2023 Neuroscience RPA Clinical-Translational Research Symposium

Program Book

2023 NEUROSCIENCE RPA CLINICAL-TRANSLATIONAL RESEARCH SYMPOSIUM

Healthy Kentucky Research Building Lobby/Rm 150

November 2, 2023 3:00pm - 6:00pm

Neuroscience Data Blitz and Networking/Social Hour

(advanced registration required - limited to grad students & above)

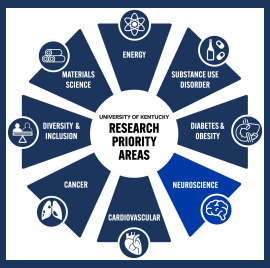
November 3, 2023 9:00am - 5:00pm

Full Day Program feat. keynote speaker, *Dr. Edward Kasarskis*

"Nutrition in ALS: Easy Question... Complicated Answer"

Register Here

NEUROSCIENCE RPA CLINICAL-TRANSLATIONAL RESEARCH SYMPOSIUM



THURSDAY, NOVEMBER 2 AGENDA

Healthy Kentucky Research Building - First Floor

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

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

Moderated by: Brian Gold, PhD

Tanner Anderson: Serotonin, Psychedelics, and Claustrum Signaling to Anterior Cingulate Cortex.

Gabriela Aparicio, PhD: Profiling Peripheral Glial Cells from Intact and Injured Human Nerves for Grafting in the Central Nervous System.

Madison Bates: How Does Hand Dominance Affect Compliance When Performing a Graded Finger Extension Task?

Ravichandra Davargaon, PhD: Energy Homeostasis within the Brain is Negatively Affected by Diabetes-Related Amylin Loss-of-Function.

Hannah Downing: Sex-Based Differences in Hippocampal Neurogenesis after TBI.

Shadan Hadi, MS: Actin Disorganization in Mammalian Auditory Hair Cell Stereocilia Shafts is a Key Difference Between Temporary and Permanent Noise-Induced Hearing Loss.

Sejuti Naurin, MS: Elucidating the Molecular Events Involved in Optic Fissure Fusion in Zebrafish Eye.

Jakob Shaykin, MS: Novel Compounds EO-139 and YZ-166 as Countermeasures for Reversing Opioid-Induced Antinociception, Motor Incapacitation, and Respiratory Depression.

Navid Tavakoli: Astrocyte Activity in the Dorsal Striatum Regulates Cue-Induced Reinstatement of Cocaine Seeking.

Velmurugan Gopal Viswanathan, DVM, PhD: Ex-vivo Model to Study Brain Capillary Mitochondrial Function and Dynamics Using Mitochondria Labeled Dentra-2.

Blaine Weiss: Localized Analysis of Vascular Astrocytes (LAVA): Measurement of Cerebrovascular Function in Live Animal Imaging.

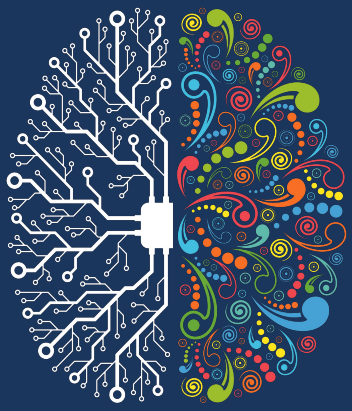
Justin Welden, PhD: Proteins Expressed from Tau Circular RNAs as New Drug Targets in Alzheimer's Disease.

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

Beer, Wine, and Light Snacks provided

Scan this QR code to register

2023 NEUROSCIENCE RPA CLINICAL-TRANSLATIONAL RESEARCH SYMPOSIUM



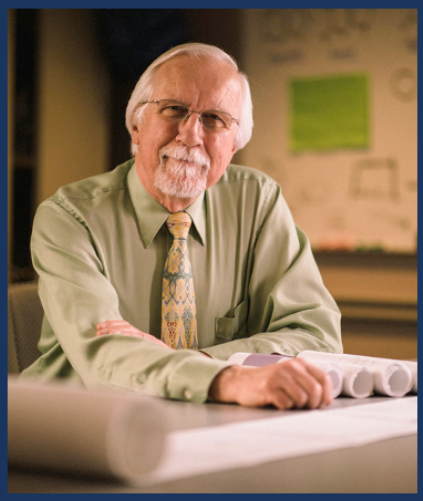
FRIDAY, NOVEMBER 3

10:00 am

Healthy Kentucky Research Building

Room 150

Keynote Speaker



Edward Kasarskis, MD, PhD

Professor, Cynthia Shaw Crispen Chair, Neurology
Medical Director of the ALS Multidisciplinary Clinic, KNI

"Nutrition in ALS: Easy Question. . . Complicated Answer"

Dr. Edward J. Kasarskis is the Medical Director of the ALS Multidisciplinary Clinic at the Kentucky Neuroscience Institute. He is also a professor in the Department of Neurology, the Cynthia Shaw Crispen Chair for ALS Research, and he has over 48 years of experience in the medical field. Dr. Kasarskis has dedicated himself to discovering the origin and cause of ALS, commonly known as Lou Gehrig's disease. His research involves ALS care, nutrition, genetics, and risk factors for ALS. There is no known cure for ALS, but he hopes that his work and that of his team will lead to breakthroughs broadly applicable to both ALS and related conditions like Parkinson's, Alzheimer's, or dementia. Dr. Kasarskis received his Ph.D. in Biochemistry and MD from the University of Wisconsin.

Register Here

FRIDAY, NOVEMBER 3 AGENDA

Healthy Kentucky Research Building (Lobby & Room 150)

9:00 - 10:00	**Complimentary Coffee and Poster Preview - HKRB Lobby**
10:00 - 10:10	**Welcome and Introduction: Larry Goldstein, MD & Linda Van Eldik, PhD**
10:10 - 10:55	**Keynote Presentation: *Edward Kasarskis, MD, PhD***
11:00 - 12:00	**Sleep & Circadian Rhythms** **Moderated by: Julie Pendergast, PhD**
	Jun Wang, MS: A Novel Protocol for Probing the Effects of Sleep and Temperature in a Mouse Model of Alzheimer's Disease. **Diane Iradukunda, MS:** Manipulation of Sleep Architecture in Mice: A Closed-Loop Thermoregulatory Approach. **Carrie Johnson:** Sleep and Circadian Rhythms, Biological Sex, and Alzheimer's Disease: A Complex Relationship. **Matt Thomas, PhD:** Feasibility of Implementing Time-Restricted Eating in Women with Mild Cognitive Impairment.
12:00 - 1:00	**Recovery After Stroke & Vascular Injury** **Moderated by: Ann Stowe, PhD**
	Hilaree Frazier, PhD: Inhibition of p38α MAPK Rescues Synaptic Function and Improves Behavioral Performance in a Mouse Model of Mixed Vascular and Amyloid Pathologies. **Katherine Cotter:** Effect of hCD20 Depletion on Operant Training After Stroke. **Christopher McLouth, PhD:** Using Penalized Regression to Identify Proteomic Predictors of Post-Stroke Recovery. **Amber Schifano & Madison Webster:** Virtual Reality Exergaming Reduces Neuroinflammation and Improves Functional Recovery in Stroke Patients: A Methodological Overview.

Scan this QR code to register

FRIDAY, NOVEMBER 3 AGENDA

1:00 - 1:30	**Lunch** (assortment of boxed lunches will be available)
1:30 - 2:30	**Poster Session - all presenting authors at their posters**
2:30 - 3:30	**Basic & Clinical Neurophysiology** **Moderated by: Sridhar Sunderam, PhD**
	Maxwell Lavin: Modeling Rat Seizure Onset Dynamics from Non-Invasive Motion Signals for Improved Seizure Screening. **Nicholas Constantino:** ATP Sensitive Potassium Channels Couple Metabolism with Neuronal Excitability. **Sarah Garcia Pava:** Graded Finger Extension Reflects Changes in the EEG: A Study in Healthy Right-hand Dominant Subjects. **Jared Rybarczyk:** Delta Focused Ictal EEG Source Imaging for Accurate Source Localization in Refractory Focal Epilepsy.
3:30 - 4:30	**Neurodegeneration** **Moderated by: Peter Nelson, MD, PhD**
	Qingjun Wang, PhD: Developing Simple Rodent Behavioral Tests for Dissecting Vision Impairment in Neurodegenerative Conditions with Cognition Decline. **Khine Zin Aung, PhD:** Alzheimer's Disease and Cancer: A Polygenic Risk Score Analysis. **Elif Coskun, MD:** Comorbid Pathology in Clinical Trial Participants: Autopsy Findings and Clinical Features. **Yang Jiang, PhD:** Working Memory related Frontal Brainwaves are Associated with Vascular and AD Plasma Biomarkers in Healthy Older Adults.
4:30	**Closing Remarks**

Scan this QR code to register

Abstract Index

Data Blitz Session

Platform Sessions

Sleep and Circadian Rhythms

Recovery After Stroke and Vascular Injury

25 Inhibition of p38α MAPK rescues synaptic function and improves behavioral performance in a mouse model of mixed vascular and amyloid pathologies.

26 Effect of hCD20 depletion on operant training after stroke.

27 Using Penalized Regression to Identify Proteomic Predictors of Post-Stroke Recovery.

28 Virtual Reality Exergaming Reduces Neuroinflammation and Improves Functional Recovery in Stroke Patients: A Methodological Overview.

Basic and Clinical Neurophysiology

30 Modeling Rat Seizure Onset Dynamics from Non-Invasive Motion Signals for Improved Seizure Screening.

31 ATP sensitive potassium channels couple metabolism with neuronal excitability.

32 Graded Finger Extension reflects changes in the EEG: A study in healthy right-hand dominant subjects.

33 Delta Focused Ictal EEG Source Imaging for Accurate Source Localization in Refractory Focal Epilepsy.

Neurodegeneration

35 Developing simple rodent behavioral tests for dissecting vision impairment in neurodegenerative conditions with cognition decline.

36 Alzheimer's Disease and Cancer: A Polygenic Risk Score Analysis.

37 Comorbid Pathology in Clinical Trial Participants: Autopsy Findings and Clinical Features.

38 Working Memory related Frontal Brainwaves are Associated with Vascular and AD Plasma Biomarkers in Healthy Older Adults.

Poster Session

Neurodegeneration

41 Extracellular Vesicles Derived from Glioblastoma Promote Microglia-Mediated Neur otoxicity.

42 In vigilo age- and sex-dependent alterations in S1 neuronal Ca2+ network dy namics may contribute to gait dysregulation.

43 Oxidative stress-associated cerebrovascular pathology in Alzheimer's disease.

44 Glial response in association to Multilumen Vascular Profiles in Neurodegenarative Dis eases.

45 GWAS of Longitudinal Cognitive Changes Related to Late-Onset Alzheimer’s Disease for Diverse Populations: A Systematic Review and Meta-Analysis.

46 Ramp sequence explains synonymous variant association with Alzheimer's disease in the Paired Immunoglobulin-like Type 2 Receptor Alpha (PILRA).

47 Participant characteristics associated with parental history of dementia in the Alzheimer’s Disease Research Center cohorts: implications for secondary analyses of National Alzheimer’s Coordinating Center’s Uniform Data Set.

Neurophysiology

49 Multichannel Electroencephalogram Database for Freewill Reaching and Grasping Tasks.

50 Neural Decoding of EEG Premovement Intention using Q-learning via Kernel Temporal Differences.

51 Regulation of neuromuscular development in C. elegans by an epidermal growth factor-like transmembrane protein.

52 Restoration of synapse development in a SNARE mutant background by a Ras GAP.

53 RAP-1 and RAC-2 GTPases contribute to PXF-1 mediated synaptic development in C. elegans.

54 A role for Rap and Ras guanine nucleotide exchange factors during synapse development in C. elegans.

55 Central processes contributing to age- and sex-dependent alterations in ambulatory stability.

56 The behavioral effects in overexpression of K2p channels in glial and motor neurons for larval Drosophila.

57 Effects of zinc on physiological processes in Drosophila and crawfish: cardiac, neural, synaptic transmission and behavioral assays.

58 The Neurophysiological effects of excess Li assessed in an authentic curriculum undergraduate research experience (ACURE).

59 The similarities and differences in the effects of bacterial endotoxin lipopolysaccharide (LPS) on synaptic transmission at glutamatergic synapses.

60 Inflammatory microRNAs are an active component of macrophage-microglia communication.

61 Investigating Parabrachial-Amygdala Circuits that Modulate Stress-Induced Anxiety.

Neurotrauma

63 Investigating mossy fiber bouton alterations after traumatic brain injury.

64 Temporal Dynamics of B Cell Diapedesis after Traumatic Brain Injury in Mice.

65 Clodronate-induced macrophage depletion following severe contusion spinal cord injury.

66 Driving reparative phenotypes of intraspinal macrophages post spinal cord injury.

67 Behavioral Impacts of PTEN Knockout Using AAVrg and Axonal Visualization via Tissue Clearing in a Lateral Hemisection Model of Spinal Cord Injury.

Other Neurologic Conditions

69 A Simplified In Vitro Platform to Study Myelin Gene Expression in Human Peripheral Glial Cells.

70 Using Single-Cell Long-Read Transcriptomics to Explore Cell-Type-Specific Signatures Across Murine Brain Regions and in Stimulated Human CD4+ T-Cells.

71 The bacterial endotoxin lipopolysaccharide (LPS) recruits synaptic vesicles for evoked transmission at glutamatergic synapses.

72 Behavioral characterization of atoxifent, a functionally selective and potent synthetic mu agonist.

73 Novel Compounds EO-139 and YZ-166 as Countermeasures for Reversing Opioid-Induced Antinociception, Motor Incapacitation and Respiratory Depression.

Stroke/Neurovascular

75 Virtual Reality as a Tool for Rehabilitation in Stroke Patients: Literature Review.

76 Rose Bengal photoinduction mediates capillary and astrocyte injury.

77 Autoimmune and Hypercoaguable Biomarkers in Moyamoya Syndrome Patients.

78 Unveiling Unique Cell Populations in Cerebrospinal Fluid of Subarachnoid Hemorrhage Patients using UMAP and Comparative Analysis with Peripheral Blood.

79 Differential Molecular Response to ELVO in Appalachian Subjects Treated with Mechanical Thrombectomy.

80 Addressing Sensitivity to Insulin and Glucose in Neurons and Astrocytes Using PercevalHR.

81 APOE4 Promotes Accumulation of Amyloid-Forming Pancreatic Amylin in Brain Parenchyma through a Blood-Brain Barrier Mediated Mechanism.

82 Aβ efflux impairment and inflammation linked to cerebrovascular accumulation of amyloid-forming amylin secreted from pancreas.

83 In vivo downregulation of pancreatic amylin improves brain function and reduces brain β-amyloid pathology in APP/PS1 mice.

Data Blitz Session

Tanner Anderson [1] • Jack Keady [2] • Judy Songrady [2] • Jill Turner, PhD [2] • Pavel Ortinski, PhD [1]
Neuroscience University of kentucky [1] • Pharmaceutical Sciences University of kentucky [2]

Serotonin, Psychedelics, and Claustrum Signaling to Anterior Cingulate Cortex

Student

The claustrum (CLA), a subcortical nucleus, has the highest density of the serotonin 2A receptor (5HT2AR) in the brain with extensive connections to other brain areas, most prominently the anterior cingulate cortex (ACC) that is involved in both cognitive flexibility and drug-seeking behaviors. Though the CLA is gaining increasing attention for its' potential importance in regulating several aspects of cognition, almost nothing is known about the role of its' robust serotonergic innervation. Here, we target several 5-HTRs in the CLA with RT-qPCR, RNAscope, and whole cell patch clamp electrophysiology to characterize the function of 5-HTRs in CLA-ACC signaling with and without cocaine self-administration.

5-HT caused dramatic inhibition in CLA-ACC neurons. Significant decreases in sEPSC frequency and amplitude were observed, as well as decreases in action potential firing rate and hyperpolarization of the resting membrane potential. Next, we used qPCR to observe the relative abundance of 13 different 5-HT receptor subtypes within the CLA, finding elevated levels of 5-HT1A, 2A, 2B, and 2C receptors. CLA-ACC neurons were then recorded in the presence of 5-HT and antagonists of each of these receptors to observe their contributions to the 5-HT effects in both cocaine and saline-yoked, and naive animals. Recordings performed in the presence of the psychedelic 5-HT2AR agonist, DOI, caused increases in sEPSC frequency and amplitude. Antagonism of the 5-HT1A receptor also attenuated the 5-HT effects on RMP in saline rats, an effect that was absent in cocaine rats. Next, we observed spike-timing dependent plasticity (STDP) in CLA-ACC neurons, revealing anti-hebbian long-term depression. DOI reversed this LTD into a robust long-term potentiation. Finally, RNA scope combined with confocal imaging was performed to interrogate the colocalization of each of the 5-HT receptor subtypes in the claustrum.

These findings provide the first evidence that the large population of CLA-ACC neurons are under inhibitory control from 5-HT, and suggest that 5-HT1 and 5-HT2 receptors are separately involved in serotonin regulation of intrinsic membrane properties and excitatory synaptic plasticity, respectively.

Gabriela Aparicio, PhD [1] • Jorge Quintero, PhD [1] • Ling-Xiao Deng, MD, PhD [2] • Kristen Wancyk, RN [3] • Michael Murphy, MD [3] • Greg Gerhardt, PhD [1] • Craig van Horne, MD, PhD [1] • Paula Monje, PhD [1]
Neurosurgery University of Kentucky, College of Medicine [1] • Neurological Surgery Indiana University School of Medicine [2] • Surgery Indiana University School of Medicine [3]

Profiling peripheral glial cells from intact and injured human nerves for grafting in the central nervous system

Other

The unique pro-regenerative capability of peripheral nervous system (PNS) cells, including repair Schwann cells (SCs) from injured nerves, has been exploited clinically in cell transplantation therapies to treat central nervous system (CNS) trauma and neurodegenerative diseases. However, the characteristics of peripheral nerve cells before and after transplantation in the CNS has not yet been addressed thoroughly in humans. Therefore, the goal of this study was to identify specific markers able to reveal the identity and stage of differentiation of cells from intact and injured human nerves before and after implantation within CNS tissues. To study injury-associated changes in the expression of SC markers, we developed an in vitro model of human nerve degeneration to be compared with injured nerve grafts and brain tissues from participants enrolled in a nerve transplantation clinical trial for Parkinson's disease (NCT02369003). Overall, our results confirmed the value of using antibodies against S100**β**, myelin protein zero (MPZ), periaxin (PRX), NGFR, and Sox10, alone and in combination with axonal markers and myelin-selective fluorophores, to identify mature (intact) and repair (injured) human SCs in relationship to axons, myelin, and nonglial cells. In particular, our histological analysis revealed that: (1) NGFR was a reliable marker to discriminate PNS-derived cells, including repair SCs, from CNS neurons and glial cells; (2) S100B and Sox10 were useful to specifically identify SCs within intact and injured nerve tissues regardless of their stage of differentiation with the caveat that they also labeled astrocytes and oligodendrocytes in the brain; and (3) MPZ and PRX were equally useful to identify human myelin derived from SCs rather than oligodendrocytes. To conclude, the abovementioned markers can be used in different combinations to reveal grafted PNS cells, mainly SCs, in the human CNS to study their survival, differentiation, and relationship to host tissue.

Madison Bates, Other [1] • Sarah Garcia Pava, Other [1] • Sridhar Sunderam, PhD [1]
F. Joseph Halcomb III, MD, Department of Biomedical Engineering University of Kentucky [1]

How does hand dominance affect compliance when performing a graded finger extension task?

Student

When drinking a cup of water or turning a doorknob with your hand, you don't often think about the finer biomechanics of motor control involved. These activities of daily living may seem simple, but the human hand and wrist movements are complex, controlled by 29 muscles as well as cortical and subcortical neurons acting in concert to perform the task satisfactorily. Due to this complexity, it can be difficult to gain a full characterization of a person's hand function, whether their hand is completely healthy or has a motor impairment (i.e., stroke patients). Hand assessments are important in determining the level of severity in a stroke survivor's impaired hand, but these assessments can be affected by compliance: i.e., the ability to perform a motor task with sufficient accuracy. Therefore, there is a need to determine a person's compliance when performing a task using sensor-based devices to accurately and quantitively track movement. In this study, we seek to determine a person's compliance as they perform a simple graded finger extension task and whether hand dominance can be a factor. With institutional approval and informed consent, we recruited able-bodied individuals (n=12, all right-hand dominant) who were prompted to extend their fingers in response to visual cues to one of four levels: low, medium, high, or "no-go" (i.e., none). Each session consisted of 12 runs of 16 trials each, alternating between the left and right hand after every run. Finger extension was monitored using a commercial motion capture system and measured in terms of the distance between the metacarpal joint and fingertips. Finger extension measurements followed the same trend in the mean as the targeted level. All subjects showed significant differences ($p<0.05$) between no-go/high and low/high targets; and a majority of participants had more significant differences between all four targets in the left hand compared to the right hand. On the right hand, it was harder to discriminate between low/medium and medium/high finger extension. These measurements showed significant overlap between targets, which points to limitations in the participants' ability to accurately perform this deceptively simple motor task. Being able to determine the level of compliance based on hand dominance can be very beneficial when quantifying and assessing hand function in motor-impaired individuals. This study was important in determining if we need to consider hand dominance as a factor in stroke assessments. In future work, we propose to extend this protocol to include clinical assessments for stroke patients in which they will wear a custom-designed sensor glove to monitor hand function objectively and quantitatively.

