity of depressive symptoms as measured by the Patient Health Questionnaire-9 (PHQ-9) score in patients three days post-stroke. In an aged rat stroke model, we observed a notable increase in the gene expression of component 5a receptor 1 (C5aR1) in blood cells (buffy coat) both acutely (5 hours) and chronically (30 days) after stroke. Preliminary findings also indicate that aged rats exhibit a significant PSD phenotype at 30 days post-transient Middle Cerebral Artery Occlusion, with elevated C5aR1 protein levels in their brains at both 3 and 30 days post-stroke. Importantly, preliminary studies with PMX205, a known antagonist of C5aR1, suggest its potential to modulate the C5aR1-mediated inflammatory response and mitigate PSD. This study is impactful as it extends our understanding of PSD pathology and explores PMX205 as a novel therapeutic target for stroke-related depression.

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Leukemia Inhibitory Factor (LIF) Improves Mitochondrial Bioenergetics after Ischemic Stroke

Staff

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Ischemic stroke is a significant cause of mortality and disability resulting from neuronal injury and mitochondrial dysfunction. Studies show that ischemic stroke injury can disrupt metabolic substrates in the tricarboxylic acid (TCA) and mitochondrial oxidative phosphorylation (OXPHOS) cycles, deplete cellular energy (a.k.a., ATP), and produce consequent mitochondria-centric dysfunction. Neuroprotection is emerging as a promising avenue for stroke injury treatment, yet there remains a critical need for therapeutic strategies that target stroke injury, particularly via mitochondrial pathways. Leukemia inhibitory factor (LIF) is a cytokine with potent anti-inflammatory and anti-oxidative properties that promotes neural cell survival. Previous studies have shown LIF's efficacy in reducing stroke-induced tissue damage and facilitates functional recovery through binding to a heterodimeric receptor comprising the LIF receptor subunit and glycoprotein 130. This binding can lead to neuroprotective cascades mediated by protein kinase Akt and subsequent transcription of antioxidant genes. This study comprehensively explores the relevant metabolic mechanisms of LIF, focusing on both acute and chronic functional and mechanistic aspects of ischemia- induced injury.To best model stroke patients, transient middle cerebral artery occlusion (tMCAO) procedures were performed on aged rats of both sexes. Our results in aged male and female rats following tMCAO injury-induced ischemic lesions revealed mitochondrial OXPHOS dysfunction in the striatum and cortex at 3 days post-stroke. Further focusing specifically on mitochondrial ETC enzyme complexes activity, we demonstrated consistent injury effects after stroke. Through western blot analysis, we confirmed that ETC protein expression remains comparable, which has further indicated that the stroke's effects are at the functional level rather than the structural level of mitochondria. Our novel findings have suggested that LIF treatment protects against mitochondrial OXPHOS without impacting ETC complexes enzyme expression and activity; further allowing us to postulate potential regulation of TCA cycle flux or other metabolic pathways by LIF. Overall, this mitochondrial specific, functional and metabolic integrated approach will be helpful in paving the way for targeting the future therapies against stroke mediated secondary injury.

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Validation of a Wearable Sensor-Based Device for Objectively Tracking Hand Function

Student

Strokes are one of the leading causes of lifelong disabilities, specifically upper extremity impairments that significantly affect hand function. Stroke survivors typically undergo extensive clinical evaluations along with physical therapy aimed at assessing impairment severity and aiding in hand motor recovery. However, these clinical assessments often rely on subjective judgments made by clinical personnel, and given these limitations, a sensor-based device could offer a valuable alternative by providing objective assessments of hand impairment and enabling the monitoring of progress during therapy.

While existing devices on the market tend to be expensive and primarily focus on finger kinematics, our wearable sensor-based device (WSBD) is designed to measure individual finger movements and the force applied at the fingertips through the use of flex sensors and force-sensitive resistors (FSRs). With IRB approval, we recruited 30 individuals, with an average age of 25.8 years (SD = 4.6) and no reported hand impairments, to assess the feasibility of using the WSBD to track hand function. Participants were prompted to perform graded extension, contraction, and applied force tasks with four distinct target levels: low, medium, high, or no-go. They received real-time feedback from a graphical user interface (GUI). Depending on whether participants were in the first group (A) of 15 or the second group (B) of 15, they were provided with slightly different interfaces. Group B had a modified GUI based on Group A's responses to a User Feedback Survey completed at the end of the session.

