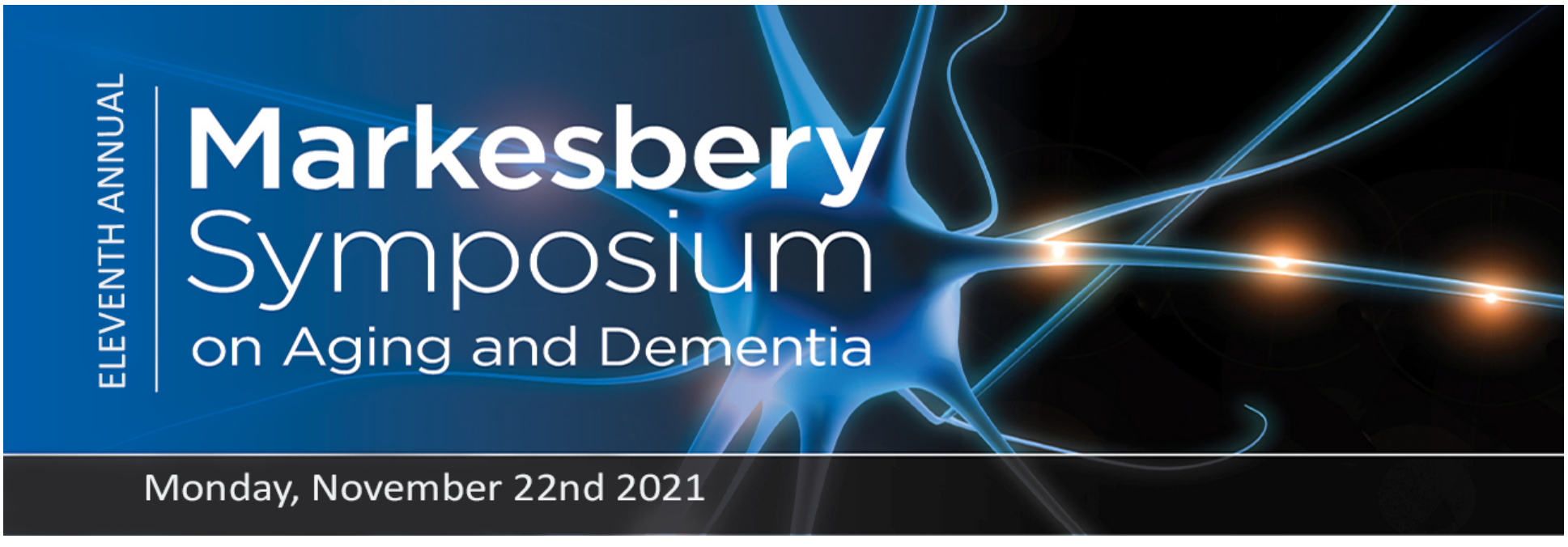
Markesbery Symposium on Aging and Dementia

Scientific & Poster Session

November 22, 2021

Sanders-Brown Center on Aging



Register for this program at https://tinyurl.com/2021-register-scientific

8:00 am	Check-in begins: Receive poster assignments. Poster presenters must have posters displayed by 8:30am.	*Lee T. Todd Lobby*
8:30 am	**Symposium Welcome** Linda Van Eldik, PhD, Director, Sanders-Brown Center on Aging and University of Kentucky ADRC **Tribute to William R. Markesbery, MD** Susanne M. Arnold, MD. Associate Director Markey Cancer Center	*Lee T. Todd Auditorium 124*
8:45 am	**35 Years of the University of Kentucky Alzheimer's DiseaseCenter: Past Discoveries and Future Directions** Frederick A. Schmitt, PhD. Professor of Neurology and Director of Outreach, Recruitment and Engagement (ORE) Core, ADRC	*Lee T. Todd Auditorium 124*
9:30am	Break	*Lee T. Todd Lobby*
9:45 am	**Investigating the role of hypusinated eIF5A in AD and relatedTDP-43 proteinopathy disorders** Maj-Linda Selenica, PhD. Assistant Professor of Molecular and Cellular Biochemistry and Sanders-Brown Center on Aging	*Lee T. Todd Auditorium 124*
	Diversity and susceptibility of microglial responses in a mousemodel of mixed etiology dementia Josh Morganti, PhD. Assistant Professor of Neuroscience and Sanders-Brown Center on Aging	
10:15 am	**Trainee Feature** Top 6 abstracts each present 8-minute talks.	*Lee T. Todd Auditorium 124*
11:15 am	**Poster Session**	*Lee T. Todd Lobby*
12:30 pm	**Boxed lunch upon departure**	*Lee T. Todd Lobby*

Keynote Speaker

35 Years of the University of Kentucky Alzheimer's Disease Center: Past Discoveries and Future Directions

FREDERICK A. SCHMITT PHD
Professor and Outreach, Recruitment, and Education Core Leader
Neurology, Sanders-Brown Center on Aging

Dr. Frederick A. Schmitt is a Professor of Neurology and Sanders-Brown Center on Aging, and holds joint appointments in the Departments of Psychiatry, Psychology, Neurosurgery, and Behavioral Science. He is also an Associate of the Spinal Cord and Brain Injury Research Center. He is a clinical scientist with a primary focus on the evaluation of the relationships between physiological changes associated with normal aging, Down syndrome, and dementia associated with neurodegenerative diseases. Dr. Schmitt has been an investigator on multiple multidisciplinary program project grants and the Alzheimer's Disease Research Center, where he currently leads the Outreach, Recruitment, and Engagement (ORE) Core. He also led the largest Alzheimer's prevention trial to date with Drs. Kryscio and Markesbery entitled PREADVISE. He has coordinated multiple studies of experimental therapies for early- stage cognitive impairment, and Alzheimer's disease. Dr. Schmitt's work currently examines age-related changes in individuals with Down syndrome, as well as developing cognitive measures sensitive for vascular cognitive impairment and dementia.

Faculty Presentations

JOSH MORGANTI, PHD
Assistant Professor, Neuroscience and Sanders-Brown Center on Aging

Dr. Morganti is an assistant professor in Sanders-Brown Center on Aging and the Department of Neuroscience at the University of Kentucky. His research interests focus primarily on neuroimmune interactions that may underly the susceptibility of the aging brain to acquire degenerative pathophysiology. Currently, his laboratory is funded by the NINDS and NIA to examine how inflammatory transcription factors regulate cross-talk between microglia and astrocytes to regulate neurodegenerative cascades in mouse models recapitulating aspects of Alzheimer's disease, mixed vascular dementia, and traumatic brain injury.