Ravichandra S Davargaon, PhD [1] • Nirmal Verma, PhD [1] • James Bain , PhD [2] • Florin Despa, PhD [1]
Pharmacology and Nutritional Sciences University of Kentucky [1] • Duke Molecular Physiology Institute Duke University, Durham NC. [2]

Energy homeostasis within the brain is negatively affected by diabetes-related amylin loss-of-function

Fellow

Introduction: Amylin is a pancreatic hormone co-expressed and co-secreted with insulin, crosses normally from blood to brain and regulates satiation; however, it forms pancreatic amyloid in persons with type-2 diabetes and co-aggregates with **β**-amyloid (A**β**) in Alzheimer disease (AD) brains. We hypothesized that amylin's loss-of-function through amyloid formation and gain-of-toxicity through accumulation in brain tissue negatively affect energy homeostasis within the brain.

Objectives: Compare metabolite fluxes within rat brain tissue associated with genetic suppression of amylin (amylin loss-of-function) vs. brain amylin amyloid accumulation (amylin toxicity).

Methods: Using non-targeted metabolomics (MBX) analysis of cerebral cortex tissues from 16-month old rats expressing human amylin in the pancreatic **β**-cells (HIP rats) and age-matched rats with deleted amylin gene (AKO rats), we analyzed metabolites involved in glucose metabolism, amino acid metabolism pathways and lipid metabolism pathways. Age-matched wild-type rats expressing non-amyloidogenic rat amylin served as control rats

Results: Compared to control WT rat brains, dysregulated metabolites involved in glycolysis (3-phosphoglyceric acid, pyruvic acid, and 2-deoxyglucose-6-phosphate) and citric acid cycles (succinic acid, malic acid) were identified in HIP rat brains, but not in the AKO rat brains. Brain amylin accumulation in HIP rats is associated with dysregulated amino acid metabolites involved in neuroprotection (alanine, serine, and histidine), neurotransmitters or their precursor (glycine, threonine, aspartic acid, glutamic acid, phenylalanine, ornithine), and neurodevelopment (methionine, and asparagine). MBX analysis of HIP rat brains also identified dysregulated lipid metabolism pathways involved in neuroinflammation (linoleic acid), immune response (arachidic acid), brain function (palmitic acid, palmitoleic acid), cognition (oleic acid), anti-oxidation (arachidonic acid), and aging (monomyristic acid). Genetic suppression of amylin in AKO rats alters the brain phenylalanine level, lipid metabolites involved in brain fueling (heptadecenoic acid) and neuroprotection (stearic acid, linoleic acid, and methyl palmitate), compared to WT rat brains.

Conclusion: Both brain amylin-amyloid accumulation (toxicity) and amylin loss-of-function affect energy metabolism within the central nervous system. Further validation is required to confirm the extent of amylin-related alteration of metabolite fluxes within the brain.

Keywords: Amylin; diabetes; metabolomics;

Hannah Downing [1]
Physiology University of Kentucky [1]
Sex-based differences in hippocampal neurogenesis after TBI
Student
Sex-based differences in hippocampal neurogenesis after TBI

Hannah C. Downing[1,2], Ashley Glover[1], & Kathryn E. Saatman[1,2]

1. Spinal Cord and Brain Injury Research Center (SCoBIRC), University of Kentucky, Lexington, KY

2. Department of Physiology, University of Kentucky, Lexington, KY

Moderate or severe contusion brain injury robustly increases cellular proliferation within the dentate gyrus, resulting in the generation of new neurons. The development, integration, and long-term survival of posttrauma-born neurons is poorly understood. To date, traumatic brain injury (TBI) neurogenesis studies have almost exclusively utilized male rodents, resulting in a significant gap in knowledge as to how this aspect of neuroplasticity may differ for females. To evaluate sex-dependent neurogenic responses to TBI, male and female Ascl1-CreERT2; R26R CAG-floxStopTom reporter mice were used to label and track neural progenitor cells (NPCs) born after injury. Mice received a lateral controlled cortical impact (CCI) followed by tamoxifen injections on days 2 and 3 postinjury to permanently label NPCs born early after TBI. Injured (n=10 male, n=9 female) and naïve (n=8 male, n=12 female) mice survived 6 weeks after receiving two tamoxifen injections. Significantly fewer tdTom+ neurons were observed in the hippocampal dentate gyrus ipsilateral to impact compared to naïve controls ($p<0.0001$). The injury-induced decrease in tdTom+ cell numbers was more pronounced in males than in females ($p<0.001$). Interestingly, CCI-injured females exhibited a significant increase in tdTom+ neuron numbers in the hippocampus contralateral to impact when compared to CCI-injured males ($p=0.0027$) or to naïve females ($p<0.005$). Sholl analysis of dendritic arbor complexity revealed a modest increase in branch complexity in neurons of female naïve mice compared to males. TBI-related changes to the dendritic arbor differed for females and males with males having a greater reduction of dendritic complexity in the medial portion of the dendritic arbor. CCI-injured males exhibited a significant reduction in total branch length compared to CCI-injured females ($p=0.036$). Numbers of mossy fiber boutons formed by tdTom+ neurons extending axons to the CA3 region were reduced after TBI, with a statistically significant reduction in CCI-injured males compared to naïve males ($p<0.0001$). This reduction in bouton density appeared to be offset by a significant increase in bouton surface volume only in CCI-injured females ($p=0.049$). TBI causes impairment in multiple aspects of hippocampal neurogenesis. Surviving posttrauma-born neurons show axonal and dendritic structural changes that suggest impaired connectivity. Responses consistent with compensatory plasticity were observed in female mice following TBI.

Shadan Hadi, MS [1] • Gregory Frolenkov, PhD [2]
Physiology University of kentucky [1] • Physiology University of Kentucky [2]

Actin Disorganization in Mammalian Auditory Hair Cell Stereocilia Shafts is a Key Difference Between Temporary and Permanent Noise-Induced Hearing Los

Student

Stereocilia bundles, mechanosensitive microvilli-like projections on the surface of the auditory hair cells, pivot around their bases upon stimulation by sound. Stereocilia shafts are composed of stable F-actin core which becomes highly dense at the base forming rootlets allowing for stereocilia's flexible pivoting while anchoring them into the cuticular plate, special gel-like actin compartment which serves as the foundation that supports stereocilia. In mammals, hair cells do not regenerate. Therefore, their normal maintenance or repair after injury is essential for life-long hearing. Damage to the stereocilia bundles by acoustic overstimulation has been recognized as the hallmark of noise-induced hearing loss (NIHL) for decades. Yet, it is still unknown exactly which ultrastructural changes in stereocilia determine whether the NIHL would be reversible (temporary) or irreversible (permanent). In this study, we explore the ultrastructural changes in the actin cytoskeleton of stereocilia, stereocilia rootlets, and cuticular plates, shortly (within ~15 min) after noise exposures that reliably generate temporary (TTS) or permanent (PTS) shifts of hearing thresholds. The noise exposures producing TTS and PTS in the adult C57Bl/6 mice in the frequency region of 16-20 kHz were determined by initial assessments of animals' hearing using auditory brainstem responses (ABRs). Then, we used focused-ion beam scanning electron microscopy (FIB-SEM) to perform 20nm serial sectioning through the hair cell's actin structures in control non-exposed and noise exposed (TTS or PTS) organs of Corti. We report disorganization of F-actin within shafts of stereocilia in PTS but not in TTS. Since this disorganization appears as "all-or-none" phenomenon in all stereocilia of a hair cell, it is likely to result from global noise-induced cellular changes, such as Ca^{2+} dependent severing of actin. Noise exposure also increased the diameter of the rootlets but in both TTS and PTS. Similarly, although noise exposure also produced the shrinkage of the actin-based cuticular plate, it was observed in both TTS and PTS, proportional to the severity of noise exposure. We conclude that actin disruption within stereociliary shafts distinguishes PTS from TTS.

Supported by NIH (R01DC014658, R01DC012564, and S10OD025130)

Sejuti Naurin, MS [1] • Oliver Voecking, PhD [2] • Jakub Famulski, PhD [3]
Biology University of Kentucky [1] • Department of Biolgy University of Kentucky [2] • Department of Biology University of Kentucky [3]

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 I 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, I 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, we have confirmed the expression of some novel target genes at OF fusion site using in situ hybridization. Confirmed targets include *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. 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.

Jocelyn Martin [1] • Jakob Shaykin, MS [1] • Emily Denehy, MS [1] • Judy Songrady [2] • Dan Luo, PhD [2] • Warren Alilain, PhD [3] • Jill Turner, PhD [2] • Michael Bardo, PhD [1] • Thomas Prisinzano, PhD [2]
Psychology University of Kentucky [1] • Pharmaceutical Sciences University of Kentucky [2] • Spinal Cord and Brain Injury Research Center University of Kentucky [3]

Novel Compounds EO-139 and YZ-166 as Countermeasures for Reversing Opioid-Induced Antinociception, Motor Incapacitation and Respiratory Depression

Student

Purpose: Unlike morphine, fentanyl causes vocal cord closure and rigidity of the chest wall muscles, an effect known as “wooden chest syndrome”, and this effect may not be fully reversed by pure mu opioid antagonists (MOR) such as naloxone or naltrexone. This study assessed the ability of two novel compounds (EO-139 and YZ-166) to serve as MOR antagonists to reverse fentanyl analgesia, as well as reverse fentanyl-induced locomotor and respiratory depression.

Methods: For the hot plate assay, male and female F1 hybrid mice (n=31) were administered fentanyl (1 mg/kg; s.c.) and then either EO-139 or YZ-166 using a cumulative dosing procedure, with nociception measured by latency to paw lick on the hot plate. For locomotion and respiration, male and female Sprague-Dawley rats (n=43) were given saline or fentanyl (200 µg/kg; s.c.) 15 min prior to a second injection of one of the following: (1) vehicle, (2) naltrexone (0.003-0.1 mg/kg; s.c.), (3) EO-139 (0.0003–0.1 mg/kg; s.c.), or (4) YZ-166 (0.003-1 mg/kg; s.c.). Rats were immediately placed into a locomotor chamber for 15 min, followed by placement into a plethysmography chamber to record ventilatory effort for 30 minutes.

Results: As expected, with the hot plate assay, both EO-139 and YZ-166 dose-dependently reversed fentanyl-induced antinociception. Unlike YZ-166, EO-139 yielded notable sex differences in the dose required to produce 50% reversal (AD_{50}). With locomotion and respiration, naltrexone as the standard MOR antagonist produced a dose-dependent reversal of the locomotor and respiratory depressant effects of fentanyl. EO-139 and YZ-166 also reversed the respiratory depressant effects of fentanyl, but not fentanyl-induced locomotor depression within the dose ranges tested. Most notable, unlike naltrexone and EO-139, YZ-166 not only reversed fentanyl-induced respiratory depression, it stimulated respiration above baseline control, suggesting “supra-antagonism”.

Conclusion: This study provides evidence that EO-139 and YZ-166 attenuate opioid-induced antinociception and respiratory depression similar to naltrexone. Moreover, YZ-166 has a profile on respiratory depression that may offer a superior countermeasure agent against exposure to high-potency synthetic opioids.

Supported by: U01 DA051377

Navid Tavakoli [1] • Samantha Malone [2] • Tanner Anderson [1] • Ryson Neeley [1] • Artin Asadipooya [1] • Pavel Ortinski, PhD [3]
Neuroscience University of Kentucky [1] • Psychology University of Kentucky [2] • Neuroscience University of Kentucky [3]

Astrocyte Activity in the Dorsal Striatum Regulates Cue-Induced Reinstatement of Cocaine Seeking

Student

Background: Recent literature has highlighted that astrocytes regulate drug-seeking behaviors. The dorsal striatum, specifically, has been shown to play a significant role in reward seeking and is influenced by astrocyte Ca2+ signaling. However, the impact of astrocyte Ca2+ on neuronal activity in the context of drug-induced plasticity remains unclear.

Methods: Animals received dorsal striatum injections of viruses driving expression of neuron-specific Ca2+ reporter, GCaMP6f, and astrocyte-specific pump, HPMCA2w/b, which suppresses astrocyte activity by extruding cytosolic Ca2+. Control animals were injected with neuronal GCaMP6f and a control, astrocyte-specific, virus. Animals then underwent jugular catheterization. Following recovery, animals underwent cocaine or saline self-administration, extinction, and cue-induced reinstatement of cocaine seeking. Subsequently, brain slices were collected from each animal for imaging of spontaneous Ca2+ signals in striatal neurons.

Results: Suppression of astrocytic Ca2+ rescued cocaine-induced decrease of neuronal Ca2+ transients. Suppression of astrocyte Ca2+ also led to decreased duration of neuronal Ca2+ events in the cocaine group. No effects of astrocyte Ca2+ suppression were observed within the saline group. Furthermore, the addition of exogenous 10 μM cocaine HCl suppressed Ca2+ transients in both HPMCA2w/b and sham animals. Together with baseline neuronal activity measures, these results suggest that cocaine self-administration impacts coupling between astrocyte and neuron Ca2+, despite preservation of sensitivity to exogenous cocaine. To explore this further, we found that application of 100nM NPS-2143, a CaSR antagonist, did not result in significant differences between groups, indicating minimal effects of astrocyte Ca2+ extrusion on extracellular Ca2+ concentrations. Therefore, suppression of astrocyte Ca2+ is likely to impact neurons via cell-intrinsic mechanisms (e.g. gliotransmission, metabolic coupling, etc). With regard to behavioral effects, we observed that suppression of astrocyte Ca2+ increased cocaine self-administration and increased cue-induced reinstatement of cocaine-seeking relative to animals injected with a control astrocyte-specific virus.

Conclusions: Our findings suggest that Ca2+ activity in dorsal striatum astrocytes regulates neuronal output via mechanisms independent of extracellular Ca2+ concentrations and that disruption of astrocyte Ca2+ is of specific relevance to generation and maintenance of cocaine-seeking.

Velmurugan Gopal Viswanathan, DVM, PhD [1] • Hemendra Vekaria, PhD [2] • Chirayu Pandya, PhD [3] • Anika Hartz, PhD [4] • Bjoern Bauer, PhD [5] • Patrick Sullivan, PhD [2] • Brad Hubbard, PhD [6]
Department of Neuroscience University of kentucky [1] • Department of Neuroscience University of Kentucky [2] • Department of Neurosurgery University of Kentucky [3] • Department of Pharmacology and Nutritional Sciences Sanders-Brown Center on Aging [4] • Department of Pharmaceutical Sciences Sanders-Brown Center on Aging [5] • Department of Physilogy University of Kentucky [6]

Ex-vivo model to study brain capillary mitochondrial function and dynamics using mitochondria labeled dentra-2

Staff

Accumulating evidence from preclinical, postmortem, and epidemiological studies demonstrates a strong link between neurovascular dysfunction and cognitive impairment. Indeed, blood-brain barrier damage and neurovascular deficits are major hallmarks of neurodegenerative diseases, such as Alzheimer's disease (AD), vascular contributions to cognitive impairment & dementia (VCID), stroke and traumatic brain injury (TBI). Oxidative stress plays a critical role in ongoing pathology that exacerbates neurovascular dysfunction. Oxidative stress is highly associated with mitochondrial dysfunction and reactive oxygen species (ROS) imbalance. To study capillary-specific mitochondrial function and dynamics in response to oxidative stress, we developed a novel *ex-vivo* model using isolated brain capillaries from transgenic mice that express photoactivatable dentdra2 green specifically in mitochondria (mtD2g). Brain capillaries were isolated using a standardized protocol and were subjected to 1. Oxidative stress using 2,2'-azobis-2-methyl-propanimidamide dihydrochloride (AAPH) a free radical generator to induce oxidative stress by lipid peroxidation. 2. Oxygen-glucose-deprivation/reperfusion (OGD-reperfusion) conditions with their respective controls. Following exposure, mitochondrial function was measured as oxygens consumption rate (OCR) using the Seahorse XFe96 analyzer and mitochondrial dynamics were measured using a confocal microscope with Imaris software. Both oxidative stress and OGD-reperfusion conditions significantly decreased OCR. Mitochondrial dynamics calculated in Z-stack demonstrated mitochondrial fission phenotype. This novel *ex-vivo* model will be a valuable tool to test pharmacological interventions to target oxidative stress/mitochondrial dysfunction in vascular pathophysiology, as a critical endophenotype of neurodegenerative disease, such as AD.

Blaine Weiss [1] • Pradoldej Sompol, PhD [1] • Christopher Norris, PhD [1]
Pharmacology and Nutritional Sciences Sanders-Brown Center on Aging [1]

Localized Analysis of Vascular Astrocytes (LAVA): Measurement of cerebrovascular function in live animal imaging

Student

Here we introduce LAVA (Localized Analysis of Vascular Astrocytes), a dedicated analytical platform tailored for investigating neurovascular coupling dynamics from awake animal two-photon brain imaging data. Neurovascular coupling, the interplay between neuronal activity and vascular responses, constitutes a fundamental aspect of brain function known to be impacted with brain aging, and the onset of disease. Developed in Matlab, LAVA facilitates precise measurements of vascular function by modeling vessels' shape in the image field of view, and creating cross sectional maps for spatial indexing of vascular tone changes in response to stimuli. After model formation and measurement of vascular activity, the software can be flipped into second channel analysis of perivascular ROIs. In its debut study, we investigated the activity of astrocyte endfeet calcium signals using Gfa104-GCaMP8f in response to air puff whisker stimulation of the barrel cortex.

An obstacle to the accurate measurement of glial cell activity during neurovascular coupling, such as with astrocyte endfeet during calcium imaging, is the relatively narrow size of perivascular ROIs, which often move along the motion of dilating and constricting vessels. LAVA overcomes these challenges by processing vessel motion data from its models into transformable vectors, which adhere ROIs to the perivascular space. It also makes scaled corrections for ROIs overlapping axial to the vessel, providing flexibility to field of view selection for 2D image stacks.The utility of LAVA extends to robust signal processing, enabling the extraction of crucial parameters, such as signal amplitudes, timing, frequency, and slopes. This facilitates a comprehensive analysis of neurovascular coupling dynamics applicable to all experimental models of cerebrovascular function.

In the context of our studies, LAVA serves as a valuable tool for characterizing cerebrovascular function impairments in live animal models of Alzheimer's disease and related dementias. (AD/ADRDs) For example, in 5XFAD mice, a model of progressive amyloid burden by familial mutations in APP and PS1, we show a significant reduction in endfoot calcium amplitudes, and slower rise time kinetics in response to whisker stimulation at 7 months of age. We also discovered a significant increase in latency between neurovascular coupling induced vasodilation, and rise in astrocyte calcium signaling compared to wild type controls.

Our findings underscore LAVA's contribution to the in-depth examination of the dynamic relationship between neuronal activity, and vascular-glial responses. The platform holds potential for advancing our understanding of brain function, shedding light on the mechanisms implicated in various neurological conditions associated with cerebrovascular dysfunction. LAVA's potential implications extend beyond the laboratory, with the prospect of informing future research and clinical approaches to cerebrovascular dysfunction.

Justin Welden, PhD [1] • Giorgi Margvelani, PhD [2] • Andrea Arizaca, MS [1] • Marie Thoele [1] • Peter T. Nelson, MD, PhD [3] • Stefan Stamm, PhD [1]
Biochemistry University of Kentucky [1] • Biochemistry University of kentucky [2] • Sanders Brown University of kentucky [3]

Proteins expressed from tau circular RNAs as new drug targets in Alzheimer's disease.

Fellow

We recently found that the human tau gene expresses at least two circular RNAs (circRNAs) [1-4]. These circRNAs are translated after undergoing adenosine to inosine RNA editing (A>I editing). The protein products of circTau RNAs promote neurofibrillary tangle formation, a hallmark of Alzheimer's disease. One of the tau circRNAs harbors a second, short open reading frame that encodes a predicted protein of 16 kDa that is similarly expressed after A>I editing. Using a peptide antiserum, we detect an increased expression of this new protein, circTau-ORF2, during Alzheimer's disease progression. CircTau-ORF2-protein colocalizes with neurofibrillary tangles at later stages of Alzheimer's (Braak VI). Our findings are the first demonstrations of proteins made from endogenous circular RNAs. Similarly, we found that A>I editing induces translation of two other Alzheimer's disease relevant circRNAs, circMAN2A1 and circMAN1A2.