Our findings revealed that, within individuals, the measurements of movement and applied force differed significantly across targets ($p < 0.05$). The output from the flex sensors strongly correlated with non-contact motion capture measurements used as a reference, while the FSR sensors' output correlated with load cell measurements. These correlations demonstrate a strong association ($|r| > 0.7$) between the test sensor and reference measurements. Additionally, the mean relative errors between the test and reference sensors were low at -0.31% for flex sensors and 3.75% for force sensors. Based on the results of this study, the WSBD shows promise as a reliable tool for consistently tracking individual finger movements and applied force. Further work must be performed to demonstrate that this device can be used as a functional assessment tool in stroke patients.

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Plasma Extracellular Vesicle-Associated Markers of Endothelial Cell Activation in Cerebrovascular Disease

Staff

The ability to differentiate cerebrovascular disease (CVD) with plasma biomarkers could be an invaluable tool. CVD encompasses a spectrum of conditions that alter intracranial blood flow and activate endothelial cells (ECs), which results in a dysregulated blood-brain barrier (BBB). Activated ECs upregulate and cleave many junctional proteins, including soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble intracellular adhesion molecule-1 (sICAM-1), and von Willebrand factor (vWF). These junctional complexes regulate BBB permeability and infiltration of leukocytes that release interferon-gamma (IFN-ɣ) for immune cell regulation. Key barriers to providing specialized therapies for CVD are a lack of biomarkers to differentiate pathologies. Extracellular vesicles (EVs) are nanoparticles released from all cells, and carry cargo specific to their parent cell's microenvironment, which may provide insight into cerebrovascular changes before overt pathological and cognitive changes are present. Therefore, we utilized the Blood And Clot Thrombectomy Registry And Collaboration" (BACTRAC; NCT03153683) and "Moyamoya and Stroke Tissue Evaluation and Repository" (MASTER) to measure plasma EV sVCAM-1, sICAM-1, vWF and IFN-ɣ concentrations from patients with aneurysms, emergent large vessel occlusions (ELVO), and Moyamoya. Moyamoya vasculopathy is a unique CVD that presents with internal carotid artery terminus stenosis and abnormal vascular collaterals. EVs were isolated using size exclusion chromatography, concentrated, and the protein isolated for analysis using MSD assays. Aneurysm (n=17, 76.5% female), ELVO (n=20, 65% female), and Moyamoya (n=21, 66.7 % female) groups did not have significant differences in sex, body mass index, hypertension, smoking status or diabetes. ELVOs were significantly older (72.75 ± 3.3 years) than Moyamoya (47.2.1 ±2.0, $p<0.0001$) and aneurysms (50.9 ± 5.5, $p=0.01$) and had significantly lower EV IFN-ɣ ($p=0.004$). In ELVOs, lower EV IFN-ɣ correlated to larger edema volumes ($p=0.023$, $r=-0.821$) and higher stroke severity (NIHSS, $r=-0.812$, $p=0.05$). Moyamoya EVs showed increased sVCAM-1 ($p= 0.069$) and sICAM-1 ($p=0.057$), compared to aneurysm, with significantly lower ($p=0.001$) vWF, compared to aneurysms and ELVOs. Higher EV sICAM-1 trended ($p=0.059$, $r=0.794$) for better cognitive outcomes following ELVO. These data suggest that the plasma biomarker profile of BBB dysregulation varies among types of CVD, and that plasma EVs may act as a liquid biopsy that provides insight into the type and severity of CVD.

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Developing Simple and Sensitive Behavioral and Vision Tests for a Mouse Model with Mild Stroke