MAJ-LINDA SELENICA, PHD
Assistant Professor, Molecular & Cellular Biochemistry and Sanders-Brown Center on Aging

Dr. Selenica joined the Center on Aging in 2019, where her independent research is focused on molecular pathways implicated in the multi-etiology of AD and related TDP-43 proteinopathy. She has established several cellular and animal models, antibodies, and molecular tools to study the impact of TDP-43 pathology in processes such as neuropathology, neuroinflammation, blood-brain barrier, and brain metabolism. Her research has successfully led to better understanding of the role of multiple misfolded proteins in neuronal toxicity; amyloid beta, alpha-synuclein, tau, and TDP-43. Other important contributions include investigating the impact of neuroinflammation in AD and related dementia pathogenesis. She has been successful in receiving support from several private and NIH agencies for her research.

ABSTRACT TABLE OF CONTENTS

Trainee Short Talks

Posters

Trainee Short Talk #1

Inflammation after mild traumatic brain injury is altered by interleukin-1 signaling

Colleen Bodnar, Emma Higgins, Kelly Roberts, Adam Bachstetter, PhD
University of Kentucky, Department of Neuroscience
Student

Objectives: Inflammation is one of the driving pathologies following mild traumatic brain injury. Interleukin-1 is a major pro-inflammatory cytokine that drives many immune responses in the brain after injury. The receptor for IL-1 in the brain, IL-1 receptor 1 (IL-1R1) is mainly expressed in endothelial cells. Along with inflammatory pathology, endothelial cells are dysregulated after injury and are a major focus of research. Thus, we wanted to investigate the role of IL-1 in the brain and endothelial cells after traumatic brain injury.

Methods: To do this, we used two different genetic manipulations of IL-1R1. First, we used a conditional global knockout of IL-1R1 (gKO). The second was a constitutive global knockout of IL-1R1 but cre-expression was used to conditionally restore IL-1R1 expression specifically in endothelial cells (eRestore). Expression of IL-1R1 was either reduced (gKO) or restored (eRestore) by driving cre-expression at 3 months of age with tamoxifen injections. Animals were injured at 4 months of age and then sacrificed at 9, 24, or 72 hours after injury. RNA was extracted and analyzed for 46 inflammatory related genes using qPCR.

Results: We found that our mild traumatic brain injury caused alterations in inflammatory genes in wild type animals, increasing many inflammatory markers. This was most profound at 9 hours after injury. IL-1R1 gKO changed this inflammatory pattern, reducing many inflammatory markers, especially at 9 hours after injury. In mice which only expressed IL-1R1 in endothelial cells (eRestore) inflammatory gene expression was similar to that of the gKO animals. This was true at all timepoints after injury.

Conclusions: Interleukin-1 receptor signaling is essential for the inflammatory response after injury. While endothelial cells do not seem to be the sole cell type driving this response, they are important in the IL-1 signaling pathway in the brain. Future work will be done to investigate further into endothelial cells and other cell types following injury.

Trainee Short Talk #2

The effect of APOE on lipid droplet dynamics in microglia

Nicholas Devanney [1], Elizabeth Allenger [1], Ana Maria Cornea [2], Katy Smith [1], Scott Gordon, PhD [3], Lance Johnson, PhD [1] Department of Physiology, University of Kentucky [1], Paul Laurence Dunbar High School [2], Department of Physiology, University of Kentucky [3]
Student

Objectives: The microglial immune response is now recognized as a significant contributor to Alzheimer's disease (AD) pathophysiology and neurodegeneration. AD brains and aged microglia have been characterized to accumulate lipid droplets (LDs), intracellular lipid storage organelles which house anti-microbial proteins on their surface, sequester cytotoxic compounds, and serve asa hub for the innate immune response. The E4 allele of Apolipoprotein E (*APOE*) is the strongest genetic risk factor for late-onset AD, and is associated with both heightened neuroinflammation and increased formation of LDs. We hypothesize that E4 microglia have increased basal LD formation as well as a higher capacity to form LDs under stress, thereby resulting in greater pro-inflammatory cytokine production. To investigate this hypothesis, we quantified LD formation in primary microglia and characterized the LD proteome of control and lipopolysaccharide (LPS) stimulated E3 and E4 mice.

Methods: Primary microglia were isolated from mice expressing human E3 or E4. Cells were plated on poly-L-lysine coated coverslips and exposed to various conditions for a 24-hour period. Control wells received DMEM/F12 1:1 supplemented with L929 conditioned media. Microglia were exposed to i) 250uM oleic acid, ii) 10ug/mL LPS, iii) dead N2A cells at a 5:1 ratio to microglia, and various combinations of i-iii. E3 and E4 expressing mice were injected with saline or LPS (5mg/kg) and perfused after lethal injection at 24h. ~400mg of liver tissue was extracted, dounced in homogenization buffer, and the LD enriched supernatant fraction was collected after centrifugation and sent for TMT quantitative proteomic analysis.

Results: Compared to E3, primary microglia from E4 mice accumulated more lipid droplets at baseline, with exogenous OA, LPS stimulation, and dead-neurons (E3 v E4 baseline $p = 0.0317$; LPS $p = 0.0032$; OA $p = 0.0277$; N2A $p = 0.0192$). Western blots on LD fractions confirm LD enrichment by surface protein, PLIN2, along with increased expression of PLIN2 (i.e. more LDs) in E4 LPS treated mice. Proteomics analysis revealed that LD fractions from E4 mice are enriched for proteins involved in innate immunity compared to E3 LDs.

Conclusion: E4 microglia accumulate lipid droplets in greater quantities compared to E3 microglia in all conditions tested. Preliminary analysis of the LD proteome supports our hypothesis that E4 is associated with increased expression of innate immune proteins. Increased lipid droplet formation in non-aged, non-diseased E4 cells may suggest preclinical dysfunction associated with the E4 risk allele. A better understanding of lipid droplet dynamics within these cells and their functional implications may provide novel targets to help improve E4-related outcomes.

Funding: The abstract presenter is supported by a diversity supplement from the National Institute on Aging (CMF –R01AG062550-03S1).