Since our data indicate that circTau RNAs could contribute to Alzheimer's disease, we tested whether circTau RNAs can be removed using siRNAs. Scanning all possible 20 siRNAs against the backsplice junction site, we identified three siRNAs with high selectivity and efficacy, demonstrating that circRNAs can be successfully targeted.

In summary, our data show that aberrant circRNA expression and translation could contribute to Alzheimer's disease, representing a new drug target.

[1] J.R. Welden, J. van Doorn, P.T. Nelson, S. Stamm, The human MAPT locus generates circular RNAs, Biochim Biophys Acta Mol Basis Dis. (2018) 2753-2760. 10.1016/j.bbadis.2018.04.023.

[2] G. Margevlani, J.R. Welden, K.A. Arizaca Maquera, S. Sardon, S. Stamm, Influence of FTDP-17 mutants on circTau RNAs, bioRXiv (2023).

[3] J.R. Welden, G. Margvelani, K.A. Arizaca Maquera, B. Gudlavalleti, S.C. Miranda Sardón, A. Campos, N. Robil, D.C. Lee, A. Gonzalo Hernandez, W. Wang, J. Di, P. de la Grange, P.T. Nelson, S. Stamm, RNA editing of microtubule associated protein tau circular RNAs promotes their translation and tau tangle formation, Nucleic Acids Res 50 (2022) 12979-12996.

[4] K.A. Arizaca Maquera, J.R. Welden, G. Margvelani, S.C. Miranda Sardón, S. Hart, N. Robil, A.G. Hernandez, P. de la Grange, P.T. Nelson, S. Stamm, Alzheimer's disease pathogenetic progression is associated with changes in regulated retained introns and editing of circular RNAs, Frontiers in Molecular Neuroscience 16 (2023). 10.3389/fnmol.2023.1141079.

Platform Sessions

Sleep & Circadian Rhythms

Jun Wang, MS [1] • Diane Iradukunda [1] • Adam Bachstetter, PhD [2] • M. Paul Murphy, PhD [3] • Bruce O'Hara, PhD [4] • Marilyn Duncan, PhD [2] • Sridhar Sunderam, PhD [1]
Biomedical Engineering University of Kentucky [1] • Neuroscience University of Kentucky [2] • Mol. & Cell. Biochemistry University of Kentucky [3] • Biology University of Kentucky [4]

A novel protocol for probing the effects of sleep and temperature in a mouse model of Alzheimer's disease.

Student

Disordered sleep, which is common in Alzheimer's disease (AD), may accelerate neuropathology, thus promoting a vicious cycle. In a previous study, we investigated the hypothesis that improving sleep through diurnal exposure to thermoneutral temperatures may slow disease progression in 3xTg-AD mice. We found that slow wave sleep (SWS) – the deepest stage of NREM sleep dominated by delta oscillations in the EEG – was significantly elevated ($p<0.05$) in the light phase for treated mice compared to controls even though the time spent in total sleep, NREM, REM, and wakefulness were unchanged. Furthermore, both Aβ40 and Aβ42 were significantly lower in the hippocampus, but not in the cortex, for the treated group. These findings imply that thermoneutral conditions might have some regional specificity in their effects, with implications for the cognitive and neuropathologic changes found in AD.

To further test whether the effect of thermoneutrality on amyloid pathology is mediated by sleep changes rather than other non-specific physiologic effects of temperature, we conducted a new study with a modified design. APP/PS1 knock-in mice (6 m.o, male) were instrumented for EEG/EMG monitoring to score sleep. After a week-long baseline recording, they were divided into four groups: 1. SE (n=8), exposed to thermoneutral temperature (30°C) during the 12-hour light period; 2. SD (n=8), which received intermittent vibratory stimuli to disrupt sleep during the light period; 3. SExSD (n=9), which received sleep disruption along with thermoneutral exposure; and 4. CTRL (n=8), which received no treatment. The SExSD condition is meant to test for any effects of temperature that are not mediated by sleep enhancement in the SE condition through sleep disruption. All animal procedures were carried out with prior approval from the Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky.

After four weeks of treatment, the animals were euthanized, and the brains removed to assay amyloid-beta (Aβ) levels using ELISA. Wake, REM, NREM, and SWS within NREM were scored from the EEG/EMG to analyze sleep. In our preliminary analysis of the data, we find that SWS was increased up to four-fold in SE mice. However, a similar increase on average was observed in SExSD mice despite the attempted sleep disruption. These outcomes will be correlated with pathology when the biochemical assays become available.

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

Manipulation of Sleep Architecture in Mice: A closed-Loop Thermoregulatory Approach

Student

Sleep is essential for physiological health and well-being. Temperature is one of many factors that play a significant role in modulating sleep. Both humans and animals exhibit changes in sleep composition and architecture in response to alterations in ambient temperature. In previous work, we have investigated the effect of both static and dynamic changes in ambient temperature on sleep in mice. However, these protocols were implemented mostly without specifically targeting periods of sleep. Here, we investigate the effect of thermoneutral temperature exposure on mouse sleep only after sleep onset.

All animal procedures received prior approval from the University of Kentucky IACUC. Wild-type mice aged 7-9 months were selected for the study. Cage temperature was manipulated using a custom-built thermostatic control system consisting of infrared heating lamps that were switched on or off in response to the error in cage temperature with respect to a setpoint. Sleep-wake state was monitored using a piezoelectric ("piezo") motion sensor (Signal Solutions, LLC) placed on the floor of the mouse cage. Sleep was detected when the mean-squared power in the piezo signal in a moving 1-second window dropped below a preset threshold. After allowing the mice to acclimate a 24-hour baseline recording was performed. For the next two days, during the 12-hour light period, the setpoint temperature was elevated to 30°C (thermoneutral for mice) when the proportion of sleep in a moving one-minute window exceeded 90% and reset to the room temperature of 22°C when it dropped below 10%. The number of sleep bouts, as well as the percent time spent in sleep were calculated as outcome measures and compared for the experimental condition against the baseline.

Our preliminary results show an increase in percent time spent in sleep and a reduction in sleep fragmentation (greater number of transitions into and out of sleep) for the experimental days compared to the baseline. Specifically, mean sleep time went from 56±12% in the baseline to 58±10%) on treatment Day 1 and 63±13% on treatment Day 2. However, the effects of similar changes in temperature without regard to vigilance state need to be considered. Furthermore, these results are based on sleep-wake discrimination using a motion sensor. Ongoing electroencephalographic recordings will provide further insights into the effect of this protocol on sleep efficiency and sub-states of sleep.

This study tests the feasibility of modulating sleep architecture in mice using a closed-loop thermoregulatory approach in preclinical animal models with potential implications for the treatment of sleep-related disorders.

Support: This study was supported by internal funds and a Jack and Linda Gill Endowment.

Carrie Johnson [1] • Valeria Buzinova [2] • Landys Guo [3] • Sarah Barth [4] • Savannah Turton [4] • Samantha Padgett [4] • Tyler Jessup [4] • Haleigh Whitlock [5] • Teresa Macheda [6] • Katharina Kohler [2] • Bruce O'Hara, PhD [3] • Sridhar Sunderam, PhD [7] • Adam Bachstetter, PhD [5] • Marilyn Duncan [5] • M. Paul Murphy, PhD [1]
Molecular and Cellular Biochemistry University of Kentucky [1] • Sanders-Brown Center on Aging University of Kentucky [2] • Biology University of Kentucky [3] • null University of Kentucky [4] • Neuroscience University of Kentucky [5] • Spinal Cord and Brain Injury Research Center University of Kentucky [6] • Biomedical Engineering University of Kentucky [7]

Sleep and Circadian Rhythms, Biological Sex, and Alzheimer's Disease: A Complex Relationship

Student

Plaques composed primarily of the amyloid-**β** (A**β**) peptide are a defining neuropathology of Alzheimer's Disease (AD). It has been known for some time that there is a connection between disturbances in sleep and circadian rhythms that relates to the development of AD, and that this relationship may be driven primarily through A**β**. Recent studies from our group have demonstrated a range of interactions between biological sex, sleep-wake rhythm fragmentation, and AD neuropathology in multiple mouse lines. In this study, we focused on an APPxPS1 knock-in (KI) line, as they express the amyloid precursor protein at normal levels under the normal pattern of expression. Sleep was recorded from wild type (WT) and KI mice, of both sexes, using PiezoSleep cages (Signal Solutions LLC) for a minimum of one week (N = 127). An additional cohort of mice (N=128) were acclimated to a 12:12 light:dark cycle for two weeks, and then switched to housing in continuous darkness (D:D) for 24-48 hours. Animals were euthanized in groups of 16 at 3 hour intervals starting after the first 24 hours in D:D. In both studies, the numbers of male and female mice, WT and KI, were approximately the same. A**β** was measured in different fractions via custom 384-well ELISAs. Female mice slept less than male mice, had higher amounts of A**β** pathology, and were more susceptible to a manual sleep fragmentation protocol, exhibiting greater amounts of rebound sleep. A daily rhythm of A**β** was detected only in the most soluble extractable fraction, reached a daily minimum at 11 am (clock time), and peaking at 11 pm. Although the A**β** rhythm was essentially identical between WT and KI mice in a less disease affected area (cerebellum), it was lost in a more disease affected region (olfactory bulb). Interestingly, the A**β** rhythm was the same in both sexes. These data suggest that although some interactions between A**β** neuropathology, sleep, and circadian rhythms are linked to biological sex, the effect may not be uniform. Because two-thirds of AD cases are women, understanding the driving factors for these differences is of critical importance for disease diagnosis and treatment. Funded by NIH (R01 AG068215, R01 AG068215-03S1, and T32 AG078110).

Matt Thomas, PhD [1] • Gregory Jicha, MD, PhD [2] • Jordan Harp, PhD [2] • Philip Kern, MD [3] • Yuriko Katsumata, PhD [4] • Julie Pendergast, PhD [5]
Biology University of Kentucky [1] • Sanders-Brown Center on Aging University of Kentucky [2] • Department of Internal Medicine University of Kentucky [3] • Department of Biostatistics University of Kentucky [4] • Department of Biology University of Kentucky [5]

Feasibility of implementing time-restricted eating in women with mild cognitive impairment

Fellow

Background: Disruption of sleep and circadian rhythms is associated with cognitive decline, preclinical Alzheimer's Disease (AD) pathology, and an increased risk of dementia. Alleviating circadian and sleep disruption could improve cognition and treat AD and related dementias (ADRD). Time-restricted eating (TRE), a circadian behavioral intervention that corrects disrupted eating rhythms by aligning food intake to the active phase, improves metabolic dysfunction, sleep quality, and health-related quality of life. Individuals who adhered to TRE were less likely to have cognitive impairment compared to those who consumed meals with no time restrictions. Although the mechanisms are unknown, prolonged overnight fasting from TRE could retain and improve cognitive function by increasing ketones, which are an energy source for the brain. However, no TRE intervention study has investigated its efficacy at improving cognition in cognitively impaired individuals. A first step toward studying TRE efficacy for cognition is to determine whether TRE is feasible in people with cognitive impairment, and to identify those who are most likely to benefit from TRE. This study will determine whether TRE can be successfully implemented in postmenopausal women with mild cognitive impairment (MCI).

Methods: Postmenopausal women, 45-95 years old, who are not taking hormones (estrogen ± progestin) and have been diagnosed with MCI will be recruited. Data will be collected for 10 weeks, which includes 2 weeks of baseline and 8 weeks of TRE. During baseline, we will collect cognitive measures, food timing and activity/sleep data, and metabolic parameters. For the duration of the study, the times of first and last meals will be collected from participants with an SMS texting system that we developed. Participants with long eating durations (≥12hours) and late eating (≥8:00pm) at baseline will be eligible for the TRE intervention. During the TRE intervention, each participant will self-select a 10-h window (last meal before 8:00pm) during which she will consume all daily calories for 8 weeks. At the end of the intervention, we will repeat baseline measures. The primary outcome of this study will be ≥70% adherence to the TRE protocol (10-h TRE window ≥5d/week).

Results: Participants will be recruited from Sanders-Brown Center on Aging Clinic. We anticipate enrolling 22 participants in baseline in order to have 15 meet the inclusion criteria for the TRE intervention.

Conclusion: This pilot study will provide insight into the feasibility of conducting TRE in women with MCI and provide preliminary data necessary to calculate sample size for a future randomized controlled trial testing the efficacy of TRE in improving, or preventing further decline, in cognition.

Recovery After Stroke & Vascular Injury

Hilaree Frazier, PhD [1] • David Braun, PhD [1] • Verda Davis, MS [1] • Meggie Coleman [1] • Colin Rogers, PhD [1] • Linda Van Eldik, PhD [1]
Sanders-Brown Center on Aging University of Kentucky [1]

Inhibition of p38α MAPK rescues synaptic function and improves behavioral performance in a mouse model of mixed vascular and amyloid pathologies

Fellow

Cerebrovascular dysfunction is frequently comorbid with Alzheimer's disease (AD), yet the mechanistic consequences of this mixed pathology remain unclear. Recent work suggests that p38 alpha (p38**α**) MAPK, a regulator of neuroinflammation, may represent an effective target for AD therapies. For example, MW150, a small molecule p38**α** inhibitor, was shown to improve cognition and decrease cytokines in amyloidogenic mice. However, p38**α** inhibition in the context of mixed vascular and AD pathologies has yet to be thoroughly characterized. We therefore tested if MW150 could reduce neuroinflammation, synaptic dysfunction, and cognitive impairment in a mouse model of mixed dementia (MD) with comorbid amyloid and cerebral small vessel disease (hyperhomocysteinemia [HHcy]). Briefly, 5xFAD mice were given a B-vitamin-deficient diet for 8-weeks to induce HHcy. Wild-type (WT) littermates were maintained on control diet. While on diet, animals received intraperitoneal injections of either saline vehicle (Veh) or MW150 (0.5 mg/kg) 3 days per week. Behavioral assessments were conducted at the end of treatment, followed by sacrifice and tissue harvest. Additional endpoints included ELISA quantification of cytokines, immunohistochemistry of glial cells and synaptic proteins, measures of amyloid and vascular pathology, and extracellular field recordings in hippocampal slices. Compared to WT Veh, MD mice had increased proinflammatory cytokines and glial cell activation, reduced cerebral vessel sizes, impaired synaptic transmission, decreased synaptic protein expression, and worsened behavioral performance. No effect of MW150 was detected on cytokine levels, the degree of amyloid or vascular pathology, or glial cell activation. Surprisingly, however, the compound rescued several measures associated with synaptic dysfunction back to levels comparable to WT Veh, including population spike thresholds, LTP maintenance, synaptic protein expression, and number of synapses in both hippocampal area CA1 and CA3. Importantly, these synaptic changes were also mirrored by improved performance on the Morris water maze test in MD MW150 mice compared to MD Veh. Our findings support further investigations of p38**α** inhibitors in the clinic, and also suggest that neuronal p38**α** signaling may mediate pathways associated with synaptic plasticity. Future work will use similar techniques in other mixed models to characterize the translatability of this approach across AD pathologies (i.e. HHcy and tau).

Katherine Cotter [1] • Mary K Malone [1] • Edric Winford [1] • Pavel Yanev, PhD [2] • Jadwiga Turchan-Cholewo, PhD [2] • Ann Stowe, PhD [2]
Neuroscience University of Kentucky [1] • Neurology University of Kentucky [2]

Effect of hCD20 depletion on operant training after stroke

Student

Background: The effect of the chronic inflammatory response after stroke on long-term cognitive functioning is not well understood. It has been shown that B cells migrate into the hippocampus, and remain there long-term, but the benefits to recovery are unclear. Autoshaping (AUTO) is a Pavlovian response learning task sensitive to lesions in the hippocampus and frontal cortex. It utilizes an unconditioned reward (peanut oil) to build an association with a conditioned stimulus.

Aim: We hypothesize that post-stroke B cell depletion results in more severe cognitive deficits in the Autoshaping task.

Methods: Female aged (16-24 month-old, n=20) hCD20$^{tamCRE/fBDNF(+/+)}$ mice will be trained on the Autoshaping (AUTO) operant touchscreen task, which tests associative learning and reward value. Both hCD20$^{tamCRE(+)/fBDNF(+/+)}$ and hCD20$^{tamCRE(-)/fBDNF(+/+)}$ mice will be used, with hCD20$^{tamCRE(-)/fBDNF(+/+)}$ serving as littermate controls. Rituximab will be injected IP for 3 days prior to beginning AUTO training, with rituximab booster doses given each week to maintain B cell depletion. Following AUTO training and 5 days of baseline acquisition, mice will undergo a bilateral prefrontal photothrombotic stroke. Mice will complete the AUTO task for 3 days on weeks 2, 4, and 6 weeks post-stroke to measure both acute and chronic post-stroke cognitive functioning. B cell depletion will be confirmed systemically using flow cytometry and in the brain using histology.

Results: We have established this method in C57BL/6 (n=6 aged 18-26 month-old, n=6 young 5-11 month-old) female mice using a peanut oil reward. Aged female mice showed a biased response to CS+ vs CS-, not significantly different from young animals (p=0.64, tray latency). Ongoing studies are using aged female hCD20$^{tamCRE/fBDNF(+/+)}$ mice.

Conclusions: Aged mice are able to be trained on an associative learning task focusing on functioning in the cortical regions (hippocampus and frontal cortex), which are associated with vascular dementia and post-stroke cognitive decline.

Christopher McLouth, PhD [1] • Will Cranford, MS [1] • Jacqueline Frank, Other [2] • Jordan Harp, PhD [3] • Keith Pennypacker, PhD [3]
Department of Biostatistics University of Kentucky [1] • Department of Neurosurgery University of Kentucky [2] • Department of Neurology University of Kentucky [3]

Using Penalized Regression to Identify Proteomic Predictors of Post-Stroke Recovery

Faculty

Stroke is a complex and debilitating neurological condition that presents a significant challenge to patients, caregivers, and healthcare providers and represents the leading cause of long-term disability worldwide. Predicting the recovery trajectory following a stroke is crucial for optimizing treatment and rehabilitation strategies. The current study focuses on data-driven analysis, demonstrating how penalized regression techniques can be harnessed to identify proteomic predictors of post-stroke recovery.

Our study leverages advanced statistical methodologies to analyze high-dimensional proteomic data from stroke patients treated with mechanical thrombectomy for an emergent large vessel occlusion. By applying penalized regression models such as Lasso, Ridge, and Elastic Net, we systematically identify key proteins and biomarker profiles associated with favorable post-stroke recovery outcomes, allowing us to gain insights into the intricate molecular pathways and protein interactions that influence recovery potential.

The results of our investigation not only enhance our understanding of the underlying mechanisms associated with post-stroke recovery but also hold immense promise for personalized medicine and targeted therapeutic interventions.

Amber Schifano, Other [1] • Madison Webster , Other [1] • Amanda Glueck, PhD [2]
Second Year Medical Student - College of Medicine University of Kentucky [1] • Neurology University of Kentucky [2]

Virtual Reality Exergaming Reduces Neuroinflammation and Improves Functional Recovery in Stroke Patients: A Methodological Overview

Student

There are many benefits exercise provides to health and patient recovery. Even though exercise is very beneficial it is difficult to get patients to complete exercises to improve their health conditions and overall wellbeing. Exergaming incorporates technology-driven activities, such as video game play, with exercise which can also be beneficial for patient rehabilitation. Several studies have concluded that exergaming using traditional gaming platforms for rehabilitation in clinical populations has reduced inflammatory markers, improved patient compliance and morale in completing rehabilitation, immobility, static and dynamic balance, and overall functional outcomes. Furthermore, with the benefits of exergaming, we propose that with the use of exercise and virtual reality gaming functional recovery with cognition, motor function, gait, and balance in stroke patients will be improved, behavioral benefits will ensue, and neuroinflammatory responses will be reduced. The proposed study is a randomized control where patients will be assigned to one of three categories: exercise, virtual reality (VR) gaming, or VR exergaming. Participants will be asked to complete up to 20 hours of exercise, gaming, or exergaming over the course of 7 weeks limiting activity to one hour per day. Prior to beginning their assigned intervention, participants will undergo pretraining, baseline neuropsychological, motor function, and balance assessments, and complete a brief symptom inventory to compare performance over the course of the study. After participants complete 20 hours of training, there will be a post-training assessment 12-36 hours following their final training session to determine neuropsychological, motor function, and balance changes. Fasting blood samples will be collected during the baseline and post-intervention assessment as well to determine the effects that the exercise, VR gaming, and exergaming have on neuroinflammatory markers. The recruitment for this study is currently ongoing. We hypothesize that participants assigned to the exergaming condition will demonstrate a cumulative effect of the gaming with exercise and thus will demonstrate significantly greater benefits on the neuropsychological measures, motor function and balance, as well as significantly lowered inflammation compared to the exercise and VR conditions.