Student

Stroke is the 5th leading cause of death and leading cause of long-term disability–including vision impairment. Characterization of the effects of various degrees of stroke injury in animal models can offer insight into understanding the injury and possible treatments. However, it is difficult to characterize the effects of mild stroke on mouse behaviors and vision. We hypothesized that stroke injuries result in altered behaviors and/or impaired vision, and that degrees of behavioral alterations and vision impairment correlate with the magnitudes of the injuries. In this project, we evaluated an array of simple behavioral and vision tests for their effectiveness in phenotyping a transient distal middle cerebral artery occlusion (dMCAO) mouse model. Our results show that several tests with selected analytical parameters provide sensitive readouts for dMCAO, including asymmetry in limb usage, grip strength/endurance, head direction, color preference, visual acuity and sensitivity to contrast, and asymmetry in visual acuity and contrast sensitivity. Our analyses of the trends of changes in these parameters along with infarct volumes further show a correlation between increasing magnitude of the dMCAO injury and increasingly altered behaviors and worsening vision. Our results provide simple and sensitive assays for quantifying outcomes of a wide range of magnitudes of strokes for preclinical studies. Based on these results, we suggest certain sensitive assays to use for mouse models of stroke. Our work not only improves our understanding of vision impairment in stroke, but also provides useful tools for evaluating treatments for long-term disability resulting from stroke.

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Utilizing Metabolic-Associated Protein Differences in Total and Astrocyte Enriched Extracellular Vesicles to Differentiate Cerebrovascular Disease

Staff

Extracellular vesicles (EVs) are an understudied, yet highly relevant, source for biomarkers of neuroinjury. EVs are small (30-150 nm) lipid particles, released from all cells, with parent cell specific surface markers. EVs are readily able to cross the blood brain barrier allowing a "non-invasive liquid biopsy" that provides a window into developing brain pathology, including cerebrovascular disease (CVD). CVD encompasses a spectrum of vascular changes that alter intracranial blood flow. Astrocytes are one of the key cells that regulate cerebrovascular blood flow and become activated when the metabolic demand within the neurovascular unit (NVU) are altered. While the brain consumes 20% of the body's total energy. Energy is primarily utilized from glucose, called glycolysis, and when glucose is low, lactate and pyruvate can be metabolized to glucose, termed 'gluconeogenesis'. Therefore, we hypothesis that total and astrocyte specific EVs (TEV and AEV)-associated metabolic proteins will differentiate pathological changes and severity of two common CVDs (carotid stenosis and emergent large vessel occlusion (ELVO)). We utilized the Blood And Clot Thrombectomy Registry And Collaboration" (BACTRAC; NCT03153683) to isolate EVs using size exclusion chromatography and concentration columns. Protein was isolated and sent to Olink for protein analysis in their metabolic panel. Preliminary data suggest carotid stenosis subjects have increased plasma TEV fructose-1,6-Bisphosphatase (FBP1), an enzyme in gluconeogenesis. Plasma collected during an acute ELVO showed lower plasma TEV FBP1 and higher TGF-beta-1 (TGF**β**1). TGF**β**-1 upregulates Hexokinase (HK), a glycolytic key enzyme and in ischemia, higher TGF**β** is associated with neovascularization. In acute ELVO subjects, higher plasma TEV TGF**β**1 was associated with lower stroke risk factors for CVD: HDL cholesterol ($p=0.03$, $r=0.633$) and triglycerides ($p=0.022$, $r^s=-0.636$), but was associated with worse cognitive outcomes, measured by the Montreal Cognitive Assessment (MoCA), ($p=0.04$, $r=-0.998$). Additionally, an enzyme associated with oxidative stress, quinoid dihydropteridine reductase (QDPR), was higher in the plasma AEVs from ELVO subjects, compared to carotid stenosis. Higher QDPR was associated with lower functional recovery following stroke (modified Rankin Score, $p=0.026$, $r=0.728$) and higher cognitive impairment (MoCA $p=0.0377$, $r=-0.8995$). These data suggest that plasma TEV and AEV metabolic proteins vary among types of CVD, and that plasma EV proteins may differentiate types of CVD and their progression.

Addendum

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Vitamin B6 homeostasis in plants and humans.

Postdoc

Dietary consumption of lysine in humans leads to biosynthesis of Δ1-piperideine-6-carboxylic acid (P6C), with elevated levels linked to the neurological disorder epilepsy. We demonstrate that P6C biosynthesis is also a critical component of lysine catabolism in plants. P6C regulates vitamin B6 homeostasis and increased P6C levels deplete B6 vitamers resulting in compromised plant immunity. We further establish a key role for pyridoxal and pyridoxal-5-phosphate biosynthesis in plant immunity. Our analysis indicates that P6C metabolism likely evolved through combining select lysine and proline metabolic enzymes horizontally acquired from diverse bacterial sources at different points during evolution.