Trainee Short Talk #3

Assessing and resolving the enigmatic role Complement Receptor 1 (CR1) plays in Alzheimer's disease

Sabrina M. Krause[1,2], J. Anthony Brandon[1,2], Kayla A. Nations[1,2], Patricia H. Doyle[1,2], Erik D. Huckvale[1,2], Elizabeth L. Vance [1,2], Madeline L. Page [1,2], Matthew Hodgman[1,2], Bernardo Aguzzoli Heberle[1,2], Tanner D. Jensen[3], Justin B. Miller[1,2,4], John D. Fryer[5,6], Mark T.W. Ebbert[1,2,4]

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Student

Complement receptor 1 (*CR1*) was implicated as a top Alzheimer's Disease (AD) risk factor in 2009, yet its role in AD remains an enigma. *CR1* encodes a transmembrane, regulatory protein with an extracellular binding domain that binds complement cascade proteins (C3b, C4b, and iC3b), and is thought to improve Aβ clearance. Oddly, while *CR1* RNA and proteins are highly expressed in various blood cells, *CR1* RNA is not expressed in brain tissue at a meaningful level, making it difficult to understand *CR1*'s involvement in AD. A study by Crehan et al. suggests CR1-homologue proteins (Cr2) are present on rat microglia, and that blocking Cr2 significantly decreases microglial phagocytosis of $A\beta_{42}$. A major challenge here, however, is whether murine *Cr2* in fact encodes a valid human *CR1* homologue.

Adding to the peculiarity of *CR1* involvement in AD, the gene has been genetically linked as a potential loss-of-function risk gene, yet certain apparent gain-of-function variants are also associated with AD risk. Specifically, CR1 variable C3b/C4b binding domains in the population, where more binding domains are associated with increased AD risk. Furthermore, we recently demonstrated the *CR1* binding domains are "camouflaged," meaning they are entirely overlooked by standard short-read sequencing methods and likely have undiscovered DNA variants. In fact, by rescuing mutations from these regions in *CR1* in the Alzheimer's Disease Sequencing Project (ADSP), we identified a rare 10-nucletotide loss-of-function frameshift deletion that could be involved in disease.

In short, significant evidence suggests *CR1* plays a major role in AD pathogenesis, yet exactly how *CR1* is involved remains an enigma, demonstrating the importance of research into *CR1*'s involvement. We propose a future study to clarify *CR1*'s apparent enigmatic role in AD. Using a combination of human tissue, cell lines, and murine models, our primary goals are to: (1) determine whether CR1 proteins are present in human brain tissue, and assess function in the brain; and (2) experimentally modify the binding domain by increasing, decreasing, and mutating the region, including the 10 bp frameshift to test its effects on AD phenotypes. We hope these experiments clarify the enigmatic nature of CR1's involvement in AD.

Trainee Short Talk #4

Enlarged perivascular spaces in the centrum semiovale are related to MoCA scores among older adults

Timothy Libecap [1], Beatriz Rodolpho, MS[1], Flavius Raslau, MD [2], Brian Gold, PhD [1]

Neuroscience, University of Kentucky [1], Radiology, University of Kentucky [2]

Student

Objectives: Cerebral small vessel disease (cSVD) is an important risk factor leading to the development of vascular contributions to cognitive impairment and dementia (VCID). cSVD is characterized by several in-vivo neuroimaging biomarkers including enlarged perivascular spaces (ePVS). PVS are fluid-filled spaces believed to play a role in the glymphatic system's removal of waste from the brain. Reduced clearance may cause backup and enlargement of the PVS and subsequent accumulation of toxic solutes characteristicof neurodegeneration, including Aβ. Evidence supports ePVS role in aging and dementia, but the relationship between ePVS and cognitive function remains unclear**.** Due to their presence in brain regions that support cognition, we hypothesized that quantitative, cross-sectional ePVS counts in older adults would predict baseline cognitive performance.

Methods/Results: We explored the relationship between ePVS and the Montreal Cognitive Assessment (MoCA) in 112 older adults ranging in age from 60-86. Participants were scanned on a 3T Siemens Prisma scanner with a 64-channel head coil. All ePVS counts were performed on T1 MPRAGE and T2 FLAIR images by an experienced rater blinded to participant demographics and under the direction of a trained neuroradiologist. In line with previously established guidelines, ePVS were defined as regions of hypointensity less than 3mm in diameter on T1 imaging and were distinguished from lacunar infarcts by the absence of T2 FLAIR hyperintensity. ePVS were individually and manually counted in a single, representative slice in the axial plane of the white matter centrum semiovale (CSePVS), basal ganglia, hippocampus, and midbrain. Linear regression analyses controlling for age, sex, intracranial volume, and education demonstrated a significant, negative relationship between total ePVS counts combined across the four regions of interest and MoCA score ($\beta = -0.353$, $P < 0.001$). The strongest relationship was found among CSePVS and MoCA ($\beta = -0.372$, $P < 0.001$)

Conclusions: These findings suggest that ePVS burden, driven primarily by CSePVS, is associated with cognitive performance at baseline. Ultimately, our results support the continued investigation of ePVS as an early neuroimaging biomarker of cSVD-related cognitive dysfunction. In particular, additional work that addresses the longitudinal relationship between ePVS and MoCA is needed to strengthen the findings that ePVS predict cognitive dysfunction.

Funding Sources: SBCoA TRIAD T32 AG057461, NIA R01 AG055449, NIA R01 AG068055, TL1 TR00199

Trainee Short Talk #5

Aβ pathology: a driver of blood-brain barrier leakage in Alzheimer's disease?

Geetika Nehra, PhD [1], Samantha Mullins [1], Bjoern Bauer, PhD [2,3]. Erin Abner, PhD [4,5], Peter Nelson, MD, PhD [6]. Sonya Anderson [1], Anika Hartz, PhD [7]

Sanders-Brown Center on Aging, University of Kentucky [1] • Pharmaceutical Sciences, College of Pharmacy University of Kentucky [2] • Department of Pharmaceutical Sciences, College of Pharmacy University of Kentucky [3] • Sanders-Brown Center on Aging,; Department of Epidemiology & Biostatistics, College of Public Health University of Kentucky [4] •Sanders-Brown Center on Aging,; Department of Epidemiology, College of Public Health; Department of Biostatistics, College of Public Health University of Kentucky [5] • Sanders-Brown Center on Aging; Department of Pathology, College of Medicine University of Kentucky [6] • Sanders-Brown Center on Aging; Department of Pharmacology and Nutritional Sciences, College of Medicine University of Kentucky [7]

Fellow

Objective: Blood-brain barrier leakage is an early pathological hallmark of Alzheimer's disease (AD). Recent studies link barrier leakage to neurodegeneration and cognitive decline but the driver of barrier leakage in AD remains unknown. In this study, we measured barrier leakage in tissue samples from AD patients and determined the relation between barrier leakage and Aβ pathology in5xFAD mice.