Basic & Clinical Neurophysiology

Maxwell Lavin [1] • Dillon Huffman, PhD [2] • Jasmine Perdeh [3] • Diane Iradukunda [4] • Bjoern Bauer, PhD [5] • Sridhar Sunderam, PhD [4]
F. Joseph Halcomb III, M.D. Dept. of Biomedical Engineering University of kentucky [1] • null Signal Solutions, LLC [2] • Dept. of Pharmaceutical Sciences University of kentucky [3] • F. Joseph Halcomb III, M.D. Dept. of Biomedical Engineering University of Kentucky [4] • Dept. of Pharmaceutical Sciences University of Kentucky [5]

Modeling Rat Seizure Onset Dynamics from Non-Invasive Motion Signals for Improved Seizure Screening

Student

Preclinical research in epilepsy is a vital step in the development of therapies that aim to reduce seizure burden. Currently, the gold standard for detecting seizures in animal models is invasive electroencephalography (EEG), which is time- and resource-intensive. Non-invasive seizure screening methods are available but lack specificity as they rely on movement and other behavioral measures of seizures rather than neuronal activity. This reliance leads to a higher false positive detection rate when compared to invasive EEG analysis. To reduce this false positive detection rate, we used the dynamics of seizure onset as captured by piezoelectric ('piezo') motion sensors to create machine learning models for a rodent model of temporal lobe epilepsy. All animal procedures performed in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Kentucky. Wistar rats (n=24) treated with lithium-pilocarpine to induce *status epilepticus* were continuously monitored for an average of nine weeks using piezo platforms (Signal Solutions, LLC) under the cage floor as well as video cameras aimed at the cages. The recorded piezo signals were processed using a line length-based algorithm that responds to fluctuations in power to produce an initial set of possible seizure instances. We then manually reviewed video data to label each of these events as true seizure (rated 3-5 on the Racine scale) or non-seizure behaviors (such as grooming). In addition, normal behaviors occurring at the same time in neighboring cages were labeled as baseline behavior samples. Thirty-second-long segments of piezo signal centered on initial event detection times were extracted for 500 random events of each mentioned label (total 1500). Four features representing measures of signal power, variance, and entropy were computed from each segment from one-second windows with a 0.5 second overlap. Two data-driven dynamical models, one a Long Short-Term Memory (LSTM) neural network, and the other a Hidden Markov Model (HMM), were fitted to a continuous-valued and discretized feature vector time series, respectively. The models were trained and tested using a 10-fold cross validation scheme and performance was evaluated in terms of F1 score – a measure of accuracy that combines precision and recall – and specificity. Mean F1 scores were 70±1% and 62±5%, while mean specificities were 82±1% and 79±6% for the LSTM and HMM, respectively. The performance of our models shows that seizure-related movement dynamics may be consistent enough for seizure detection in preclinical models of epilepsy. We plan to compare our models against static classifiers to test whether these results translate into gains in specificity and precision, as well as apply the models to continuous, chronic data rather than isolated segments.

This work was supported by National Institutes of Health Grant No. NS107148 (to SS) and R01NS079507, R01AG075583, and R21NS131903 (to BB).

Nicholas Constantino [1] • Caitlin Carroll [2] • Holden Williams, PhD [3] • James Snipes [3] • Nicholas L'Italien [3] • Diksha Satish [3] • Carla Yuede, PhD [2] • Robert Gould, PhD [4] • Lance Johnson, PhD [3] • Shannon Macauley [3]
Neuroscience University of Kentucky [1] • null Washington University School of Medicine, St. Louis [2] • Physiology University of Kentucky [3] • null Wake Forest University School of Medicine [4]

ATP sensitive potassium channels couple metabolism with neuronal excitability

Student

ATP sensitive potassium (KATP) channels act as metabolic sensors to regulate cellular excitability throughout the body. We previously demonstrated that neuronal KATP channels are composed of Kir6.2 subunits and are highly expressed on excitatory and inhibitory neurons (Grizzanti et al, 2022). Therefore, the goal of this study was to explore how Kir6.2-KATP channel deletion impacted cerebral metabolism and neuronal activity. Using a mouse model lacking Kir6.2-KATP channel activity, we investigated how KATP channel deletion impacts cerebral glucose utilization and metabolic flux using stable isotope-resolved metabolomics (SIRM). We found that Kir6.2-/- mice had alterations in brain glucose utilization compared to WT mice. In Kir6.2-/- mice, labeled glucose was shunted toward metabolic pathways, including glycolysis, and away from neurotransmitter synthesis. Given this change in neurotransmitter synthesis, we explored the impact of this glucose shunt on neuronal activity. We developed a novel approach to simultaneously record electroencephalography/electromyography (EEG/EMG) and metabolic rhythms via intracranial biosensors to measure sub-second oscillations of brain interstitial fluid (ISF) glucose, ISF lactate, and sleep/wake states. When examining absolute EEG power, a measure of the strength of neuronal activity, we found a dampening of EEG power in Kir6.2-/- mice relative to WTs across all frequency bands; suggesting decreased neuronal activity. Given that metabolism and activity change across a diurnal cycle, we explored whether these changes in metabolic and neuronal activity altered daily metabolic rhythms and sleep/wake cycles. Both Kir6.2-/- and WT mice have diurnal fluctuations in brain ISF glucose and lactate, with increased levels of ISF glucose and lactate during the dark cycle (when mice are awake) and decreases in the light cycle (when mice are asleep). Interestingly, Kir6.2-/- mice have a 4hr delay in the rise of ISF lactate during the light-dark transition, suggesting changes in arousal or sleep/wake cycles. Therefore, we explored whether Kir6.2-/- mice have alterations in sleep/wake patterns compared to WT mice. Kir6.2-/- mice spend more time awake during the light period compared to WT mice and their relative EEG power spectra shift towards higher frequency activity. Behaviorally, we observed increased activity during the light period but a decrease in their startle response. Together, we hypothesize that KATP channel activity is necessary for arousal and state shifts by coupling metabolic information with the demands of neuronal excitability.

Sarah Garcia Pava [1] • Sridhar Sunderam, PhD [2] • Madison Bates [1]
Biomedical Engineering University of Kentucky [1] • Biomedical Engineering University of Kentucky [2]
Graded Finger Extension reflects changes in the EEG: A study in healthy right-hand dominant subjects.
Student
Keywords: event-related desynchronization, brain rhythms, brain-computer interfaces, finger extension, EEG

Abstract:

Modern neuroscience relies on electroencephalography (EEG) to non-invasively explore intricate neuronal communication patterns. In particular, EEG is used to study the brain's response to specific tasks, stimuli, or events, revealing unique brain rhythms such as the beta (13-30 Hz) and mu (7-11 Hz) rhythms, critical to movement research. Active or passive limb movements lead to an attenuation in these frequency ranges, a phenomenon called event-related desynchronization, widely used in neural and motor rehabilitation. Despite EEG's effectiveness in motor rehabilitation, limitations arise when trying to capture diverse commands or activities non-invasively, particularly in brain-computer interfaces.

In our study, we aimed to distinguish between distinct levels of finger extension in 14 right-hand-dominant, healthy individuals. The subjects matched their finger movements to visual cues indicating no-go (no movement), low, medium, and high target extensions. Each session involved 12 runs (alternated between both hands) with 16 trials and was recorded using an EEG device with 32 active channels following the international 10-20 system. Each trial had a prep period during which a fixation cross was shown to get the subject's attention, followed by the plan period indicating the desired target extension, a task period during which the subject would extend their fingers, and a rest period. The signals were pre-processed offline, and the mu-beta band power was extracted. The mu-beta strength increased monotonically from low to high finger extension in 8 participants on the right hand, a proportion greater than chance ($p<0.05$), and in 7 participants on the left hand. This trend reflects a progressive increase in cortical recruitment correlated with extensor activity and indicates the feasibility of deriving a graded volitional signal that represents fine motor control. This insight paves the way for the potential inclusion of more nuanced commands in future brain-computer interface developments, offering individuals with motor injuries the opportunity to regain greater control over their bodily functions.

Jared Rybarczyk [1] • Jordan Clay, MD [2] • Flavius Raslau, MD [3] • Jihye Bae, PhD [1]
Electrical and Computer Engineering University of Kentucky [1] • Neurology University of Kentucky [2] • Neurology, Radiology, Neurosurgery University of Kentucky [3]

Delta Focused Ictal EEG Source Imaging for Accurate Source Localization in Refractory Focal Epilepsy

Student

20-40% of the 50 million people diagnosed with epilepsy eventually become refractory, where antiepileptic drugs (AEDs) are no longer a viable means for controlling seizures (French, 2007). For these patients, resecting or disconnection of the seizure onset zones (SOZs) is a common option used to control or eliminate seizures. Accurate localization of the SOZs, ideally to the lobar and sub-lobar level, is essential to the success of such procedures. Based on the patient's electroencephalogram (EEG) and anatomical model, EEG source imaging (ESI) can be carried out to estimate SOZs. Interictal EEG data has been conventionally used in ESI for its consistently high congruence with clinically derived SOZs, largely due to localizing interictal epileptiform discharges (IEDs) (Mouthaan et al, 2019). However, the usage of ictal EEG data in ESI has shown potential for improvement upon the interictal case in both localization accuracy and sensitivity/specificity (Sharma et al, 2019). Our study aims to validate an ictal ESI strategy with a specific focus on delta waves . We evaluated 2 specific delta bands of 0.3-4Hz and 1-4Hz across 4 different ictal windows, including durations from ictal initiation to 2 seconds elapse, 10 seconds, and until the termination of the seizure. Structural magnetic resonance imaging (MRI) and EEG data from 10 patients with focal refractory epilepsy are provided by the University of Kentucky's HealthCare Epilepsy Center. The structure MRI is processed computationally to produce an individualized patient's head model using boundary element method (Gramfort et al, 2010). In addition, minimum norms estimate (MNE), standardized low resolution electromagnetic tomography (sLORETA), and equivalent current dipole model (ECD) are applied to produce estimates of the SOZ. These methods have been chosen due to their well-documented accuracy in solving the ESI inverse problems (Mierlo et al, 2020). The estimated SOZs from our proposed ictal ESI are compared to clinically defined SOZs to evaluate their validity. Our results support the possible use of delta waves in ictal ESI to accurately estimate SOZs, with regionally concordant and lateralizing ESI. Specifically, we observe considerable concordance between computational and clinical estimates in seizures exhibiting delta initial rhythms.

This project was supported by the College of Medicine Alliance Initiative and the Neuroscience Research Priority Area at the University of Kentucky.

Neurodegeneration

Qingjun Wang, PhD [1] • Kathleen West [1] • Athena Mandzy [2] • Kelby Kane [1] • Lincoln Nichols [1] • Robert Kline IV [3] • Christopher McLouth, PhD [4]
Ophthalmology and Visual Sciences University of Kentucky [1] • Ophthalmology and Visual Sciences Math Science Technology Center (MSTC), Dunbar High School [2] • Rodent Behavioral Core (RBC) University of Kentucky [3] • Biostatistics University of Kentucky [4]

Developing simple rodent behavioral tests for dissecting vision impairment in neurodegenerative conditions with cognition decline

Faculty

Kathleen C. West[1], Athena Mandzy[1,2], Kelby M. Kane[1], Lincoln J. Nichols[1], Robert H. Kline IV[3], Christopher J. McLouth[4], **Qingjun Wang[1,*]**

[1] Department of Ophthalmology and Visual Sciences, University of Kentucky

[2] Math Science Technology Center (MSTC), Dunbar High School

[3] Rodent Behavioral Core (RBC), University of Kentucky

[4] Department of Biostatistics, University of Kentucky

* Presenter

CLN3 disease (*aka.*, juvenile Batten disease or juvenile neuronal ceroid lipofuscinosis) is a devastating pediatric neurodegenerative disease with progressive vision impairment, cognitive decline, and motor failure prior to premature death. Our published work shows that *Cln3*-deficient (*Cln3*KO) mice displayed vision impairment, as detected by electroretinography (ERG), with pathologies in both neural retina and retinal pigment epithelium. Our preliminary data also show that *Cln3KO* males exhibited a cognitive deficiency in the Morris Water Maze task. However, it is unclear if vision impairment, despite detectable by ERG, prevents *Cln3*KO from fulfilling visual tasks, and if such vision impairment contributes to reduced performance in cognition tasks. To determine visual behavioral phenotype for *Cln3*KO, and to untangle the contributions of vision impairment to reduced performance in cognition tasks, we designed several preference tests based on our initial observation of strong preference of wild type (WT; C57B6/J) mice for light (*vs.* dark) blue objects. For this work, we have used 3D-print to generate objects with different colors, shades of grey, and stripes. First, we will test WT's preferences for different colors, shades of grey, and stripes. Next, we will select the most sensitive preference test(s) to evaluate visual behavioral phenotype in *Cln3*KO (*vs.* WT) mice. In addition, we will determine if the preference test results correlate with classical OptoDrum test results. The simple preference tests that we are developing here, if work, will be useful for dissecting vision impairment in CLN3 disease and other neurodegenerative conditions with cognition decline, such as Alzheimer's.

Acknowledgement: NRPA Pilot Awards and CCTS Innovation and High Impact Pilot Award (to QJW), VPR support (to the RBC), and Natalia S. Mandzy.

KHINE ZIN AUNG, MD, PhD [1] • Xian Wu, PhD [1] • Erin Abner, PhD [2] • David Fardo, PhD [1] • Yuriko Katsumata, PhD [1]
Department of Biostatistics, College of Public Health Sanders-Brown Center on Aging [1] • Department of Epidemiology, College of Public Health Sanders-Brown Center on Aging [2]

Alzheimer's Disease and Cancer: A Polygenic Risk Score Analysis

Fellow

Background: In recent years, an inverse association between cancer and Alzheimer's disease (AD) has been reported; various factors such as common signaling pathways, hormonal systems, and genetic predispositions have been identified as important contributing factors. However, exact mechanisms are still unknown. Research suggests that patients receiving anti-cancer therapy are likely to have cognitive deficits during their treatment course, and identifying underlying mechanisms is warranted. Polygenic risk scores (PRS) are valuable for determining individual genetic risk for disease, and its implementations for cancer screening were conducted in research studies. To date, there have been no published findings connecting cancer and AD through PRS. Here, we investigate the genetic connection between various types of cancer and dementias using PRS. Our research framework is based on (a) association of cancer PRS with dementia-related phenotypes including clinical diagnosed and autopsy-confirmed AD, frontotemporal dementia (FTD), hippocampal sclerosis, and Lewy body dementia, cognitive test scores, and biomarkers; (b) which types of cancer are more likely to be linked to the inverse association by examining correlation between each PRS of cancer type and AD; and (c) which underlying and interconnected mechanisms are contributing to these associations.

Method: Data were obtained from the National Alzheimer's Coordinating Center (NACC), the Alzheimer's Disease Genetics Consortium (ADGC), and the UK biobank. We included various malignancies and analyzed their associations with AD by using PRS. For differentiation between various types of dementia and assessing the severity of cognitive impairment, scores from neuropsychiatric testing (MMSE, MoCA, Boston Naming Test, Digit Span Forward and Backward Test, WAIS-R Digit Symbol, Category Fluency vegetables and animals, Trail Making Test Part A and B, Wechsler Memory Scale-Revised (WMS-R) Logical Memory – immediate and delayed, Craft Story immediate recall, Craft Story delayed recall tests) were used. AD diagnosis criteria are based on clinical scores and neuropathology scores (Thal phase, Braak stage, CERAD score, and NIA-AA Alzheimer's disease neuropathologic change). Both Cross-sectional and longitudinal analysis will be applied.

Results and Conclusion: We expect individuals having higher cancer polygenic risk scores are associated with lower risk of AD and other dementia-related phenotypes. Underlying shared mechanisms will be explored. In conclusion, we hope that our results will help to access individuals' genetic risks to both AD and cancers for early detection and prevention. Moreover, we believe that our research can contribute to precision medicine for treatment and lower the burdens encountered during anti-cancer treatment.

Funding: P01AG078116

Elif Coskun, MD [1] • Justin Barber [1] • Erin Abner, PhD [1] • Brian Gold, PhD [1] • Richard Kryscio, PhD [1] • Frederick Schmitt, PhD [1] • Linda Van Eldik, PhD [1] • Peter Nelson, MD, PhD [1] • Gregory Jicha, MD [1]
Sanders-Brown Center on Aging University of Kentucky [1]

Comorbid Pathology in Clinical Trial Participants: Autopsy Findings and Clinical Features

Faculty

Background: The presence of multiple comorbid pathologic features in late onset dementia has been well documented across cohort studies that incorporate autopsy evaluation. It is likely that such mixed pathology may also be common in Alzheimer's disease and related dementias (ADRD) clinical trial participants, potentially confounding the results of interventional trials that are designed to target a solitary pathophysiologic mechanism.

Methods: The UK ADRC autopsy database was screened for participants that had previously engaged in therapeutic interventional trials for Alzheimer's disease, vascular cognitive impairment and dementia, and/or ADRD prevention trials from 2005 to present. Seventy-five cases (out of a total n=546) had engaged in clinical trials for the prevention or treatment of ADRD. Pathologic features studied included b-amyloid, tau, a-synuclein, TDP-43, and cerebrovascular disease using conventional rating scales.

Results: Autopsy cases for those that previously engaged in clinical trials did not differ significantly from those that were trial-naïve in respect to demographic, genetic (ApoE), or clinical characteristics with the exception of CDR global scores that were lower for past trial participants ($p<0.05$). Comorbid pathologies were common across study types with a mean of 3 pathologic features/participant, 18% with quadruple misfolded proteinopathy (QMP; Ab, tau, a-synuclein, & TDP43), and only 34% of MCI/AD trial participants demonstrating a "pure" disease state that was targeted by the study intervention.

Conclusions: In our study, approximately 2 out of 3 ADRD trial participants had comorbid mixed pathologic features. Understanding the heterogeneity of pathologies seen in clinical trial participants may allow improved I/E criteria based on clinical features and the rational use of antemortem biomarkers to stratify the likelihood of mixed comorbid pathology that may be unwanted or may be the target for future interventional strategies. Such insights may also enable improved power analyses and statistical designs that may be able to adjust for the confounds of such mixed pathologic disease states that are common in ADRD clinical trials.

Working Memory related Frontal Brainwaves are Associated with Vascular and AD Plasma Biomarkers in Healthy Older Adults
Faculty

Yang Jiang1,2, Yuriko Katsumata2, Kate E. Foley2, Baoxi Wang1, Jennifer A Neal1, Tiffany L Sudduth2, Christopher M. Norris1,2, Gregory A Jicha1,2 & Donna M Wilcock1,2

1. University of Kentucky College of Medicine. 2. University of Kentucky Sanders Brown Center on Aging

Background: We have previously reported distinct frontal signature of working memory related potentials (WMRP) in older adults with Normal Cognition (NC) versus age-matched older adults with Mild Cognitive Impairment (MCI) (Li et al., 2017). Our Kentucky cohort demonstrates that asymptomatic older individuals with MCI-like frontal brainwave patterns convert to MCI within a short 5-year period, as opposed to individuals with NC-like patterns that remain normal 10 years later (Jiang et al., 2021). Leveraging blood-based Vascular/AD biomarkers, we test the hypothesis that WMPR is associated with preclinical AD/ADRD pathologies.

Method: 33 (19 women) cognitively intact older volunteers, average age 79 (SD 8.53) years old, from a longitudinal cohort followed by University of Kentucky ADRC participated. Each participant was assessed by electroencephalogram (EEG) recording (64- or 14-channels headset) during a visual working memory task and vascular/AD plasma markers (Wilcock et al., 2022). Multiple linear regression analyses adjusting for age and sex were applied for examining several frontal WMRP difference waves (Target items held in working memory – Nontargets) and multiple vascular/AD plasma biomarkers A**β**42/40, pTau181, and GFAP.

Results: We examined the association between WMRP and the percentage changes of A**β**42/40, pTau181, GFAP levels from plasma Vascular/AD biomarkers. The decrease of WMRP levels (MCI-like) was significantly associated with increased level of pTau181 at the left frontal F7 site after adjusting for age and sex (b = -0.035 and p = 0.043). GFAP (astrocyte reactivity) had negative correlations with WMRP (-0.33).A**β**42/40 showed weak positive correlations.

Conclusion: Our results indicate that predictive working memory related brainwaves are associated with tau pathologies. Working memory-related EEG neuromarkers and plasma AD biomarkers are sensitive and affordable screening tools to predict continuous cognitive decline risk in healthy normal older adults.

References

Jiang, Y., Li, J. Schmitt, F. A., Jicha, G. A., Munro, N. B., Zhao, X., Smith, C, Kryscio, R, Abner, E. L. (2021). Memory-Related Frontal Brainwaves Predict Transition to Mild Cognitive Impairment in Healthy Older Individuals Five Years Before Diagnosis. *J. Alzheimer's Dis.* 79, 531–541.