Methods: *Postmortem Study:* Samples from AD patients and cognitively normal individuals (CNI) with CAA pathology were used to measure S100β and claudin-5 levels in plasma by ELISA. Levels of both barrier leakage markers were correlated with age, diffused plaque (DP) scores, neuritic plaque (NP) scores, neurofibrillary tangle (NFT) scores, mini-mental state examination (MMSE) scores, and clinical dementia ratings (CDRs) that were provided by the UK-ADC Neuropathology. *In Vivo Study:* Wild type (WT) and 5xFAD mice were aged to 9, 12, and 16 months before they underwent cranial window surgery. Barrier leakage of fluorescent-labeled dextrans (3 kDa, 70 kDa) was visualized by in vivo two-photon imaging. Zen Intellesis software was used for machine-learning-based image analysis to determine the mean leakage index (extravascular-to-intravascular mean fluorescence intensity ratio). We also measured S100β, claudin-5, hAβ40, and hAβ42 levels in mouse plasma samples by ELISA.

Results: *Postmortem Study*: We found increased S100β levels (2.7-fold; p = 0.0497) in plasma samples from AD patients compared to CNI. S100β plasma levels correlate with NFT scores (r = 0.959, p = 0.001) and inversely correlate with age (r = -0.92, p = 0.001). We also detected 1.9-fold higher (p = 0.0198) claudin-5 levels in plasma samples from AD patients compared to CNI. Increased claudin-5 levels correlate with NP scores (r = 0.896, p = 0.006) and inversely correlate with DP scores (r = -0.859, p = 0.013). Our data indicate that the blood-brain barrier is leaky in AD patients and that barrier leakage markers detected in plasma samples from AD patients correlate with Aβ pathology. *In Vivo Study:* We found no signs of barrier leakage in 9-month-old 5xFAD mice, but 12- and 16-month-old 5xFAD mice had a 1.5-fold higher leakage index for 3 kDa dextran compared to age-matched WT mice. 16-month-old 5xFAD mice also had a 1.7-fold higher leakage index for 70 kDa dextran compared to age-matched WT mice indicating that leakage is age-dependent. Our data further indicate that the barrier is leaky to small molecules in mice at 12 months of age but becomes leaky for large molecules in mice at 16 months of age. We detected no significant differences in S100β and claudin-5 plasma levels across ages but there was an age-dependent trend of high plasma levels for both markers.

Conclusion: Our findings indicate that barrier Aβ pathology drives barrier leakage in AD patients and future studies are needed to discern the mechanism underlying barrier leakage in AD.

Funding: R01 AG039621; PI: Hartz

Trainee Short Talk #6

Utilization of gabapentin in older adults with different levels of cognitive status from 2006 to 2019

GYeon Oh, PhD [1], Daniela Moga, MD, PhD [1,2,3], Erin Abner, PhD [1,2,4]

[1]Sanders-Brown Center on Aging, University of Kentucky; [2]Department of Epidemiology, University of Kentucky; [3]Department of Pharmacy Practice and Science, University of Kentucky; [4]Department of Biostatistics, University of Kentucky

Fellow

Objectives: The off-label use of gabapentin has been increasing in the general population, and even in older adults, without reliable evidence of efficacy and safety profile. To our knowledge, the utilizations of gabapentin in older adults across different levels of cognitive status havenot been evaluated. Therefore, the objective of this study is to examine trends of gabapentin use among older adults with different levels of cognitive status and to assess the potential risks of using gabapentin inappropriately.

Methods/Results: Data were extracted from National Alzheimer's Coordinating Center (NACC) Uniform Data Set (2006-2019). We included participants age 65+ with any medications reported at the time of visit. Within each study year, we estimated the prevalence of gabapentin use among the participants who reported any medication use, both overall and within cognitively [normal cognition, mild cognitively impaired(including impaired other), and dementia] and demographically [age and sex] defined subgroups. Additionally, we assessed prevalence of concurrent use of gabapentin with contraindicated medication classes, including opioid, combined opioid and benzodiazepine, antidepressant, and antipsychotic. Overall, reported gabapentin use nearly tripled (2.2% to 6.1%) from 2006 to 2019. Further, prevalence increased over this period in every participant subgroup. Among gabapentin users, the concurrent use of gabapentin with antidepressants or antipsychotics was more common in participants with dementia than those with normal cognition throughout the study period. Over half of gabapentin users with dementia also reported use of antidepressants. The frequency of concurrent use of gabapentin and opioid medications (ranged from 10 to 30%) was also relatively common both overall and within every subgroup throughout the study period. By contrast, concurrent use of gabapentin, benzodiazepine, and opioid medications was relatively rare (below 8% in all groups) throughout the study period.

Conclusions: Given increasing use among older adults, including those with cognitive impairment, rigorous studies are needed to examine the safetyof gabapentin in this population, especially when using gabapentin with other central nervous system medications concurrently.

Funding: This study was supported by NIH T32 AG057461: "Training in Translational Research in Alzheimer's and Related Dementias (TRIAD).

Poster #1

The Polygenic Risk Score Knowledge Base: a centralized online repository for calculating and contextualizing polygenic risk scores

Madeline Page[1], Elizabeth Vance [1], Matthew Cloward [2], Ed Ringger [2], Louisa Dayton [2], Mark Ebbert, PhD [1] for the Alzheimer's Disease Neuroimaging Initiative [3], Justin Miller, PhD [1], John S. K. Kauwe, PhD [2]

Biomedical Informatics, University of Kentucky [1], Biology, Brigham Young University [2] • [3]

Staff

Objectives: Genome-wide association (GWA) studies identify correlation between genetic variants and phenotypes, and these findings can be used to calculate polygenic risk scores, which aggregate the genetic risk for disease across all associated loci compared to the general population. Tools such as PRSice2 and PLINK can be used to calculate polygenic risk scores but require users to provide the necessary GWA study data files for calculations. While there are copious amounts of GWA studies available, gathering the data for multiple studies can be time-consuming. Additionally, score contextualization against large cohorts is necessary to conceptualize the relative risk but requires access to genetic data from those cohorts and additional time and resources to calculate polygenic risk scores for each individual in the cohort. The purpose of this study was to create a tool for polygenic risk scores that facilitates the calculation of risk scores across many diseases and studies and allows for score contextualization against other populations.

Methods/Results: We developed a centralized polygenic risk score calculator, The Polygenic Risk Score Knowledge Base (https://prs.byu.edu), containing over 2,300 GWA studies from the NHGRI-EBI GWAS Catalog. Polygenic risk scores are calculated from user-uploaded data using various user-defined parameters across many diseases or studies. We report study-specific polygenic risk score percentiles across the U.K. Biobank, 1000 Genomes, and the Alzheimer's Disease Neuroimaging Initiative (ADNI) for users to employ for comparing and contextualizing their data. As proof of utility, we additionally identify elevated genetic risk for other diseases in ADNI cases.