Li, J., Broster, L, Jicha G, Munro,N, Schmitt, F, Abner, E, Kryscio, K, Smith C, Jiang, Y. (2017). A cognitive electrophysiological signature differentiates amnestic mild cognitive impairment from normal aging. *Alzheimer's Research & Therapy*, 9, 3.

Wilcock D, Jicha G,… MarkVCID Consortium. (2021). MarkVCID cerebral small vessel consortium, *Alzheimers Dement.* 2021 Apr;17(4):704-715.

Poster Session

Neurodegeneration

Sara Palacio [1] • Nicole Rummel, PhD [2] • James Campbell [1] • Allan Butterfield, PhD [2] • Subbarao Bondada, PhD [3] • Heidi Weiss, PhD [4] • John Villano, MD, PhD [5] • Ines Batinic-Haberle, PhD [6] • Daret St. Clair, PhD [1] • Luksana Chaiswing, PhD [1]
Toxicology and Cancer Biology University of Kentucky [1] • Chemistry University of Kentucky [2] • Microbiology, Immunology and Molecular Genetics University of Kentucky [3] • Internal Medicine University of Kentucky [4] • Neuro-Oncology University of Kentucky [5] • Radiation Oncology Duke University [6]

Extracellular Vesicles Derived from Glioblastoma Promote Microglia-Mediated Neurotoxicity

Student

Little is known about the underlying mechanisms of glioblastoma (GBM) and/or therapy-derived cognitive impairment (CICI). 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 (RT). Importantly, these EVs (named Redox EVs), contain high levels of highly reactive **α**,**β**-unsaturated aldehyde 4-hydroxynonenal (HNE), which participates in multiple pathological processes. 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 and monitoring them for 6h, 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 ROS release from microglia cells, we treated HMC3 cells with Redox EVs and monitored H_2O_2 levels in the medium. Results showed that Redox EVs from LN18, GBM-PDX cells (G44 and G84), and GBM patients; caused a significant increase 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. 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. More importantly, the viability of HCN2 cells was rescued by pre-treating them with catalase. Next, we tested if altering the microglial redox state using BMX-001 (an MnSOD mimetic, currently in clinical trials for high-grade gliomas), could mitigate glial cells activation. Adding BMX-001 in combination with RT increased the levels of 4HNE-adducted proteins in GBM cells but decreased in microglial cells. Cytokines like TNF**α**, IL6 and TIM4 were also measured as markers of microglia activation and inflammatory response. Overall data and the change in these markers suggest that H_2O_2 released from microglia could be a key for Redox EV-mediated neuronal injury and that BMX-001 could reduce GBM damage to non-cancer cells such as microglia and neurons.

Ruei-Lung Lin, MS [1] • Sami Case, PhD [1] • Olivier Thibault, PhD [1]
Pharmacology & Nutritional Sciences University of Kentucky [1]

In vigilo age- and sex-dependent alterations in S1 neuronal Ca2+ network dynamics may contribute to gait dysregulation

Staff

Over the past 30 years, the calcium (Ca^{2+}) hypothesis of brain aging has provided clear evidence that hippocampal neuronal Ca^{2+} dysregulation is a key biomarker of aging. Indeed, age-dependent Ca^{2+}-mediated changes in intrinsic excitability, synaptic plasticity, and activity have helped identify some of the mechanisms engaged in memory and cognitive decline. However, much of this work has been done at the single-cell level, mostly in slice preparations, and in restricted structures of the brain. Recently, our lab identified age- and Ca^{2+}-related neuronal network dysregulation in the cortex of the anesthetized animal. Still, investigations in the awake animal are needed to test the generalizability of the Ca^{2+} hypothesis of brain aging and dementia. Here, we used *in vigilo* two-photon (2P) imaging in ambulating mice, to image GCaMP8f in the primary somatosensory cortex (S1), during ambulation and at rest. In order to investigate aging- and sex- related changes in the neuronal Ca^{2+} network, a continuous wavelet transform (CWT) analysis was developed (MATLAB) to extract measures of network communication while also addressing pair-wise correlations at single-cell resolution. Following imaging, gait behavior was characterized to test for changes in locomotor stability. During ambulation and compared to rest, in both young (4 months) and aged mice (22 months), an increase in connectivity and synchronicity was noted. An age-dependent increase in network synchronicity was seen in ambulating aged males only. Additionally, females displayed a greater number of active neurons, area-under-curve, and neuronal activity compared to males, particularly during ambulation. These results suggest S1 Ca^{2+} dynamics and network synchronicity are likely contributors of locomotor stability. We believe this work raises awareness of central elements at play in S1 where neuronal Ca^{2+} network dysregulation is seen with aging, perhaps highlighting potential therapeutic targets that may help offset age-dependent increases in falls.

Moltira Promkan, PhD [1] • Susan D. Kraner, PhD [1] • Irina A. Artiushin [1] • Peter T. Nelson, MD, PhD [1] • Pradoldej Sompol, PhD [1]
Sanders-Brown Center on Aging University of Kentucky [1]

Oxidative stress-associated cerebrovascular pathology in Alzheimer's disease

Staff

Oxidative stress is a key mechanism in pathogenesis and pathophysiology of neuronal disorders such as Alzheimer's disease (AD) and also plays roles on vascular injury. Cerebrovascular lesions highly comorbid with AD pathology and may worsen disease progression and reduce treatment efficacy. Oxidative stress markers including nitration of macromolecules are increased in AD. Here, we aim to investigate nitration status of fibronectin, a multifunction extracellular matrix protein that present in blood stream and brain parenchyma where it maintains vascular and perivascular integrity. Immunolabelling was performed to investigate levels of fibronectin and nitrotyrosine in postmortem AD brain specimens confirmed with vascular pathology obtained from UK-ADC brain bank. We found several lesions that link to different stages of vascular and brain pathology. Immunoreactivity of fibronectin and nitrotyrosine surrounding multiple arterioles and venules indicates acute vascular leakage. Levels of fibronectin and nitrotyrosine are increased in reactive astrocytes surrounding the vessels suggesting that oxidative stress is involve in astrocyte activation. Our results provide evidence that oxidative damage of fibronectin could be used as a new biomarker and strengthen association of oxidative stress and vascular complication in AD which could benefit future studies of the combined pathology.

Ryan Shahidehpour , MS [1] • Eseosa Ighodaro, MD, PhD [2] • Peter Nelson, MD, PhD [1] • Adam Bachstetter, PhD [3]
Sanders-Brown University of Kentucky [1] • Neurology Emory University [2] • Spinal Cord and Brain Injury Research Center University of Kentucky [3]

Glial response in association to Multilumen Vascular Profiles in Neurodegenarative Diseases

Student

With aging and disease, cerebrovascular structures show remarkable remodeling and formation of vascular pathologies, however, little is known about many of the subtypes of vascular pathology. One such subtype, known as multi-lumen vascular profiles (MVPs), are described as multiple vessel lumens enclosed within a single vascular space, and are commonly observed in aged human brains. While these vascular features are surprisingly common, little is known about glial responses in the presence of MVPs and disease.

In order to assess glial reactivity in proximity to MVPs, a within-subject comparative approach was utilized involving 13 human tissue samples containing the amygdala or hippocampus, originating from the UK-ADC cohort and featuring MVPs. Tissue was stained using our method of multiplexed serial staining (QUIVER). Vascular structure profiles were first visualized by staining for CD34, from which 20 single vascular profiles (SVPs) and 20 MVPs were randomly identified in each case, employing CD34 reactivity exclusively for vascular profile identification. Subsequent serial staining using Iba1, Ferritin and GFAP was completed, and after each round, sections were digitized in entirety using a Zeiss Axio scanner. Using HALO software, these images were registered, deconvolved and merged into a single pseudo-colored image. Three concentric rings at 50um intervals were generated away from each vascular profile's periphery and the glial response was assessed at each interval using the Area Fractionator and Object colocalization algorithms in HALO software.

Analysis of approximately 40 profiles (SVPs and MVPs) for each of the 13 cases was used for quantification of staining, which demonstrated that the extent of glia reactivity within a 150μm radius from the vascular profile was similar between the SVPs and MVPs. Slight differences can be seen in ferritin activity in close proximity to MVPs compared to SVPs, however, little differences in astrocytic or microglial reactivity.

MVPs are a surprisingly common pathological feature associated with age and disease. In our study we found little evidence to suggest that MVPs are associated with large changes in glial reactivity within the hippocampus/ amygdala of diseased individuals, yet more information is needed to understand the link between MVPs, glia and neurodegenerative disease.

Xian Wu, PhD [1] • Khine Zin Aung, MD, PhD [1] • David Fardo, PhD [1] • Erin Abner, PhD [2] • Yuriko Katsumata, PhD [1]
Department of Biostatistics, College of Public Health Sanders-Brown Center on Aging [1] • Department of Epidemiology & Environmental Health, College of Public Health Sanders-Brown Center on Aging [2]

GWAS of Longitudinal Cognitive Changes Related to Late-Onset Alzheimer's Disease for Diverse Populations: A Systematic Review and Meta-Analysis

Other

Alzheimer's disease (AD), which affects cognitive functioning over time, is the leading cause of dementia in older people. Neurocognitive tests are low-cost ante-mortem examinations and, thus, commonly used to trace cognitive changes in AD. Heritability estimates of Late-Onset AD (LOAD) are high – about 60~80% according to twin studies. Although there is a considerable number of studies on associations between genetic risk factors and LOAD-related cognitive decline, these studies often showed inconsistent findings. Moreover, racial disparities have had a significant impact on research, diagnosis, treatment, and support in AD. For example, AD research cohorts consist of mostly White participants, despite clinical and epidemiologic research showing that Black populations experience a higher burden of AD.

To fill the gap, address health equity disparities, and improve research on diverse populations in AD, the current study will conduct a systematic review and meta-analysis to determine the associations between genetic risk factors and longitudinal cognitive changes for diverse populations.

Systematic literature searches were conducted in PubMed, PsycINFO, Scopus, EMBASE, Web of Science, and Google Scholar. Search terms included a combination of GWAS terms (e.g., genome-wide association), cognitive terms (e.g., cognitive change/decline /impairment, global function, memory, mini-mental state examination [MMSE], and Montreal cognitive assessment [MoCA]), and Alzheimer's terms. The complete index of search terms and inclusion/exclusion criteria have been established and applied. Through systematic literature searches, 1,499 references were included in the title/abstract screening after duplicates were removed. Among them, 719 references were included in the full-text screening. Approximately 130 references will be coded for data extraction and involved in the meta-analysis.

Two reviewers will extract data from relevant papers separately according to a coding sheet with an expectation of interrater correlation above .80. A summary table will be generated across studies, including gene symbol, single nucleotide polymorphism (SNP) ID, chromosome, position, cognitive function, cognitive test, effect size, and study/sample characteristics (e.g., race/ethnicity). Pooling effect sizes and 95% confidence interval (CI) will be estimated and forest plots will be created by each of the diverse populations. Publication bias will be assessed.

The current study will provide important information regarding genetic risk factors and longitudinal cognitive changes in AD and the differences among diverse populations. Findings will elucidate differential biological mechanisms, which can in turn guide the development of new therapeutics for diverse populations. Limitations in the existing literature will be discussed and recommendations for future research will be suggested.

Supported by the DREAM Scholar program and P01AG078116

Hady Sabra, MS [1] • Justin Miller, PhD [2]
Microbiology, Immunology and Molecular Genetics University of Kentucky [1] • Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington, KY 40506, USA Sanders-Brown Center on Aging, University of Kentucky, 800 South Limestone St. Lexington, KY 40536-0230, USA Division of Biomedical Informatics, Department of Internal Medicine, University of Kentucky, Lexington, KY 40506, USA Department of Microbiology, Immunology, and Molecular Genetics, University of Kentucky, Lexington, KY 40506, USA University of Kentucky [2]

Ramp sequence explains synonymous variant association with Alzheimer's disease in the Paired Immunoglobulin-like Type 2 Receptor Alpha (PILRA)

Student

Synonymous variant NC_000007.14:g.100373690T>C (*rs2405442:T>C)* in the Paired Immunoglobulin-like Type 2 Receptor Alpha (*PILRA*) gene was previously associated with decreased risk for Alzheimer's disease (AD) in genome-wide association studies, yet the biological implications of this mutation are largely unknown. Since *rs2405442:T>C* has no impact on protein primary structure and is in high linkage disequilibrium with a common missense variant, *rs1859788:A>G*, its association with AD has largely been ignored. However, we found that *rs2405442:T>C* alone decreases codon efficiency at the 5' end of *PILRA*, which destroys a ramp sequence that we predict would increase mRNA and protein levels by limiting downstream ribosomal collisions. We experimentally validated the predicted effects of *rs2405442:T>C* with quantitative polymerase chain reactions (qPCR), and enzyme-linked immunosorbent assays (ELISA). Using Chinese hamster ovary (CHO) cells harboring the synonymous variant compared to wildtype cells without the variant, we show that both mRNA (P=3.2222 x 10^{-7}) and protein (P=0.01296) levels are significantly decreased in the mutant versus the wildtype. We show that tRNA pools in various cells and tissues influence the effects of *rs2405442:T>C* on ramp sequences, which likely impacts overall mRNA and protein levels across those cell types and tissues as well. *rs2405442:T>C* alone directly impacts *PILRA* mRNA and protein levels in the direction that we predicted based on the ramp sequence. This study is the first time that ramp sequences have been used to prioritize disease-associated variants for biological validation. We propose that since *rs2405442:T>C* is well-tolerated in the general population (minor allele frequency>0.35) and directly decreases both mRNA and protein levels, it might be a viable therapeutic target to mitigate risk for AD by reducing ramp-mediated *PILRA* expression without altering the protein product.

Chad Guilliams, Other [1] • Erin Abner, PhD [2]
College of Public Health University of Kentucky [1] • Epidemiology University of kentucky [2]
Participant characteristics associated with parental history of dementia in the ADRC cohorts: implications for secondar
Student

Background:

The characteristics associated with parental history of dementia for participants in the National Alzheimer's Coordinating Center (NACC) are not well documented. Using NACC data, we conducted a cross-sectional analysis to evaluate the association between parental history of dementia and selected participant characteristics: *APOEe4* allele dose, demographics, diagnosis of cognitive impairment, and age of onset of cognitive impairment.

Method:

Included participants (*n=27,723*) were stratified by parental history of dementia and frequencies and means were tabulated by factors of interest. Associations of *APOE4* dose with maternal and paternal dementia history were assessed using multinomial regression analysis. We used binary logistic regression to estimate the association between participant cognitive status (cognitive impairment vs. no significant cognitive impairment) and parental history of dementia, adjusting for *APOEe4* dose, race, age at visit, years of education, and sex. Among those with diagnosed cognitive impairment, we used multiple linear regression to test the hypothesis that participants with parental history of dementia would have earlier age at onset, adjusting for *APOEe4* dose, race, years of education, and sex. The above analyses were performed using the full sample of participants and again stratified by sex.

Result:

Parental history of dementia was commonly reported: no history of parental dementia (53%, *n=14,803*), maternal (29%, *n=8,033*), paternal (12%, *n=3,256*), and both parents (6%, *n=1,631*). The highest frequency of cognitive impairment diagnosis was found among those reporting no history of parental dementia (61%), and the lowest frequency was found among those with a history of both parents (50%). In the logistic regression analysis, Black race (vs White) was associated with decreased adjusted odds of having a diagnosis of cognitive impairment, OR=0.55, [95% CI: 0.51-0.59]. Participants reporting both parents with dementia had lower adjusted odds of cognitive impairment (OR 0.64, 95% CI: 0.56-0.72) compared to those with no reported parental history.

Conclusion:

Our results suggest substantial heterogeneity between ADRC participants with and without any reported parental history of dementia, and our results indicated differential reporting by race and sex. Overall and within strata of sex, Black participants were much less likely than White participants to report any type of parental history. Participant characteristics associated with patterns of parental dementia history suggest different selection may lead to participation, most evident in the group with no parental history but with the highest proportion of diagnosed cognitive impairment. Although the risk of misclassification based on self-reported parental history is high, our results suggest that *APOE* is not sufficient for capturing genetic risk of dementia and that parental history should also be included in any secondary analyses

Neurophysiology

John Boggess [1] • Bhoj Raj Thapa [2] • Jihye Bae, PhD [2]
CPE University of Kentucky [1] • ECE University of Kentucky [2]

Multichannel Electroencephalogram Database for Freewill Reaching and Grasping Tasks

Student

Brain-machine interfaces (BMIs) using electroencephalogram (EEG) signals are a widely utilized tool for neurorehabilitation. Notably, BMIs have found application in diverse technological platforms such as assistive exoskeletons and prosthetics (Al-Quraishi et al. 2018) as well as assistive mobile robots (Krishnan et al. 2016). The development of such systems commonly requires the training of machine learning algorithms using reliable datasets. While numerous open-source EEG datasets are currently available, those datasets are mainly for motor imagery tasks where the target imagination is provided by the experimenter. These datasets fail to address person's free will. To overcome this gap, we collected multichannel EEG dataset for freewill reaching and grasping task. In the experiment, we recruited right-handed, neurotypical young adults, age ranged 18-35 years. 32 EEG electrodes were placed on the scalp, with a ground electrode at FPz and a reference electrode at Cz. Additionally, 4 electrooculogram (EOG) electrodes were used to monitor eye movements along with an accelerometer on the right wrist, to detect movement onset. Participants were instructed to perform a reaching and grasping task involving 4 cups placed on a table. Of these cups, 2 were filled with water, and 2 were empty, serving to study the impact of movement intention on different task objectives. For the water filled cups, the participants were instructed to grasp the cup, sip the water, and place the cup at a predefined location on the table. Whereas for the empty cups, participants had to grasp the cup and place it in the same predetermined position. Each experimental trial began with an auditory "start" cue, where participants were asked to maintain an idle posture until the auditory signal is ceased entirely. Participants selected a cup to grasp by their own will after the start cue. An auditory "end" cue played at the end of the trial, 12 seconds after the start cue, affording participants a 7-second interval to place the cup in its original location and return their hands to the idle position before the initiation of the next trial. Each recording day encompassed 5 to 7 sessions, with each session comprising 30 individual trials. Each participant was recorded for a minimum of 2 days. Currently, our dataset comprises a total of 126 session recordings collected from 8 male and 4 female participants, and it is expected to obtain recordings from 8 more participants by early next year. Our unique multichannel EEG database allows investigations of techniques essential to design real-time BMIs, and the inclusion of freewill planning and execution of arm and hand movement offers the potential for the development of realistic neurorehabilitation devices.

This work has been supported by College of Engineering Research Mini Grant Program and Dr. Jihye Bae's Start-Up Funds provided by Department of Electrical and Computer Engineering at the University of Kentucky.

Jenna Kim [1] • Jihye Bae, MD, PhD [1]
Electrical and Computer Engineering University of Kentucky [1]

Neural Decoding of EEG Premovement Intention using Q-learning via Kernel Temporal Differences

Student

Brain-Machine Interfaces (BMIs) have shown promising advancements within neurorehabilitation efforts, with emphasis on utilizing neural intentions related to movement determination to assist upper limb rehabilitation. Recent studies show that electroencephalogram (EEG) based BMIs can be integrated in the use of robot assistance in neurorehabilitation, and its feasibility on robot therapy within upper limb rehabilitation has been reported in (Bhagat et al., 2016).

Investigation of EEG characteristics and subsequent neural decoding methods can increase the applicability of BMIs. In this study, we investigate an EEG premovement intention, which occurs prior to the actual movement (Schurger, Hu, Pak, & Roskies, 2021). A proper decoding of the premovement intention offers a more efficient initiation of assistive devices for rehabilitation to adjust and reinforce the user's needs.

Reinforcement learning (RL) has shown advantages in BMIs, with the ability to continuous interact between BMI users and an external device using a trial-and-error approach. RL's greatest benefit would be that it does not require an explicit desired signal, instead it uses implicit information, called reward, which can be modeled based on the users' neural intention (Poole & Lee, 2021). Both these factors have great implications with practical uses of BMI in neurorehabilitation, with greater ease of use and accessibility to help patients during therapy.

In this study, we examine the efficacy of an RLBMI algorithm called Q-learning via kernel temporal differences (Q-KTD) to decode EEG premovement intention. KTD is an incremental learning model that utilizes TD learning and kernel expansions (Thapa, Tangarife, & Bae, 2022). KTD has been integrated in Q-learning to provide nonlinear approximation of the action value function, Q. A publicly available EEG dataset of right-left key pressing tasks was used to validate Q-KTD's applicability (Kaya, Binli, Ozbay, Yanar, & Mishchenko, 2018). The adaptability and the applicability demonstrated within this study has shown the Q-KTD algorithm's capability to effectively classify premovement EEG data. Our results have shown the average success rates converged to 100% after enough iterations of learning. Furthermore, we evaluate the feasibility of transfer learning to overcome the common issue of RL, slow learning rates in the initial learning stage. Investigation of these RL techniques can bring a significant advantage for rehabilitation (Botvinick et al., 2019).