Conclusions: Calculating the genetic predisposition for various diseases can help to identify potential correlations between seemingly unrelated diseases and provide additional avenues of study for disease research. Scores can be used to classify disease subtypes, stratify populations, influence clinical and personal disease interventions, and determine causal genetic relationships through Mendelian randomization studies. To facilitate these types of analyses, we introduce the first streamlined analysis tool and web interface to calculate and contextualize polygenic risk scores across various studies. Our centralized database enables users to quickly and easily calculate scores for multiple individuals across a comprehensive list of studies. We anticipate that the PRSKB will enable a wider adaptation of polygenic risk scores in disease research, leading to additional mechanisms for disease diagnosis, treatment, and prevention.

Poster #2

Age-related increases in neuronal Ca2+ networks are sensitive to insulin and L-VGCCs and reflect on locomotor stability

Sami Lin Case, Ruei-Lung Lin, Hilaree N. Frazier, Katie L. Anderson, Adam O. Ghoweri, Olivier Thibault
University of Kentucky, Department of Pharmacology and Nutritional Sciences
Student

Despite falls accounting for the greatest cause of injury-related morbidity and mortality among older adults, few strategies are available to prevent these occurrences. Interventions such as physical therapy and exercise appear capable of reducing fall frequency but are only moderately effective, and there are currently few pharmacologic therapies targeting these events. To characterize potential central mechanisms underlying age-dependent changes in locomotor stability, we tested for the presence of neuronal Ca2+ network dysregulation *in vivo* in the primary somatosensory cortex of young and aged Fisher 344 rats using single-cell resolution techniques and associated these findings with ambulatory performance. Compared to young, aged animals displayed decreased ambulatory speed and increased overall activity and connectivity of the network. In aged animals, intranasal insulin increased ambulatory speed as well as network synchronicity. In young animals, delivery of the L-VGCC modifier Bay-K 8644 altered network properties and enabled the development of the aged phenotype, reinforcing the role of L-VGCC-mediated Ca2+ dynamics in altering neuronal network properties.

These results suggest that Ca2+ dysregulation, often rooted in the hippocampus, may be generalizable to other areas, such as the somatosensory cortex, and engage modalities that are associated with locomotor stability. Further, given the safety profile of intranasal insulin in the clinic and the evidence presented here showing that this central dysregulation is sensitive to insulin, we suggest that these processes can be targeted to potentially increase motivation and coordination while also reducing fall frequency with age.

Significance Statement

The National Council on Aging has reported that falls are the leading cause of fatal injury among older adults, causing one older adult death every 19 minutes. Therefore, it is clear that investigations into the links between Ca2+ and gait dysregulation in S1 with age are vitally needed. The work presented here addresses the significant knowledge gap surrounding the central mechanisms of age-related gait deficits by focusing on the links between ambulatory behavior, neuronal Ca2+ network dynamics, and L-VGCC and insulin modulation within S1 in intact aging rats.

Poster #3

Microglial INPP5D isoform expression and Alzheimer's disease

Diana Zajac[1,2], James Simpson[1,2], Joshua Morganti[2,3], Steve Estus[1,2]

[1]Department of Physiology, College of Medicine, University of Kentucky, Lexington, KY; [2]Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY; [3]Department of Neuroscience, College of Medicine, University of Kentucky, Lexington, KY

Student

Background: *INPP5D* (Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1), the gene encoding SHIP1, contains single nucleotide polymorphisms (SNPs) that are strongly associated with Alzheimer's Disease (AD) risk. In the brain, *INPP5D* is expressed asseveral isoforms, mostly in microglia. Full-length SHIP1 is encoded by 27 exons, including an amino-terminal SH2 domain followed by the phosphatase domain. Truncated isoforms lacking the SH2 domain begin from internal transcription start sites. Whether these isoforms are relevant to the SNP's actions in AD is unclear. To better understand the function of *INPP5D* in the human brain, we investigated *INPP5D* isoform expression as a function of AD status, and AD-associated SNPs.

Methods: Single-cell RNAseq was performed on APP/PS1 mice to understand the relationship between *INPP5D* expression and microglial activation. The expression of microglial *INPP5D* isoforms was analyzed using quantitative polymerase chain reaction on RNA from AD and non-AD anterior cingulate human brain samples. Samples were genotyped for the AD-associated SNPs rs35349669 and rs10933431 using TaqMan SNP kits. Isoform expression results were analyzed as a function of microglial gene expression (ITGAM and AIF1), total *INPP5D* expression, AD status, and SNP status. In addition, reporter SNPs in the 5' UTR region were used to detect unequal allele expression in the two full-length isoforms of *INPP5D* as a function of AD SNP status.

Results: APP/PS1 mice showed an increased expression of disease-associated microglia compared to controls. *INPP5D* was expressed at equivalent levels in both homeostatic and disease-associated microglia. Expression of each *INPP5D* isoform correlated strongly with microglial gene expression and increased with AD neuropathology but did not show a significant association with AD SNPs. Sequencing of samples heterozygous for both AD SNPs provided preliminary evidence for unequal allele expression modulatedby SNP status.

Conclusion: In summary, *INPP5D* expression is uniform in both disease-associated and homeostatic microglia. Expression of *INPP5D* isoforms is increased with AD neuropathology. The mechanisms whereby AD genetics influence *INPP5D* expression is not clear, but the rs35349669 allele that increases AD risk may be associated with allelic expression. Our next step is to quantify the unequal expression using next generation sequencing.