Avanti Sawardekar [1] • Salvatore Cherra, PhD [1]
Neuroscience University of Kentucky [1]

Regulation of neuromuscular development in C. elegans by an epidermal growth factor-like transmembrane protein.

Student

Epidermal growth factor promotes the development of many tissues, including neurons and glial cells. In mammals, epidermal growth factor receptor signaling coordinates the development and function of central and peripheral neuron synapses. Previous research suggests that epidermal growth factor-like proteins promote synapse development. Using the *Caenorhabditis elegans* (*C. elegans)* neuromuscular junction, we have identified a transmembrane epidermal growth factor domain-containing protein that reduces synapse formation. Using fluorescent synaptic markers, we observed that mutant animals produce more synapses than wild type animals at each developmental stage. Moreover, these extra synaptic connections were functional and produced an increase in neuromuscular activity as measured using *in vivo* calcium imaging. Although expressed in multiple tissues, this epidermal growth factor-like protein is only required in the epidermis of *C. elegans* to reduce synaptic connectivity of motor neurons. To reveal the underlying molecular mechanisms, we investigated potential functional domains beyond the epidermal growth factor domains. We identified a critical carboxy terminus peptide that is essential for protein function. Based on predicted protein interaction motifs, we performed an RNA interference-based screen and identified two genes that contain complementary interaction domains. Knockdown of one of these genes recapitulated the phenotype caused by mutations in the epidermal growth factor-like protein. Knockdown of the second gene restored development to normal in the epidermal growth factor-like mutants. These results suggest our RNAi screen has uncovered a new positive and negative of this epidermal growth factor-like signaling pathway. We propose that this epidermal growth factor-like signaling pathway mediates an interaction between the epidermis and motor neurons to prevent excessive synapse formation at the neuromuscular junction. Elucidation of the molecular mechanisms of this new signaling pathway will provide additional insights into how epidermal growth factor domain-containing proteins may bidirectionally modulate synapse formation and function during development.

Donya Vaez [1] • Salvatore Cherra, PhD [1]
Neuroscience University of Kentucky [1]

Restoration of synapse development in a SNARE mutant background by a Ras GAP

Student

SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors) are presynaptic proteins that drive neurotransmitter exocytosis. Mutations in SNAREs, like SNAP-25, VAMP, or syntaxin, and their binding proteins lead to deficits in synaptic transmission and are associated with neurodevelopmental disorders, like autism spectrum disorders and epilepsy. In *C. elegans*, mutations in SNAREs and their binding proteins result in an uncoordinated locomotor phenotype due to deficits in synaptic transmission. Restoration of synaptic function in SNARE mutants represents the ideal outcome when developing novel therapeutic targets for SNARE-related disorders. Here, we used *C. elegans* in an attempt to identify genes that would restore synaptic development or function in SNARE mutant backgrounds. We found that mutations in a SNAP-25 homolog reduce synaptic vesicle abundance in motor neurons. Although mutations in a syntaxin-binding protein homolog result in locomotor deficits, they do not result in changes to synaptic vesicle abundance. Therefore, we focused on the SNAP-25 homolog to identify genes the restore synaptic vesicle abundance in these mutants. Interestingly, we uncovered a loss of function mutation in a Ras GTPase activating protein (GAP) that was sufficient to restore synaptic vesicle abundance in the SNAP-25 homolog mutants. We tested whether Ras GAP mutants were sufficient to restore locomotor function in syntaxin-binding protein mutants; however, there was no discernable differences in locomotion in these mutants in the presence and absence of Ras GAP mutants. These findings suggest that mutations in different SNAREs or binding proteins may cause synaptic transmission deficits and neurological phenotypes through divergent mechanisms even though this complex of proteins acts together to coordinate the release of neurotransmitters. Future studies will determine how Ras GAP signaling influences synapse development in SNAP-25 homolog mutants and whether mutations in Ras GAP prevent synaptic impairments caused by mutations in other SNARE proteins.

Reagan Lamb [1] • Salvatore Cherra, PhD [1]
Neuroscience University of Kentucky [1]

RAP-1 and RAC-2 GTPases contribute to PXF-1 mediated synaptic development in C. elegans

Student

The family of Rho GTPases is comprised of important modulators of cellular and molecular signaling. Of these Rho GTPases, the Rac *Caenorhabditis elegans* orthologues, RAC-2, CED-10, and MIG-2, are known to have a wide range of functions within neurons including axonal guidance, mechanisms governing forgetting, and cell migration. While they are found to be triply redundant in some pathways, new studies have shown that they also may act independently of one another in cell and tissue specific manners. We previously found that the *C. elegans* PDZ-GEF orthologue, PXF-1, promotes the development of synapses at the neuromuscular junction. We found that *pxf-1(gk955083)* mutants animals exhibited decreased synaptic vesicle intensity, frequency of calcium transients, and perisynaptic filamentous actin. Based on the canonical role of Rho GTPases in regulating cytoskeletal development, we hypothesized that PXF-1 may be acting within a GTPase signaling pathway upstream of a known RAC protein. To determine this, we again used the neuromuscular junction of *C. elegans* as our model circuitry system. We first screened multiple GTPases mutants for their sensitivity towards the acetylcholinesterase inhibitor, aldicarb, and found that *rac-2(ok326)* and *ced-10(n1993)* mutant animals displayed resistance to aldicarb as compared to wild type animals. Focusing on RAC-2, we next examined the synaptic vesicle intensity of *rac-2(ok326)* and *pxf-1(gk955083)* single and double mutant animals using mCherry::RAB-3. We found that each single mutant and the double mutants all displayed decreased mCherry::RAB-3 intensity. We also found that mutations in the canonical RacGEF, TIAM-1, using *tiam-1(1556)* caused a similar decrease in mCherry::RAB-3 intensity in the presence and absence of the *pxf-1(gk955083)* mutation. Using a GFP::utrophin-calponin homology domain (GFP::ut-CH) as a marker for filamentous actin, we measured perisynaptic GFP::ut-CH intensity in *rac-2(ok326)* and *pxf-1(gk955083)* single and double mutants and found again a significant decrease in GFP::ut-CH intensity in all three mutants compared to wild type. Finally, to better determine whether *pxf-1* mediated defects are caused by a decrease in RAC-2 activity, we pan-neuronally expressed wild type RAC-2 and constitutively active RAC-2 G12V in the *pxf-1(gk955083)* mutant background. We found that expression of constitutively active, but not wild type, RAC-2 was able to restore synaptic vesicle intensity to wild type levels. Overall, these findings indicate that RAC-2 may act downstream of PXF-1 to mediate its effects on synapse development. These findings will contribute to a greater understanding of neuronal function and developmental signaling.

Michael Scales [1] • Salvatore Cherra, PhD [1]
Neuroscience University of Kentucky [1]

A role for Rap and Ras guanine nucleotide exchange factors during synapse development in C. elegans

Student

Small GTPases promote the development and organization of all tissues, including the nervous system. Mutations in GTPases or their regulatory proteins have been associated with many neurological disorders. For example, defects in Ras or Rap GTPase signaling have been linked to autism-spectrum disorders, schizophrenia, and epilepsy. We previously found that mutations in a Rap guanine nucleotide exchange factor (GEF), PXF-1, causes deficits in synaptic development and neuronal function in *C. elegans*. In mice, knockout of the PXF-1 homolog, RapGEF6, causes similar deficits in neuronal activity in the amygdala. However, it's not completely known how Rap GEF function promotes synapse function. We performed a candidate-based genetic screen to identify genes that function in the PXF-1 pathway to promote synapse development. Unexpectedly, we found that activation of a *C. elegans* Ras GTPase was sufficient to restore synapse development in *pxf-1* mutant animals. To determine how Ras GTPase signaling was modulated downstream of the Rap GEF, PXF-1, we tested candidate Ras GEF proteins and identified a putative Ras GEF that is essential for synapse development. We created double mutant animals between *pxf-1* and the putative Ras GEF mutant to determine if these two genes function in the same pathway. We found that double mutants caused a decrease in synaptic development to the same level as either single mutant, indicating that this putative Ras GEF acts in the same pathway as PXF-1. Based on these results we propose that precise regulation of Ras activity is necessary for synapse development and function. Overall, this study suggests that disease-associated mutations that disrupt Rap or Ras signaling may influence a linear signaling pathway to cause nervous system deficits.

Olivier Thibault, PhD [1] • Sami Case, PhD [1] • Nicholas Wright, MS [1] • Leopoldine Galopin, MS [1] • Rei-Lung Lin, MS [1] • Sophiya Sims, Other [1] • Jacquelyn Rhinehart [1]
Pharmacology and Nutritional Sciences University of kentucky [1]

Central processes contributing to age- and sex-dependent alterations in ambulatory stability

Staff

Recently published work from our lab has identified primary somatosensory cortex (S1) network dysregulation in aged mice, particularly during ambulation, and mostly in males. This analysis was conducted on calcium transients (gCamp8f) obtained in awake ambulating and stationary animals (Neurotar) undergoing two-photon imaging. While network alterations based on pairwise correlation coefficients were noted using a continuous wavelet transform, analysis of individual neuronal calcium transients also showed sex-dependent increases in activity and area-under-the-curve in aged ambulating females. Here therefore, we tested the hypothesis that several calcium-fluxing proteins might be able to reflect on these alterations in individual transients. We used S1 samples in combination with Western blot analyses to quantify L-VGCC (CaV1.2), NMDAR (NR2B) and ryanodine receptors (RyR2) across 43 samples from young-adult (4 months) and aged (22 months) C57BL/6J males and females. Using standard SDS-PAGE protocols in combination with primary and secondary antibodies we report here on age-dependent decreases in NR2B proteins in males only, along with a trend in CaV1.2 proteins increasing in aged males only. RyR2 also showed a strong trend for an age-dependent increase in both sexes. Along with these results, it is interesting to note that females displayed better performance on the walking task (stride length, stride time deviance index) compared to males. The results appear to be clinically relevant based on evidence for a decreased risk of falls, along with lower stride time deviance and greater stride length, in older females, compared to older males. We hope our work also raises awareness about the central components of gait dysregulation with age, focusing on network alteration in S1 and the influence of neuronal calcium-centric processes.

Alaina Taul, Other [1] • Elizabeth Elliott, Other [2] • Robin Cooper, PhD [2]
Biology, Neuroscience University of Kentucky [1] • Biology University of Kentucky [2]

The behavioral effects in overexpression of K2p channels in glial and motor neurons for larval Drosophila

Student

The two-P-domain K+ channels (i.e., K2P), previously known as leak channels, are responsible for maintaining the resting membrane potential of cells. There appear to be 15 known types of K2P channels in humans, and 11 known types in *Drosophila*. There are six subfamilies (TWIK, TREK, TASK, TALK, THIK, and TRESK). Some research indicates that altered expression of TASK-3 misexpression is related with cancerous tissues and forms of epilepsy. However, little is known about the expression of these subtypes in various animal tissues and the impact of altered expression on cellular physiology. It is well established that glial cells within the nervous system play an important role in the development and function of the nervous system. Glia cells release gliotransmitters and cytokines which, when altered, can influence neural circuits and behavior of animals. The influence and role of K2P channel on neuronal function and behavior (via these glial cells) have yet to be investigated. Additionally, selective misexpression in subsets of neurons is feasible in the Drosophila model to address the effects in the particular cell type, as well as the whole animal. We are examining the impact of over-expression of a subtype of K2P channels selectively in pan-glial cells, motor neurons, and cardiac neurons to assess the effects on behavior and physiology in larval Drosophila. Body wall movement (crawling) and mouth hook movement behaviors of larval *Drosophila* are commonly used to assess neuron dysfunction. These assays were also run at higher temperatures to further exacerbate potential impacts. In most genetically altered *Drosophila* lines, overexpression of these K2P channels has a statistically significant effect on behavior compared to the parent UAS-ORK1 line.

Elizabeth Elliott [1] • Sydney Speed [2] • Dylan Crawford [2] • Mikey Datta [2] • Jack Hirtle [2] • Abigail Leach [2] • Rebekah McIntosh [2] • Katy Roemer [2] • Lauren Sotingeanu [2] • Alaina Taul [2] • Breanna Vessels [2] • Kaitlyn Brock [2] • Sean Altenburg, Other [3] • Sonya Bierbower, PhD [3] • Jeremy Nadolski, PhD [4] • Robin Cooper, PhD [5]
University of Kentucky [1] • undefined University of Kentucky [2] • Chemistry and Life Science West Point [3] • Mathematical and Computational Science Benedictine University [4] • Biology University of Kentucky [5]

Effects of zinc on physiological processes in Drosophila and crawfish: cardiac, neural, synaptic transmission and behavioral assays

Student

Zinc (Zn^{2+}) is an essential element that affects proper organ function, cell growth, and immune function. However, it can also be present in too great a quantity, with zinc toxicity resulting in both minor and major physiological effects. This study examined the effects of $ZnCl_2$ on both nervous and cardiac function through a number of different experimental methods, using crawfish and *Drosophila* as model organisms. Cardiac function, activity at the neuromuscular junction, and survival were all assessed following the organisms' exposure to zinc. Function of the crawfish sensory muscle receptor organ (a proprioceptive organ analogous to the mammalian muscle spindle) was also examined. Dose-response was used to examine any changes in *Drosophila* larval crawling rates, mouth-hook movement rates, and touch sensitivity. Increased exposure to zinc generally led to decreased survival rate, in both crawfish and *Drosophila*; similarly zinc exposure led to depressed or even eliminated heart rates in both models. The neuromuscular junctions in both organisms saw varying effects: high concentrations (1mM, 10mM; n=6, p>0.05) eliminated synaptic transmission without affecting spontaneous quantal events, while lower concentrations (0.1mM, 0.5mM) merely depressed that transmission. At the crawfish MRO, high concentrations of $ZnCl_2$ (that is, 5mM; n=6, p>0.05) depressed neural activity (but this returned during washout), while lower concentrations (0.1mM and 1mM) saw MRO activity increase. No consistent effect was yet observed in *Drosophila* behavior with differing zinc concentrations.

Tessa Burnette [1] • Terra Yarbrough [1] • S.O. Nwadialo [1] • A. Asadipooya [1] • D. Bocook [1] • K.E. Brock [1] • I.V. Chauhan [1] • B. Chhadh [1] • R. Crane [1] • E.R. Elliott [1] • A. Glover [1] • J. Griffith [1] • J.A. Hudson [1] • H. Kashif [1] • D.M. Neely [1] • A. Nukic [1] • D. Patel 1 • G.L. Ruschman [1] • J.C. Sales [1] • A.C. Taul [1] • Robin Cooper, PhD [2]

undefined University of Kentucky [1] • Biology University of Kentucky[2]

The Neurophysiological effects of excess Li assessed in an authentic curriculum undergraduate research experience (ACURE)

Student

Animals are exposed to lithium (Li+) in the natural environment as well as by contact with industrial sources and therapeutic treatments. Low levels of exposure over time and high volumes of acute levels can be harmful and even toxic. The following study examines the effect of high-volume acute levels of Li+ on sensory nerve function and nerve conduction. A proprioceptive nerve in the limbs of a marine crab (Callinectes sapidus) was used as a model to address the effects on stretch activated channels (SACs) and evoked nerve conduction. The substitution of Li+ for Na+ in the bathing saline slowed nerve condition rapidly; however, several minutes were required before the SACs in sensory endings were affected. The evoked compound action potential slowed in con-duction and slightly decreased in amplitude, while the frequency of nerve activity with joint move-ment and chordotonal organ stretching significantly decreased. Both altered responses could be reversed with the return of a Na+ containing saline. Thus, perhaps subtle long-term exposure to Li+ may alter the function of SAC in organisms related to proprioception and nerve conduction.

Kaitlyn Brock [1] • Rebekah McIntosh [1] • Robin Cooper, PhD [1]
Biology University of Kentucky [1]

The similarities and differences in the effects of bacterial endotoxin lipopolysaccharide (LPS) on synaptic transmission at glutamatergic synapses

Student

Statistics show that over 1.5 million people in the US are hospitalized yearly due to sepsis. Gram-negative bacteria contain the endotoxin lipopolysaccharides (LPS) responsible for triggering the associated immune response in humans. Little is known about the direct action of LPS on cells or how LPS may affect other targets independent of the secondary immune response. LPS is known to alter glutamatergic synaptic communication in mammals and invertebrates. Crustaceans and insects are susceptible to bacterial septicemia by injury or through a leaky gastrointestinal tract just as humans. *Serratia marcescens* is known to play a role in septicemia in humans and other animals. The focus of this study was on the acute responses of LPS and associated peptidoglycans from this strain. High concentration of LPS from *Serratia marcescens* increased synaptic efficacy at glutamatergic synapse at the crayfish neuromuscular junction (NMJ) but depressed synaptic efficacy at glutamatergic synapse at the larval *Drosophila* NMJ. Both preparations resulted in transient hyperpolarization. At the *Drosophila* NMJ, quantal responses and evoked excitatory junction potentials (EJPs) decreased in amplitude. It appears that postsynaptic glutamate receptors were blocked. However, this was not the case at the crayfish NMJ, despite both receptor subtypes at the NMJs being pharmacologically classified as quisqualate receptors. Doxapram is a therapeutic compound used clinically to improve respiratory drive of carotid bodies by blocking the pH sensitive K2p channels (TASK subtypes). Doxapram (10 mM) depolarizes the larval *Drosophila* muscle in all preparations, but not as substantially for the crayfish muscle (1 to 10 mV) for all preparations. Doxapram at the crayfish NMJ does not cause the NMJ to spontaneously fire action potentials as it does at the larval *Drosophila* NMJ. Doxapram does not block the LPS response on glutamate receptors in Drosophila, but does block the hyperpolarization induced by LPS. The hyperpolarization due to LPS then appears to be a result of transiently activated K2p potassium channels at the Drosophila NMJ. Investigations at the crayfish NMJ is still underway. Perhaps doxapram could be used to block the LPS direct responses during Gram-negative bacterial septicemia.

Paresh Prajapati, PhD [1] • Urim Geleta [1] • Wang-Xia Wang, PhD [2]
Sanders-Brown Center on Aging Sanders-Brown Center on Aging [1] • Pathology and Laboratory Medicine Sanders-Brown Center on Aging [2]

Inflammatory microRNAs are an active component of macrophage-microglia communication

Staff

Effective intercellular communication between peripheral immune cells and the central nervous system (CNS) is essential for the brain's response to microorganism invasion and other pathological alterations. MicroRNAs (miRNAs), a class of small noncoding RNAs are known to be secreted/released by many cell types and often enclosed within extracellular vehicles (EVs). These miRNAs are then distributed in extracellular fluids, allowing them to reach distant target cells, in which they modulate protein expression. Previously, we reported that several inflammatory miRNAs are differentially enriched in CD11b+ cells isolated from brain tissue and bone marrow. Specifically, miR-223-3p and miR-155-5p were found to be enriched in bone marrow CD11b+ cells, whereas miR-146a-5p and miR-150-5p were enriched in brain CD11b+ cells. In our recent study, we co-cultured wildtype (WT) bone-marrow-derived macrophages (BMDM) with microglia devoid of miR-223-3p isolated from miR-223-3p knockout (KO) mice. This experiment revealed that macrophage-enriched miR-223-3p and miR-155-5p were actively secreted and readily detected in total exosomes of culture media. Further, we observed clear detection of miR-223-3p in miR-223-3p KO microglia after 48-hour incubation, suggesting a transfer of miR-223-3p from BMDM to microglia. Similarly, miR-155-5p appeared to be transferred between macrophage/microglial cells. Notably, this led to a reduction in the expression of several inflammatory and immune genes in KO microglia. These included miR-223-3p targets such as *NLRP3, CTSE*, MHCII molecules (*H2-Aa, H2-Eb1*), *CD86, TNFα*, and *CCL17*. Nevertheless, some other miR-223-3p targets remained elevated in miR-223-3p KO cells even following co-incubation. We conclude that inflammatory miRNAs are an active component of macrophage-microglia communication, and suggest a novel role for miRNAs that can be passed between cells in EVs.

Alex Do [1] • Jordan B. Tyree [1] • Anel A. Jaramillo, PhD [2]
Pharmaceutical Sciences University of Kentucky [1] • Pharmaceutical Sciences University of Kentucky College of Pharmacy [2]

Investigating Parabrachial-Amygdala Circuits that Modulate Stress-Induced Anxiety

Student

Anxiety disorders affect behavioral and physiological responses to life stressors. Chronic stress causes individuals to be more susceptible to developing psychiatric disorders such as anxiety. It is proposed that the bed nucleus of the stria terminalis (BNST) and parabrachial nucleus (PBN) play a role in modulating stress-related anxiety disorders. The BNST has a role in modulating anxiety-like behavior. The PBN acts as an alarm that detects threats. The PBN contains CGRP neurons that project to the BNST. This research aims to understand underlying neural mechanisms of the BNST and PBN in stress-related anxiety. We hypothesize that responses to stress and anxiety are driven by PBN CGRP neurons in the BNST. First male C57BL/6J mice were exposed to 4 days of repeated forced swim stress (FSS) or restraint. Next, anxiety-like behavior was measured by latency to consume food in the novelty suppressed feeding task (NSFT). Lastly, we utilize fluorescent immunohistochemistry to quantify cFOS, a marker for neuronal activity colocalized to CGRP in the BNST. Results demonstrate stress increased latency to feed in NSFT (2-way ANOVA stress $p=0.0476$). The data indicates that CGRP drives anxiety-like behavior following repeated stress. Understanding the BNST and PBN neural circuit will be key in identifying neural mechanisms related to anxiety and developing therapeutic agents for treatment. Future studies will utilize cFOS to identify specific cell types in the BNST that express CGRP receptors.

Neurotrauma

Ashley Glover [1] • Hannah Williams [1] • Kathryn Saatman, PhD [1]
College of Medicine University of Kentucky [1]
Investigating mossy fiber bouton alterations after traumatic brain injury
Student
Investigating mossy fiber bouton alterations after traumatic brain injury

Ashley Glover1, Hannah C. Downing1,2, & Kathryn E. Saatman1,2

1. Spinal Cord and Brain Injury Research Center (SCoBIRC), University of Kentucky, Lexington, KY

2. Department of Physiology, University of Kentucky, Lexington, KY

Traumatic brain injury (TBI) is a significant public health concern, often resulting in long-term cognitive and motor impairments. Within the hippocampus, the mossy fiber pathway has long been recognized for its significance in memory formation and information processing. This pathway, comprised of axons of granule cell neurons (GCNs) in the dentate gyrus (DG), serves as a connection between DG and the CA3 pyramidal neurons. Changes to synaptic connections of GCNs within the hippocampal DG after TBI are poorly understood. To evaluate how TBI alters mossy fiber synapses or boutons (MFB), Ascl1-CreERT2; R26R CAG-floxStopTom reporter mice were used to label granule cell neurons and their presynaptic connections. 8-week old mice were injected with tamoxifen and injured (n=3 females, n=2 males) mice received a controlled cortical impact brain injury 6-weeks after tamoxifen injection and were euthanized at 3 days postinjury. Images of tdTomato+ pre-synaptic terminals in the CA3 region were acquired as a z-stack (0.5um step size) at 40x magnification using a confocal microscope. Image stacks from each animal were imported into Imaris for 3D visualization and reconstruction. MFB numbers and surface volume were quantified in three brain sections per mouse. Significantly fewer MFB were observed in the CA3 region of the hippocampus ipsilateral to impact compared to the contralateral CA3 region ($p=0.039$). However, the surface volume of the MFB did not change 3 days postinjury. Surviving mature granule cell neurons have significant alterations to the mossy fiber pathway 3 days post injury suggesting impaired connectivity acutely after TBI.

Alyssa Franklin [1]
Physiology University of Kentucky [1]
Temporal Dynamics of B Cell Diapedesis after Traumatic Brain Injury in Mice
Student
Temporal Dynamics of B Cell Diapedesis after Traumatic Brain Injury in Mice

Alyssa M. Franklin[1], Pavel Yanev[1], Jack B. Miller[1], Brittney A. Williams[1], Anthony J. DeSana[1,2], Ann M. Stowe[3], and Kathryn E. Saatman[1,2]

[1] Spinal Cord and Brain Injury Research Center, [2]Department of Physiology, and [3]Departments of Neurology and Neuroscience, University of Kentucky, College of Medicine, Lexington, Kentucky

TBI is a leading cause of mortality and morbidity for young adults. Survivors of moderate to severe TBI often face persistent cognitive and neurobehavioral deficits. Repeated failed clinical trials targeting neuronal injury mechanisms have motivated expanded efforts to understand the roles of other cell types in the complex secondary injury cascade following trauma. The roles of astrocytes and microglia in driving neuroinflammation are now 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. Although clinical studies describe engagement of systemic adaptive immunity, a significant gap in knowledge exists regarding the timing and extent of B cell diapedesis into the brain after TBI, as existing studies in experimental TBI are limited largely to a single timepoint. The role of B cells in posttraumatic neurodegeneration or neuroplasticity remains unknown. We hypothesize that TBI triggers delayed B cell diapedesis into the cortex following a cortical contusion injury. To test this hypothesis, tissues collected from adult mice euthanized 1, 3, 7, 14 or 28 days after receiving controlled cortical impact TBI or sham injury were immunolabeled with the B cell antibody B220. Our data demonstrates a small number of B220+ B cells within the contused cortex at 1 and 3 days, increased numbers at 7 and 14 days, and fewer cells at 28 days. B cells can be detected within the parenchyma at a distance from hemorrhage sites by 7 days and as late as 28 days after injury. Future studies will characterize morphological and phenotypic characteristics of B cells within the injured brain to gain insight to their potential function.

Funding: This work supported by Kentucky Spinal Cord and Head Injury Research Trust grant 22-4, the University of Kentucky Neuroscience Research Priority Area, and the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR001998. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Sajeev Kaur, PhD [1] • Fernanda S. Franca, PhD [1] • Reena Kumari, PhD [2] • Samir P. Patel, PhD [1] • John C. Gensel, PhD [1]
SCoBIRC, Department of Physiology University of Kentucky [1] • SCoBIRC, Department of Physiology University of kentucky [2]

Clodronate-induced macrophage depletion following severe contusion spinal cord injury

Fellow

Spinal cord injury (SCI) leads to intraspinal inflammatory response including infiltrating blood leucocytes and resident glia. 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, liposome-encapsulated clodronate aka clodronate liposomes, depletes monocyte-derived intraspinal macrophages and several independent laboratories have reported therapeutic effects. To date, few studies have examined the extent to which clodronate liposomes are effective across different spinal levels and severities of SCI. In this study, we investigated the effect of clodronate liposomes on macrophage depletion following severe high thoracic contusion SCI. We hypothesize that intravenous clodronate liposome, delivered after T3 contusion SCI, will reduce intraspinal macrophage activation. 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). At 7 days post-injury, blood was collected from heart prior to transcranial perfusion for IDEXX. Spinal cords were isolated and analysis for depletion is ongoing. This pilot run will help us understand the potency of clodronate to cause depletion in our T3 severe contusion SCI model.

Reena Kumari, PhD [1] • Hemendra J. Vekaria, PhD [2] • Olivia H. Wireman, Other [1] • William M. Bailey, Other [1] • Steven M. MacLean, Other [1] • Andrew N. Stewart, PhD [1] • Ethan P. Glaser, PhD [1] • Patrick G. Sullivan, PhD [2] • Samir P. Patel, PhD [1] • John C. Gensel, PhD [1]

undefined Spinal Cord and Brain Injury Research Center and Department of Physiology, University of Kentucky, College of Medicine, Lexington Kentucky, United States [1] • undefined Spinal Cord and Brain Injury Research Center and Department of Neuroscience, University of Kentucky, College of Medicine, Lexington Kentucky, United States [2]

Driving reparative phenotypes of intraspinal macrophages post spinal cord injury

Staff

Keywords: Immunometabolism, microglia, neurotrauma, contusion, mitochondria

Spinal cord injury (SCI) activates resident microglia and recruits peripheral monocytes (collectively, CNS macrophages) into the injured nervous system that play roles in neuropathology by exacerbating neurodegeneration but also promoting repair. The average age at the time of SCI has increased to a median age of 50.5 years old in the US. Age is a key determinant of functional recovery following both clinical and experimental SCI. Sustained pro-inflammatory macrophage activation contributes to these age-related SCI deficits. Here, we hypothesize that injury-induced impairments in macrophage metabolism, and specifically oxidative phosphorylation (OXPHOS), drive pro-inflammatory macrophage activation after SCI and that age-dependent impairments in macrophage metabolism drive sustained pro-inflammatory macrophage activation. We tested this hypothesis by analyzing the bioenergetic profiles of intraspinal macrophages after T9 contusion SCI and comparing young (4-month-old; MO) versus aged (14-16 MO) mice. Spinal cords were collected at 7 days post-injury (dpi) and subjected to a magnetic bead sorting protocol to isolate intraspinal macrophage/microglia cells (CD11b+). Viable cells were plated at 50,000 cells/ well and subjected to Seahorse XF (Agilent) assay analysis and real-time levels of oxygen consumption rate (OCR) were determined.

Treatment with dichloroacetate (DCA 25mM-3 hrs)- a pan pyruvate dehydrogenase PDK inhibitor- significantly increased basal OCR and ATP-linked OCR in cultured macrophages and SCI macrophages isolated and treated ex-vivo. Additionally, basal respiration and ATP synthesis-linked respiration of intraspinal CD11b+ cells were significantly lower in aged mice compared to young mice. Our observation indicates SCI causes metabolic dysfunction in macrophages by decreasing OXPHOS which can be improved by DCA treatment. We further show that age causes metabolic dysfunction limiting macrophages' abilities to shift from glycolysis to OXPHOS. Recent advances in macrophage metabolism highlight efficient OXPHOS as key for sustaining anti-inflammatory/reparative functions. Therefore, our findings implicate macrophage metabolism as a potential contributor to pro-inflammatory activation with age.

Funding sources

University of Kentucky-SCoBIRCEndowment#5, R01NS091582, EpiMet pilot

Christopher Bosse-Joseph [1] • Kennedy Stewart, Other [2] • John Gensel, PhD [3] • Andrew Stewart, PhD [2]
Department of Neuroscience University of kentucky [1] • Department of Neuroscience University of Kentucky [2] • Department of Physiology University of Kentucky [3]

Behavioral Impacts of PTEN Knockout Using AAVrg and Axonal Visualization via Tissue Clearing in a Lateral Hemisection Model of Spinal Cord Injury

Student

Spinal cord injury poses multiple regeneration barriers, including neuronal-intrinsic and extrinsic factors. Overcoming these barriers has been a longstanding challenge in neuroscience. One well-studied mechanism to promote spinal cord regeneration involves activation through the Akt/mTorc1 pathway by knocking out phosphatase and tensin homolog protein (PTEN). PTEN knockout (KO) via adeno-associated virus (AAV) viral vector has shown encouraging results but also some potential deleterious effects. In this study, we utilized a mouse model of T9 hemisection and administered cre-recombinase (Cre) and a red fluorescent protein via AAV-retrograde (AAVrg) to induce PTEN knockout. Our primary objectives were three-fold: 1) Determine and compare the extent to which PTEN-KO using AAVrg's affects locomotor function in both the ipsilateral and contralateral leg after lateral hemisection. 2) Assess some potential deleterious effects outcomes as a result of PTEN KO on locomotor function. 3) Directly compare the extent that AAVrg's can transduce spared versus damaged axons in spinal-projecting neurons throughout the brain and brainstem. To evaluate the behavioral recovery of the mice, we utilized weekly Basso Mouse Scale (BMS) locomotor rating scale scoring to evaluate the effectiveness of PTEN-KO in promoting functional recovery. Furthermore, we optimized tissue-clearing techniques and employed confocal microscopy to visualize and reconstruct three-dimensional images of the brain and spinal cord, aiming to compare axon sprouting and transduced neuronal populations quantitatively. Our results shed light on the potential for AAVrg's to induce genetic knockouts in both damaged and spared axons and provide valuable insights into the intricate relationship between PTEN-KO and locomotor function. By understanding the extent of behavioral recovery and assessing the spatial distribution of newly generated axons within the cord, this study contributes to spinal cord regeneration knowledge and suggests novel therapeutic approaches. The findings will further our understanding of the utility of viral vectors for genetic manipulation in spinal cord injury.

Other Neurologic Conditions

Paula V. Monje, PhD [1] • Gabriela Aparicio, PhD [2] • David Sant, PhD [3] • Gaofeng Wang, PhD [4]
Dept neurosurgey University of Kentucky [1] • Dept. of Neurosurgery University of Kentucky [2] • undefined Noorda College of Osteopathic Medicine, Utah [3] • undefined University of Miami, Miami, Florida [4]

A SIMPLIFIED IN VITRO PLATFORM TO STUDY MYELIN GENE EXPRESSION IN HUMAN PERIPHERAL GLIAL CELLS

Faculty

Pharmacological manipulation of intracellular cAMP is a useful tool to induce the differentiation of rodent Schwann cells (SC) *in vitro*, but achieving the same manipulations in human SCs has proven challenging. Here, we utilize controlled cAMP stimulation to optimize cell-based assays of myelin gene expression using isolated human SCs obtained from nerve tissues. To assess the cellular and molecular changes associated with cAMP-induced differentiation, we employed a combination of immunofluorescence microscopy, live cell video-imaging microscopy, qRT-PCR, and next-generation transcriptome profiling (RNA-seq). Our results revealed that treatment of human SCs with nonhydrolyzable analogs of cAMP halted cell proliferation and promoted differentiation, as evidenced by morphological changes and the corresponding expression of numerous SC-specific, myelin-related markers. Time course analysis showed a robust and fast (within 1 day) upregulation of various transcripts encoding for myelinating SC-specific proteins (e.g., Egr2, MPZ, PLP1, GAL3ST1 and MBP) along with a concomitant downregulation of immature SC genes (e.g., cJun, Sox2, NES, and NGFR). RNA-seq analysis uncovered that surprisingly, many genes typically associated with peripheral neuropathies were transcriptionally modulated by cAMP. These results indicate that it is feasible to manipulate myelin gene expression in neuron-free human SC cultures. We propose that when used in a controlled setting, cAMP modulators can be exploited to develop scalable *in vitro* platforms to assess the potency of donor-derived SCs and test the effect of drugs targeting myelin genes.

Patricia Doyle [1] • Samantha Hart [2] • Brendan White [1] • Madeline Page [1] • Jason Brandon, PhD [1] • Barbara Nikolajczyk, PhD [3] • Mark Ebbert, PhD [4]
Neuroscience Sanders-Brown Center on Aging [1] • Biochemistry University of Kentucky [2] • Biochemistry Department of Pharmacology and Nutritional Sciences [3] • Neuroscience, Internal Medicine Sanders-Brown Center on Aging [4]

Using Single-Cell Long-Read Transcriptomics to Explore Cell-Type-Specific Signatures Across Murine Brain Regions and in Stimulated Human CD4+ T-Cells

Student

Background

Single-cell studies provide insight into cellular diversity and mechanisms underlying disease, offering novel therapeutic targets that may be obscured by bulk sequencing. Single-cell RNA sequencing (scRNA-seq) approaches require fresh tissue, a limitation that prevents it from being used with many clinical brain samples, which are typically frozen. Although single-nucleus RNA-sequencing (snRNA-seq) is used as an alternative to scRNA-seq that can be used with frozen tissue, some cell-types do not survive freeze-thaw cycles. Additionally, cytoplasmic signatures and RNA molecules in the cytoplasm that provide crucial information about cell state may be lost in snRNA-seq. Our understanding of isoform-level expression in different cell populations is currently limited due in part to the absence of single-cell approaches for long-read sequencing. scRNA-seq studies typically use short-read sequencing, which collapses all measures of single-gene isoform variants into a single gene expression measurement and, due to insufficient depth and/or mapping quality, cannot truly detect isoform-level expression. Long reads provide broad, isoform-length coverage of transcripts that may provide insight into functional variations in the resultant protein and require fewer reads (~1/3), allowing researchers to sequence greater numbers of cells at a lower depth with the same clarity. Recent studies combine single-cell and long-read sequencing to find RNA isoform-level changes in bacteria, humans, and mice, which will inform novel disease mechanisms and drug targets. Our objective from this pilot is to establish effective use of our novel long-read scRNA-seq preparation with fresh murine brain tissue and stimulated human T-Cells. We plan to use this technique on clinical samples in the future to distinguish isoform expression signatures between brain regions and cell types.

Methods

Our pilot used stimulated CD4+ T-Cells from young, healthy males and fresh murine cortex and cerebellum to identify gene and isoform expression signatures between different cell populations and regions. We adapted Particle-templated Instant Partition Sequencing (PIP-seq) for us with long-reads instead of customary 10X Genomics methods. PIP-seq offers fast, instrument-free cell preparation, a critical advantage for collecting fresh clinical samples, which may become available unpredictably. The adapted protocol yields high-quality, large-fragment cDNA (>500bp) with minimal fragmentation from ~10k cells.

Conclusion

Overall, we establish a novel utilization of the PIP-seq protocol for long-read single-cell sequencing. In the future, we plan to collect multiple regions of rapid post-mortem brain tissue and Peripheral Blood Mononuclear Cells (PBMCs) from Alzheimer's disease (AD) patients and cognitively normal controls. Our objective for future studies is to inform novel genetic markers and isoform risk factors for disease-associated cellular phenotypes between regions and cell types.

Rebekah McIntosh [1] • Kaitlyn Brock [2] • Robin Cooper, PhD [2]
Biology University of Kentucky [1] • Biology University of Kentucky [2]

The bacterial endotoxin lipopolysaccharide (LPS) recruits synaptic vesicles for evoked transmission at glutamatergic synapses.

Student

The endotoxin lipopolysaccharides (LPS), secreted from Gram-negative bacteria, has direct effects on synaptic transmission independent of systemic secondary cytokine responses. High concentration of LPS (500 µg/mL) from Serratia marcescens increased synaptic efficacy at glutamatergic synapse at the crayfish neuromuscular junction (NMJ) (N=6; $P<0.05$). LPS appears to promote vesicles in the reserve pool to the readily releasable pool. The action of LPS at the glutamatergic synapses of the crayfish neuromuscular junction is unique in promoting synaptic transmission as compared to other glutamatergic synapses in Drosophila and mammals, where synaptic transmission is depressed. Through quantal analysis of evoked and spontaneous quantal events, we can also address if all the effect is presynaptic in recruiting vesicles only for evoked responses or randomly. By analysis in the shape of the quantal events the postsynaptic receptor sensitivity to glutamate is being examined. This content is being addressed with intracellular recording of the muscle at NMJ as well as focal macropatch recordings over defined synaptic varicosities. To date, it appears evoked responses increase quantal content N=6 ($P<0.05$) without significant effects of the occurrences of spontaneous quantal events. This will help to address the direct effect of LPS on synaptic transmission.

Kai Johnson [1] • Dan Luo, PhD [1] • Judy Logan [1] • Jill Turner, PhD [1] • Thomas Prisinzano, PhD [1]
Pharmaceutical Sciences University of Kentucky College of Pharmacy [1]

Behavioral characterization of atoxifent, a functionally selective and potent synthetic mu agonist

Student

The opioid class of drugs are the strongest analgesics available and have been invaluable for the treatment of pain. Synthetic opioids, like meperidine and fentanyl, are typically easier to produce than semi-synthetics and can be more potent analgesics. Unfortunately, while these synthetics have increased analgesic efficacy, they also have the potential to elicit opioid side effects such as respiratory depression, constipation, addiction, and dependence. The current model of agonism recognizes drugs can signal different intracellular pathways, which underlie their effects on various physiological and behavioral changes after administered. My research characterizes atoxifent, a synthetic opioid which has less analgesia potency than fentanyl, but more than morphine, in mice evaluated in the hotplate test. This finding is expected given fentanyl and atoxifent have similar levels of *in vitro* G-protein signaling, a pathway involved in opioid induced analgesia. Other similarities between atoxifent and fentanyl include their reversibility by opioid antagonists and ability to increase locomotor activity. However, unlike fentanyl which robustly activates **β**-arrestin mediated signaling, atoxifent shows very little *in vitro* activity in a **β**-arrestin functional assay. Additionally, **β**-arrestin has been implicated in the unwanted side effects, most notably the development of tolerance to opioids. Interestingly, atoxifent has a significantly longer duration of action in mice. My next experiments will elucidate this difference as well as test opioid tolerance development and addiction liability by atoxifent, which could help clarify the role of **β**-arrestin *in vivo* and hopefully contribute to the development of a more efficacious opioid analgesic.

Jocelyn Martin [1] • Jakob Shaykin, MS [1] • Emily Denehy, MS [1] • Judy Songrady [2] • Dan Luo, PhD [2] • Warren Alilain, PhD [3] • Jill Turner, PhD [2] • Michael Bardo, PhD [1] • Thomas Prisinzano, PhD [2]
Psychology University of Kentucky [1] • Pharmaceutical Sciences University of Kentucky [2] • Spinal Cord and Brain Injury Research Center University of Kentucky [3]

Novel Compounds EO-139 and YZ-166 as Countermeasures for Reversing Opioid-Induced Antinociception, Motor Incapacitation and Respiratory Depression

Student

Purpose: Unlike morphine, fentanyl causes vocal cord closure and rigidity of the chest wall muscles, an effect known as “wooden chest syndrome”, and this effect may not be fully reversed by pure mu opioid antagonists (MOR) such as naloxone or naltrexone. This study assessed the ability of two novel compounds (EO-139 and YZ-166) to serve as MOR antagonists to reverse fentanyl analgesia, as well as reverse fentanyl-induced locomotor and respiratory depression.