Funding: RF1AG059717

Poster #4

Cell-specific APOE allele wwitching as a therapeutic approach for Alzheimer's disease: development of novel transgenic mouse models

Lesley Golden[1,2], Elizabeth Allenger[1,2], Katy Smith[1,2], Gabriela Hernandez[1,2], Jing Chen[1,4], Haining Zhu[1,4], Corinne Cherfils[5,6], Randa Khani Bittar[5,6], Dominique Bonnefort-Rousselot[5,6], Kumar Pandya[9], Josh Morganti[1,3,7], Scott Gordon[1,2,8], Lance A. Johnson[1,2,3]

(1) University of Kentucky, (2) Department of Physiology, (3) Sanders-Brown Center on Aging, (4) Department of Molecular and Cellular Biochemistry, (5) La Litie Salpetriere-Charles Foix University Hospital, (6) Department of Metabolic Biochemistry, (7) Department of Neuroscience, (8) Saha Cardiovascular Research Center, (9) University of North Carolina Chapel Hill

Student

Background: Individuals homozygous for the ε4 allele of Apolipoprotein E (*APOE*) face up to a 15-fold increase in Alzheimer's Disease (AD) risk. In comparison, those carrying two ε2 alleles have nearly a 99.6% reduction in risk. Given *APOE*'s strong risk profile and multitude of effects, directly targeting *APOE* itself has emerged as a promising therapeutic strategy. Studies have used viral overexpression and exogenous administration of *APOE2* as a promising therapeutic strategy for e4 carriers. However, *APOE* is expressed by multiple cell types from development through late life, meaning the continued presence of endogenous ApoE4 during *APOE*2 viral delivery could limit effects of the therapy. CRISPR-Cas9 technology has been used to successfully edit *APOE in vitro,* where iPSC-derived glia and neurons edited from ε4 to ε3 show pronounced transcriptional and phenotypic changes. However, whether the putatively beneficial effects of *APOE* switching holds true in *in vivo* models has yet to be determined.

Results: To overcome these limitations, we developed a novel transgenic mouse model, the *APOE* "switch mouse" (4S2). This model allows for inducible and cell-type specific transition from expression of ApoE4 to ApoE2. Preliminary data from proteomic, western blotting, and ELISA analyses confirms that the 4S2 mice synthesize a full-length human APOE4 identical to the ApoE protein synthesized by commonly used transgenic 'targeted-replacement' (TR) APOE4 mice. Physiological phenotyping, gene expression measures, immunohistochemistry, and proteomic analyses further show that following tamoxifen injection, the inducible switch successfully results in efficient (>98%) recombination and expression of human ApoE2 in various cell types of interest (dependent on the Cre promoter): i) all cells, ii) astrocytes and iii) microglia. Ongoing studies aim to determine whether cell-specific replacement of ApoE4 with ApoE2 will rescue E4 associated metabolic dysfunction, disease associated gene signatures, and AD pathology.

Conclusions: Our preliminary studies have leveraged this new mouse as a promising model to assess the feasibility and therapeutic window of replacing ApoE4 with ApoE2, along with any potential off-target effects. We hope that this new, exciting model will be a valuable resource for the AD/APOE research community.

Poster #5

Long-read sequencing: resolving genomic structural variation through new technologies

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Student

Objectives: Next-generation sequencing, or short-read sequencing has provided a remarkable improvement on DNA and RNA sequencing, but still faces complications. Short-read sequencing works by fragmenting DNA into millions of short segments. These fragments are like tiny puzzle pieces. To get them in the right order, a reference genome is used which is like the picture on the puzzle box. A problem with short-read sequencing is that in repetitive areas of the genome, or genes that have been duplicated or inverted, the short fragments arenot unique. Since multiple puzzle pieces appear identical, the algorithm cannot determine which fragment goes where. We call genes like this dark or camouflaged. CR1 is a dark gene that is one of the top five risk genes for Alzheimer's Disease. Some structural variants in CR1 cannot be detected by short-read sequencing, but can be detected by long-read sequencing.

Methods/Results: The Bionano Saphyr allows us to take images of an entire genome. Green fluorophores bind to specific sequences on the DNA. The DNA is imaged and provides us with an optical map of full chromosomes, telomere to telomere. The location of these fluorophores can be compared to a reference genome, allowing us to identify large structural variants in a person's genome that are overlooked by short-read sequencing.
Oxford Nanopore's PromethION can use the optical map created by Saphyr and sequence the structural variants it identified. This is not possible for short-read sequencing technologies if the gene of interest is a dark gene. The PromethION reads DNA fragments that are up to 2 million bases long compared to short-read sequences which uses fragments of about 200 bases. The longer fragments make for fewer puzzle pieces that are more unique, so structural variants in dark genes like CR1 can be detected.

Conclusions: Both the Saphyr and PromethION have been recently purchased by the Ebbert lab. With this equipment, we can conduct novel research that identifies structural variants in disease-causing genes that could not be studied with short-read sequencing. CR1 is a dark gene of major interest in our lab since it is a top 5 Alzheimer's Disease risk gene. We are in a unique position as we are one of the only labs that are using these long-read technologies to study dark genes related to Alzheimer's Disease. Using long-read sequencing technology, we hope to identify structural variants that can be detected before disease symptoms develop. Recent studies suggest that Alzheimer's pathology begins long before the first symptoms are noticed. Our goal is to use these findings to develop the first screening tool for Alzheimer's disease before symptom onset.

Poster #6

Protective Destruction: How synonymous variant rs2405442:T>C destroys a ramp sequence in PILRA, which protects against Alzheimer’s disease

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Faculty

Objectives: The Paired Immunoglobulin-like Type 2 Receptor Alpha (*PILRA*) gene recognizes specific O-glycosylated proteins on various cell types. *PILRA* also contains a ramp sequence in the reference gene and has previously been associated with Alzheimer’s disease through genome-wide association studies. Here, we describe how synonymous variant rs2405442:T>C, which is in high linkage disequilibrium with the genome-wide associated nonsynonymous variant *rs1859788*, might be driving the disease association by destroying the ramp sequence. Ramp sequences are gene regulatory regions that counterintuitively increase gene expression by slowing initial translation, which evenly spaces ribosomes and decreases downstream ribosomal collisions. We assessed the transcriptional and translational effects induced by *rs2405442:T>C* destroying a ramp sequence to better understand how ramp sequences might affect Alzheimer's disease.

Methods: Cultured Chinese hamster ovary (CHO) cells were transfected with plasmids to establish control (wild type) and mutant cells. mRNA and total protein were isolated 24 hours post-transfection. We established the transcriptional efficiency of mutant cells versus wild-type cells through four sets of three quantitative polymerase chain reaction (qPCR) replicates, normalized by the number of cells in each replicate. We then performed eight sets of four enzyme-linked immunoassay (ELISA) replicates on both the *PILRA* wild type and mutant, which were normalized to the total protein of each replicate. Total protein of each replicate was determined by bicinchoninic acid (BCA) assay.

Results: We found that mRNA levels in the mutant gene were significantly less expressed than the wild type (p-value=4.45 x 10-9). We also show a significant decrease in relative protein quantity in the *PILRA* mutant versus control (p=0.0130) as determined by ELISA.

Conclusions: Our results support our hypothesis that synonymous variant *rs2405442:T>C* directly impacts transcription and translational efficiency in *PILRA* without altering the protein product by destroying a ramp sequence. The destruction of a ramp sequence significantly lowers *PILRA* gene expression, which has been shown to be protective against Alzheimer's disease.
Therefore, we determined a likely mechanism for *rs2405442:T>C* association with Alzheimer's disease and propose that ramp sequences, in general, may be underappreciated regulatory regions that directly impact mRNA and protein levels.

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Poster #7

Alpha adaptins show isoform-specific association with neurofibrillary tangles in Alzheimer's disease

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Faculty

Aims: The heterotetrameric assembly protein complex 2 (AP-2) is a central hub for clathrin-dependent endocytosis. The AP-2 α-adaptin subunit has two major isoforms, encoded by two separate genes: *AP2A1* and *AP2A2*. Endocytosis has been implicated in the pathogenesis of neurodegenerative disease, and recent studies linked α-adaptins (gene variants, splicing defects, and altered expression) with late-onset Alzheimer's disease (LOAD) risk. Here, we used multiple antibodies to investigate α-adaptin isoforms and their localization in human brains.

Methods: The specificities of ten different α-adaptin antibodies were evaluated using immunoblots after human *AP2A1* and *AP2A2* plasmid transfection in cultured cells. Additional immunoblot analyses were then performed on protein homogenates from control and LOAD subjects. Formalin-fixed, paraffin-embedded brain sections from control and LOAD subjects were immunohistochemically stained, andimmunofluorescence experiments were performed for quantitation of colocalization with digital image analysis.

Results: Eight of the ten evaluated antibodies recognized transfected α-adaptin proteins on immunoblots. The α-adaptin subspecies were relatively uniformly expressed in five different human brain regions. The α-adaptins were present in the detergent-insoluble fraction fromcognitively impaired, but less so in control, brains. Immunohistochemical analyses showed colocalization of AP2A1 with tau pathology in LOAD brains. By contrast, AP2A2 colocalized with microglial cells.

Conclusions: These observations provide evidence of isoform-specific changes of α-adaptins in the brains of LOAD subjects. Antibodies that were verified to recognize AP2A1, but not AP2A2, labelled neurofibrillary tangles of LOAD patients. The findings extend our understanding of AP-2 proteins in the human brain in healthy and diseased states.

Poster #8

Does a mild closed head injury early in life have lasting neuropathological changes in an Alzheimer's disease-relevant mouse model?

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Student

A traumatic brain injury (TBI) is a well-established environmental risk factor for developing age-related neurodegenerative diseases, including Alzheimer's disease (AD). The strongest epidemiological evidence shows an association between moderate-to-severe TBI and increased risk for AD. However, it is unclear if a mild TBI early in life could also interact with AD-related pathology. Therefore, we hypothesized that a mild TBI caused by a closed head injury (CHI) in mice would lead to chronic gliosis that would interact with amyloid-beta plaques – one of the hallmark AD pathologies. To test our hypothesis, we used the APP/PS1 knock-in (KI) mice, which express the mutated human form of the gene for the amyloid precursor protein and presenilin 1. The KI or wild-type (WT) mice received a CHI at 4 months of age. At 10 months post-injury (14 months of age), we evaluated by histology the effect of the CHI on amyloid-beta plaques, microgliosis, and reactive astrogliosis. Across multiple brain regions, we found a primary effect of genotype, with the KI mice having more amyloid pathology and gliosis than the WT mice. However, in both the WT and the KI mice, we found no effect of the CHI. While additional endpoints are being evaluated to look for other possible injury effects, our results suggest that with sufficient time the effects of a mild CHI resolve and do not have a lasting impact on the brain.

Poster #9

The serotonin 2A receptor regulates synaptic plasticity of anterior cingulate cortex projecting claustrum neurons, impacting cocaine-induced cognitive flexibility deficits

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Objectives: The claustrum (CLA) has long been speculated to be fundamental in a variety of brain functions such as attention salience and cognitive flexibility. The CLA boasts being the most densely connected structure in the brain, emerging as a preeminent orchestrator of cortical neurotransmission. Receiving extensive serotonergic innervation, the CLA also has the highest density expression of the serotonin 2A receptor (5HT2AR), the main excitatory receptor subtype for serotonin (5HT), in the brain. Recently the CLA, 5HT and the 5HT2AR have all been implicated in facilitating cognitive flexibility, the ability to adapt behavior to environmental changes, though these relationships remain largely uninterrogated. Cognitive flexibility deficits are a prominent symptom of substance use research, as many drugs of abuse, including cocaine, may render users more likely to relapse to or less likely to abstain from continued substance use. In this project, we investigate the relationship between serotonergic transmission in CLA neurons projecting to anterior cingulate cortex (ACC), to establish synaptic and circuit mechanisms underlying cognitive deficits that develop from cocaine use.

Methods/Results: First, we observed the effects of cocaine and the potent 5HT releasing agent, MMAI, on rat cognitive flexibility performance using a set-shifting task. Both cocaine and MMAI had profound negative effects on task performance. Next, we used whole cell electrophysiological recordings to observe effects of 5HT on neuronal activity in CLA-ACC cells. CLA-ACC neurons were recorded in the presence of 5HT and the 5HT2AR antagonist, ketanserin. Spontaneous excitatory post-synaptic current (sEPSC) frequency and amplitude were analyzed, along with other membrane properties. 5HT caused a drastic inhibitory response. Significant decreases in sEPSC frequency and amplitude were observed in CLA-ACC neurons after application of 5HT, accompanied by decreased action potential firing rate and hyperpolarized resting membrane potential. Increases in sIPSC frequency and amplitude were also observed, indicating an increase in CLA GABAergic tone with 5HT exposure. Blockade of the 5HT2AR with ketanserin eliminated the synaptic effects of 5HT, suggesting a regulatory role of the 5HT2AR in claustrocortical signaling. Last, we observed spike-timing dependent plasticity (STDP) in CLA-ACC neurons, revealing anti-hebbian LTD that is reversed with $GABA_A$ receptor blockade. Future experiments will probe the specific effects of 5HT and 5HT2AR in the claustrum on long-term plasticity and cognitive flexibility following drug self-administration.

Conclusions: These findings provide the first physiological evidence that the large population of CLA-ACC neurons are under strong inhibitory control from 5HT and the 5HT2AR. Our behavioral data also confirms that disruption of serotonergic brain signaling can negatively affect cognitive flexibility performance.