Methods: For the hot plate assay, male and female F1 hybrid mice (n=31) were administered fentanyl (1 mg/kg; s.c.) and then either EO-139 or YZ-166 using a cumulative dosing procedure, with nociception measured by latency to paw lick on the hot plate. For locomotion and respiration, male and female Sprague-Dawley rats (n=43) were given saline or fentanyl (200 µg/kg; s.c.) 15 min prior to a second injection of one of the following: (1) vehicle, (2) naltrexone (0.003-0.1 mg/kg; s.c.), (3) EO-139 (0.0003–0.1 mg/kg; s.c.), or (4) YZ-166 (0.003-1 mg/kg; s.c.). Rats were immediately placed into a locomotor chamber for 15 min, followed by placement into a plethysmography chamber to record ventilatory effort for 30 minutes.

Results: As expected, with the hot plate assay, both EO-139 and YZ-166 dose-dependently reversed fentanyl-induced antinociception. Unlike YZ-166, EO-139 yielded notable sex differences in the dose required to produce 50% reversal (AD_{50}). With locomotion and respiration, naltrexone as the standard MOR antagonist produced a dose-dependent reversal of the locomotor and respiratory depressant effects of fentanyl. EO-139 and YZ-166 also reversed the respiratory depressant effects of fentanyl, but not fentanyl-induced locomotor depression within the dose ranges tested. Most notable, unlike naltrexone and EO-139, YZ-166 not only reversed fentanyl-induced respiratory depression, it stimulated respiration above baseline control, suggesting “supra-antagonism”.

Conclusion: This study provides evidence that EO-139 and YZ-166 attenuate opioid-induced antinociception and respiratory depression similar to naltrexone. Moreover, YZ-166 has a profile on respiratory depression that may offer a superior countermeasure agent against exposure to high-potency synthetic opioids.

Supported by: U01 DA051377

Stroke/Neurovascular

Madison Webster [1] • Amber Schifano [1] • Amanda Glueck, PhD [2]
College of Medicine University of Kentucky [1] • Neurology University of Kentucky [2]
Virtual Reality as a Tool for Rehabilitation in Stroke Patients: Literature Review
Student
Millions of patients are afflicted by strokes each year, and many of these patients are left with disabilities following the stroke. Rehabilitation is necessary to help patients regain function after stroke, and it is most beneficial when performed frequently and with active concentration. The combination of repetition and focus allows for synaptic rewiring to take place based on the principle of neuroplasticity. A limitation of traditional therapy is that patients often report boredom and poor compliance. One rehabilitation aid that has garnered positive outcomes in terms of compliance and health benefits is virtual reality. When added to traditional therapy, virtual reality is engaging and incorporates goal-oriented aspects of gaming leading to better compliance and engagement. To explore the benefits of virtual reality-based rehabilitation in post-stroke patients, a search was performed in the Academic Search Complete Database through UKY Libraries using keywords “virtual reality,” “rehabilitation,” “stroke,” and “compliance.” The results of this search yielded fifteen studies demonstrating the benefits of virtual reality for rehabilitation, including better compliance, increased motivation, and individualized training. Additionally, virtual reality can provide objective feedback that can provide patients with an objective measurement of their progress. Importantly, virtual reality rehabilitation demonstrated improvements in motor function, sometimes greater than traditional therapy. Furthermore, there is some evidence that patients who had reached a plateau during traditional therapy were able to achieve additional improvements with virtual reality therapy. However, technology in general, and virtual reality in particular, are often under-utilized in physical therapy. There is still more work to be done to determine why this is so, including research on barriers and facilitators for therapists recommending the use of virtual reality as treatment for stroke patients. In addition, more research is required to determine types of exercises and other elements of rehabilitation that can make it both effective and engaging for patients.

Kristin Drummond [1] • Moltira Promkan, PhD [2] • Pradoldej Sompol, PhD [2]
undefined University of Cincinnati [1] • Sanders-Brown Center on Aging University of Kentucky [2]

Rose Bengal photoinduction mediates capillary and astrocyte injury

Other

Cerebrovascular pathology is highly found in the brains of Alzheimer's disease (AD) and related dementias. Diabetes, hypertension, and obesity are the major causes of cerebrovascular pathology which is widely believed to exacerbate AD pathophysiology and complicate anti-AD therapeutic strategies. The study of microvascular pathology and function in AD has been limited, in part, because factors such as the magnitude, timing and localization of pathology are not well-controlled. Here, we develop a local oxidative stress-induced vascular damage technique that makes it easier to assess the real-time development of functional changes in the precise vicinity of vascular injury. The major advantage of this model is that vessel stalls, occlusion, and microhemorrhage can be followed in single capillary in living mice, in real time. Moreover, behavior of perivascular cells such as astrocytes, glia cells that maintain cerebrovascular integrity and brain function, at physiological and pathological conditions can be investigated

Min Jian Hou Ma, Other [1]
College of Medicine University of Kentucky [1]
Autoimmune and Hypercoaguable Biomarkers In Moyamoya Syndrome Patients
Student
AUTOIMMUNE AND HYPERCOAGUABLE BIOMARKERS IN MOYAMOYA SYNDROME PATIENTS

Min Jian Hou Ma[6,7], Christopher J McLouth[5,6], Laura Whitnel[1,6], David Dornbos III[1],

Justin F. Fraser[1,2,3,4,6],Jill Roberts[1,6]

Departments of Neurosurgery[1], Neurology[2], Neuroscience[3], Radiology[4], Biostatistics[5], Center for Advanced Translational Stroke Science[6], University of Kentucky College of Medicine[7], University of Kentucky, Lexington, KY

Moyamoya is a rare condition that involves stenosis of the intracranial internal carotid arteries (ICA) that leads to the formation of abnormal collateral arteries, both of which result in ischemic and hemorrhagic stroke. However, the etiology of moyamoya is currently unknown. The incidence of moyamoya syndrome in Kentucky is nearly 10 times the expected amount for the American population. This may be due to higher rates of inflammation, especially amongst females in comparison to males, as the majority of moyamoya patients are female and inflammation is thought to be a contributing factor. We have enrolled 28 patients into our Moyamoya And Stroke Tissue Evaluation and Repository (MASTER) study to learn more about this patient population and identify possible biomarkers for this condition. We hypothesize moyamoya syndrome patients have elevated levels of autoimmune and coagulable factors. Our patients were diagnosed and enrolled into our prospective study. Blood was collected and analyzed, and all patients were assigned a Suzuki score of pathological severity based on angiography. The median age of our subjects was 46, and 68% were female. Approximately 46% had bilateral pathology and 71% had a Suzuki score of 5-6 (most severe). From the data collected, we found that there were no significant differences between males and females in the autoimmune and coagulable factors tested. However, the average erythrocyte sedimentation rate (ESR) for females was above the normal value (20mm/hr.), and factor VIII levels in males were on average higher than the expected range (56-191%). While some of our patients exhibit high levels of markers such as ESR, Factor VIII, Protein S and Prothrombin time, this was not significantly related to increased Suzuki scores. In addition, 31.3% of our females and 37.5% of the males showed a speckled ANA test. These data provide valuable information to help understand the etiology of moyamoya and assist in finding future biomarkers and potential therapeutics.

Thomas Ujas [1] • Jadwiga Turchan-Cholewo, PhD [2] • Kevin Hatton, MD [3] • Ann Stowe, PhD [2]
Neuroscience University of Kentucky [1] • Neurology University of Kentucky [2] • Anesthesiology University of Kentucky [3]

Unveiling Unique Cell Populations in Cerebrospinal Fluid of Subarachnoid Hemorrhage Patients using UMAP and Comparative Analysis with Peripheral Blood

Student

Background:

We used flow cytometry to identify an active presence of immune cells in cerebrospinal fluid (CSF) of patients with aneurysmal subarachnoid hemorrhage (aSAH). These cell populations were compared with those found in the systemic blood of the patients. The purpose of this study was to identify novel leukocytes using an un-biased algorithm that self-identifies unique populations through clustering in high-dimensional space.

Methods:

Data was gathered from aSAH patients (n = 12), of which eight had an external ventricular drain (EVD) placed and CSF samples (n = 29) were obtained at days 3, 5, 7, and 10 post-aSAH. Additionally, blood samples (n = 37) were obtained from these patients at days 3, 5, 7, 10, and 14 to compare immune populations between the CSF and blood. All samples were processed and stained using a general immunophenotyping panel. Additionally, blood samples were analyzed using a second more specific B and T cell panel. Cell populations were identified using phenotypic characteristics in FlowJo.v10. All CD45+ live cells were analyzed using uniform manifold approximation projection (UMAP). Then FlowSOM ran an unsupervised clustering algorithm that serves as a visualization aid to gain insight on subpopulations.

Results:

Using our primary flow cytometry panel, unsupervised gating identified 8 main cell populations. Using a mixed-effects statistical model, we saw that CD8 T cells were significantly higher in the blood than CSF ($p = 0.0417$). Our second FACS panel showed a unique population of CD8+ CD154+ cells that remained low at day 3 but trended higher at days 5-14. CD19+ B cells populations decreased from day 3 to day 10 in the CSF ($p = 0.0381$), and were found at much lower levels in the CSF than blood ($p = 0.0044$). Our second FACS panel showed a unique CD19+ CD11c+ population that continued to increase through day 14. Additionally, our second panel identified a unique CD19+ CXCR5+ subset of B cells present in the blood samples. There were no differences in CD4 T cell populations between CSF and blood. Conclusions: Currently, we are looking at unique patient information to further identify trends in immune cells present following aSAH.

Keith Pennypacker, PhD [1]
Neurology University of Kentucky [1]

Differential Molecular Response to ELVO in Appalachian Subjects Treated with Mechanical Thrombectomy

Faculty

The Appalachian region of North America is a subpopulation of the country that has gathered significant attention due to its healthcare accessibility,, health disparities, and health outcomes. Individuals from Appalachia exhibit a higher likelihood of experiencing stroke-related comorbidities including diabetes, obesity, and increased tobacco usage. Moreover, rural patients in this region are less likely to receive thrombectomy treatment, leading to poorer clinical outcomes compared to their urban counterparts. The objective of this study was to identify inflammatory proteomic biomarkers of stroke specific to stroke patients residing in Appalachian counties.

Eighty-one subjects met inclusion criteria for this study and underwent mechanical thrombectomy for large vessel occlusion, and intracranial blood samples underwent proteomic analysis. Statistical analyses were employed to examine whether the relationship between protein expression and outcomes differed by Appalachian status for post-stroke function (NIH Stroke Scale; NIHSS and Modified Rankin Score; mRS), cognition (Montreal Cognitive Assessement; MoCA) and mortality.

No significant differences were found in demographic data nor co-morbidities when comparing Appalachia to non-Appalachia subjects. Last know normal (time of stroke till treatment; LKN) was significantly longer with patients from Appalachia. Significant differences occurred in NIHSS, MoCA and mRS scores at discharge and infarct and edema volumes between the two populations. Comprehensive analysis of 184 cardiometabolic and inflammatory proteins revealed seven proteins were predictive of function and 14 predictive of cognition. Another six proteins were associated with mRS and 7 proteins related to mortality. All these proteins were differentially correlated with these functions dependent on whether the patient was from Appalachia or non-Appalachian county.

Our study utilizes an ELVO tissue bank and registry to investigate the proteomic environment occurring at time of thrombectomy. We found that patients presenting from Appalachia-associated areas have a different proteomic response at the time of MT when compared to patients presenting from non-Appalachia areas. These differentially expressed proteins relate to stroke outcome and could be used as prognostic biomarkers, or as targets for novel therapies. Lastly, the identification of a disparate proteomic response in Appalachian patients suggests a plausible connection with environmental exposures. Nevertheless, further investigations through community-based studies are imperative to elucidate the underlying causes of this differential response.

Sophiya Sims [1] • Sami Case, PhD [2] • Ruei-Lung Lin, MS [1] • Nicholas Wright [1] • Leopoldine Galopin [1] • Jacquelyn Rhinehart [1] • Olivier Thibault, PhD [1]
Pharmacology University of Kentucky [1] • Biomedical Sciences Colorado State University [2]

Addressing Sensitivity to Insulin and Glucose in Neurons and Astrocytes Using PercevalHR

Student

Brain homeostatic equilibrium is a well-maintained and orchestrated metabolic process which, when lost, is associated with brain aging or neurodegenerative diseases, and is often detected as hypometabolism in aging and AD. It is therefore, integral to maintain this homeostasis on a second-to-second basis and across multiple cell types. Major energetic processes can be addressed through the measurement of ATP levels within a single cell. In addition to the well-established role of glucose metabolism in the CNS, more recently, insulin has also been recognized to play an essential role in the regulation of cognitive function, particularly in the hippocampus, where it can ameliorate spatial memory recall. Using mixed primary hippocampal cultures (neurons and astrocytes), we tested the hypothesis that PercevalHR, an ATP:ADP biosensor, could reliably quantify bioenergetics with single cell resolution. Embryonic rat hippocampi (E17) were extracted and maintained for 12-16 days *in vitro* (DIV). Cultures were treated with lentivirus (Human Ubiquitin C promoter) containing the PercevalHR nanosensor.

To control for PercevalHR's pH sensitivity, some experiments were conducted concomitantly with the intracellular pH sensor pHrodo. We attempted to normalize glucose transporter function following ~12 days in high glucose concentration (30 mM), by returning the cells to a serum-free 5.5 mM glucose media ~24 h prior to imaging. PercevalHR emission was filtered at 525 nm and pHrodo emission at 580 nm. After 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 resulted in rapid decreases in ATP:ADP ratios. Insulin only demonstrated a slight drop in ATP:ADP. PercevalHR seems to reliably report on cell energetics in mammalian cultures and surprisingly, appears to indicate that neurons display higher baseline ATP:ADP compared to astrocytes. These data help evaluate bioenergetic status in two closely associated cell types that are known to share intermediates. Ongoing studies are investigating PercevalHR imaging in astrocytes using *in vivo* 2P microscopy in a mouse model of amyloidosis during ambulation (i.e., awake).

Noah Leibold, Other [1] • Gopal Velmurugan Viswanathan, PhD [1] • Laura Radulescu, Other [1] • Deepak Kotiya, PhD [1] • Nirmal Verma, PhD [1] • Florin Despa, PhD [1]
Dept. of Pharmacology & Nutritional Sciences University of Kentucky [1]

APOE4 PROMOTES ACCUMULATION OF AMYLOID-FORMING PANCREATIC AMYLIN IN BRAIN PARENCHYMA THROUGH A BLOOD-BRAIN BARRIER MEDIATED MECHANISM

Student

Aims. We tested the hypothesis that ApoE4 genotype augments the deposition of amyloid-forming amylin in brain parenchyma and microvessels in a humanized ApoE4-human amylin mouse model.

Methods. Based on the innate biochemical differences between human amylin (amyloidogenic) and rodent amylin (non-amyloidogenic), we generated humanized mouse models expressing human amylin in the pancreas along with human ApoE4 or ApoE3 protein in the brain and peripheral tissues (ApoE4HIP and ApoE3HIP mice). Male mice, 6 months old, were tested for brain function using novel object recognition and open field tests followed by endpoint biochemical analyses of amylin levels in isolated brain microvessel lysates and brain tissue homogenates using ELISA. ThioflavinS staining was used to visualize pancreatic amyloid burden. GFAP and amylin colocalization in the brain microvasculature was quantified using IHC.

Results. Brain parenchyma, but not microvessels, from ApoE4HIP animals had significantly more amylin than those in ApoE3HIP. Pancreatic amylin immunoreactivity signal intensity was significantly increased in ApoE4HIP vs. ApoE3HIP mice. IHC analysis of brain tissue revealed significantly increased GFAP and amylin immunoreactivity signal intensity in ApoE4HIP brains. Behavior deficits were increased in ApoE4HIP compared to those in ApoE3 HIP mice.

Conclusions. Our data suggest that amyloid-forming pancreatic amylin interacts with parenchymal ApoE and exacerbates amyloid pathology differentially in ApoE4HIP vs. ApoE3HIP mice. Increased astrogliosis in ApoE4HIP mice may suggest neurodegenerative injury as a result of increased parenchymal amylin accumulation. Additionally, elevated amylin amyloid burden may underlie the neurological deficits apparent in ApoE4HIP mice. Our data suggest a role of ApoE4 in the increased amylin-induced BBB dysfunction and brain amylin amyloid accumulation. Further studies to delineate mechanisms by which amylin and ApoE interact at the BBB and promote parenchymal amylin amyloid deposition are necessary.

Nirmal Verma, PhD [1] • Gopal Viswanathan Velmurugan [2] • Edric Winford [2] • Han Coburn [2] • Deepak Kotiya [2] • Noah Leibold [2] • Laura Radulescu [2] • Sanda Despa [2] • Kuey C. Chen [2] • Linda J. Van Eldik [2] • Peter T. Nelson [2] • Donna M. Wilcock [2] • Gregory A. Jicha [2] • Ann M. Stowe [2] • Larry B. Goldstein [2] • David K. Powel [2] • Jeffrey H. Walton [2] • Manuel F. Navedo [2] • Matthew A. Nystoriak [2] • Andrew J. Murray [2] • Geert Jan Biessels [2] • Claire Troakes [2] • Henrik Zetterberg [2] • John Hardy [2] • Tammaryn Lashley [2] • Florin Despa [2]

Pharmacology and Nutritional Sciences University of Kentucky [1] • undefined undefined [2]

Aβ efflux impairment and inflammation linked to cerebrovascular accumulation of amyloid-forming amylin secreted from pancreas

Faculty

Impairment of vascular pathways of cerebral **β**-amyloid (A**β**) elimination contributes to Alzheimer's disease (AD). Vascular damage is commonly associated with diabetes. Here we show in human tissues and AD-model rats that bloodborne islet amyloid polypeptide (amylin) secreted from the pancreas perturbs cerebral A**β** clearance. Blood amylin concentrations are higher in AD than in cognitively unaffected persons. Amyloid-forming amylin accumulates in circulating monocytes and co-deposits with A**β** within the brain microvasculature, possibly involving inflammation. In rats, pancreatic expression of amyloid-forming human amylin indeed induces cerebrovascular inflammation and amylin-A**β** co-deposits. LRP1-mediated A**β** transport across the blood-brain barrier and A**β** clearance through interstitial fluid drainage along vascular walls are impaired, as indicated by A**β** deposition in perivascular spaces. At the molecular level, cerebrovascular amylin deposits alter immune and hypoxia-related brain gene expression. These converging data from humans and laboratory animals suggest that altering bloodborne amylin could potentially reduce cerebrovascular amylin deposits and A**β** pathology.

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

In vivo downregulation of pancreatic amylin improves brain function and reduces brain β-amyloid pathology in APP/PS1 mice

Staff

Background:

Amylin is a systemic hormone that is co-secreted with insulin from pancreatic **β**-cells. Amylin co-aggregates with brain parenchymal and vascular **β**-amyloid in persons with Alzheimer's dementia. Cross-sectional data show higher CSF and blood amylin levels are associated with increased frequency of cognitive impairment. The present study sought to determine how *in vivo* downregulation of amylin influences brain function during the development of A**β** pathology.

Method:

Because mouse amylin is nonamyloidogenic, we developed APP/PS1 mouse model in which the mouse amylin gene is replaced by the human amylin gene and is conditionally i) upregulated and ii) downregulated by tamoxifen (TMX) injection, intraperitoneally. At 3 months of age, male mice were randomly assigned to either amylin downregulation (maintained amylin expression) or upregulation group (no amylin expression) (n=10/group). Two months later, we assessed brain function with the novel object recognition test and performed comparative immunochemical A**β** analyses of hippocampal tissue by using MSD ELISA and immunohistochemistry (IHC). We measured the level of of amylin and proinflammatory cytokines in plasma and in brain.

Result:

Mice with downregulated human amylin show enhanced recognition memory index ($p < 0.001$) and lower plasma/brain amylin levels ($p < 0.001$) along with lower proinflmmatory cytokines level ($p < 0.05$) compared to those that continued to express human amylin. This was associated with decreased hippocampal levels of A**β**42 ($p < 0.05$) measured by MSD ELISA and decreased number of plaques ($p < 0.01$) measured by IHC. The opposite results were observed in the reverse model of upregulating human amylin as compared with the mice expressing no amylin.

Conclusion:

Amylin downregulation in APP/PS1 mice improves memory. Molecular processes associated with improved memory involved decreased hippocampal A**β** pathology and decreased level of proinflammatory cytokines. Further studies are needed to understand how altered secretion of pancreatic amylin may influence the balance between brain A**β** accumulation and A**β** elimination from the brain.

Acknowledgments: Funding

NIH: Programming amylin secretion to slow brain aging - an animal model (5R01AG057290-03)

NIH: Role of Systemic Amylin Dyshomeostasis in Alzheimer's Disease (5R01AG053999-03)

Research Alliance for Reducing Diabetic Microvascular Dysfunction