Funding: NIH DA04041513

Poster #10

Using gold to improve mitochondrial bioenergetics after traumatic brain injury in mice

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Fellow

Objectives: Traumatic brain injury (TBI) is a serious health concern resulting from an initial bump, blow, or jolt to the head that impairs normal brain function and affects millions of people yearly in the US alone. There are currently no FDA approved treatments available most likely due to the complexity and heterogeneity of TBI. Mitochondrial dysfunction is one of the hallmarks of TBI and an enticing target for therapeutic intervention. Here we utilize the organometallic gold(III) compound, AuPhos-89, which has shown to increase mitochondrial Complex-I mediated respiration independent of ATP synthesis to improve mitochondrial bioenergetics, learning and memory, and histological outcomes after controlled cortical impact (CCI) in mice.

Methods/Results: One cohort of mice was divided into 3 groups (n=7) for bioenergetic measures: CCI/vehicle, CCI/AuPhos-89, and sham/vehicle. Mice received vehicle (1% DMSO + 10% kolliphor; i.p.) or AuPhos-89 (10mg/kg; i.p.) 15 minutes and 47 hours after surgery. Total mitochondria were isolated by differential centrifugation (DC) from the cortical injury epicenter and ipsilateral hippocampus 48 hours post-surgery. The results showed impaired cortical mitochondrial respiration in the vehicle-treated mice after CCI relative to sham, and no significant difference between the AuPhos-89-treated mice relative to sham. There were no significant injury effects seen in the hippocampi of either the vehicle- or AuPhos-89-treated groups relative to sham. Another cohort of mice was utilized for behavioral studies (n=6): CCI/vehicle and CCI/AuPhos-89. These mice were given treatment 15 minutes, 2 days, and 4 days post-surgery. The novel object recognition (NOR) assay was performed 1, 2, and 3 days post-surgery to assess memory recall in the injured mice. The results showed the AuPhos-89-treated mice had a recognition index score of ~60% while the vehicle-treated mice scored ~40% indicating poorer memory recall in the vehicle-treated mice. The mice were perfused with 4% paraformaldehyde 7 days post-surgery and brains were fixed, sliced, mounted and stained with cresyl violet to assess lesion volume, and white matter sparing. The results showed there were no improvements in cortical lesion volume or corpus callosum sparing in the AuPhos-89-treated mice compared to vehicle.

Conclusions: Though AuPhos-89 improved acute mitochondrial bioenergetics after CCI and mild improvements in NOR performance, it did not promote cortical or white matter sparing.

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Poster #11

Comparison of treatment patterns and disease impact between those with posttraumatic vs. idiopathic knee osteoarthritis

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Objectives: There is increasing evidence that the pathophysiological mechanisms underlying posttraumatic osteoarthritis (PTOA) may differ from those of primary knee OA. In addition, patients with end-stage PTOA demonstrate significantly worse post-operative outcomes following total knee arthroplasty when compared to those with primary OA. Anecdotally, those with PTOA are often offered similar treatments as patients with primary OA despite potentially different underlying mechanisms. The purpose of this study was to compare patient demographics, and the treatments utilized by PTOA and OA patients. We hypothesized that PTOA patients will be significantly younger and will have sought a greater number of knee treatments than those with primary OA.

Methods/Results: We prospectively collected and analyzed questionnaire data from 99 participants utilizing a Redcap survey through ResearchMatch, which is an organization funded by the NIH that allows people to list their medical history and demographic data and then contacted by prospective researchers. To qualify, participants had to be between 18 and 95 years old, have a self-reported physician-diagnosed knee osteoarthritis, and be a member of ResearchMatch. Incomplete surveys were excluded. Participants reporting a prior knee injury or surgery in their osteoarthritic knee were designated as the PTOA group while those not reporting injury orsurgery history were designated as the OA group. The survey included a detailed list of questions covering general demographic data, knee injury or surgery history, and a list of treatments the participants had utilized and the treatment characteristics.
Of the 99 eligible participants, 84 fully completed the survey and were included in the analyses. Of the 84 participants, 36 report that their knee OA was the result of a previous injury. The most common injuries were meniscus and ACL tears. Demographically, we found no differences. We did find that the total number of treatments each group utilized did not differ (PTOA = 5.3$\pm$3.1, OA=4.1$\pm$3.2, p=0.09).A greater percentage of the PTOA group utilized self-directed exercise (PTOA=69.4% vs. OA=37.5%, p=0.004), acetaminophen (PTOA=63.9% vs. OA=35.4%, p=0.01), and knee braces (PTOA=55.6% vs. OA=31.3%, p=0.025) than the OA group.

Conclusion: Overall, both groups utilized similar treatments. However, PTOA patients may be more amenable to utilizing self-directed treatments such as exercise and knee braces. As a result, these patients may be better suited for combination therapies that pair intra-articular treatments with exercise modification.

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Poster #12

Characterization of age-associated B cells in aged mice

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Objectives: Age-associated B cells (ABCs) have been extensively studied in autoimmune diseases such as multiple sclerosis & Lupus but not in terms of ischemic stroke or brain. ABCs accumulate with age & are stimulated by a combined activation of BCR & TLR7/9 plus IL-21 & IFN-γ. ABCs also have the ability to secrete potentially autoreactive antibodies. With my thesis project, I intend to characterize ABCs in the context of aged brain & periphery in mice that have undergone a transient middle cerebral artery occlusion (tMCAo). **I hypothesize that ABCs formed in the periphery migrate to the brain to promote detrimental neuroinflammation post-stroke, with aged females having a higher number of ABCs due to loss of estrogen.** Preliminary data is still being collected.

Methods: Subjects: Male and female C57BL/6 mice aged 22-24 months. Starting n=6 per group, with sham, healthy, & post-tMCAo groups. The same experiments will be performed on human post-stroke blood samples. tMCAo: This surgery creates a unilateral ischemic stroke (blocking blood flow by 80%), followed by reperfusion of blood back to 60% baseline. A suture is tied around the common carotid artery (CCA) to occlude blood flow followed by a threading of a silicon-tipped filament into the CCA to the middle cerebral artery (MCA) origin for occlusion for 60 minutes. The suture is removed & reperfusion measured. Permanent strokes (no reperfusion) or early reperfusion (back to 100% baseline) will not be included based on laser doppler flowmetry. A successful reperfusion is considered a return to at least 50% of baseline blood flow. Histology: I will perfuse, fix with paraformaldehyde, & section brains to a thickness of 30μm. Cortical, hippocampal, & cerebellar floating sections will be stained for CD19, T-Bet, and DAPI to identify ABCs. Immunocytochemistry will be used on cytospins of isolated B cells to stain ABCs & other B cell migration markers. Flow Cytometry: FACS will be utilized to quantify ABC populations using both extracellular and intracellular antibody panels. Brains, spleens, & cervical lymph nodes will be processed. Proliferation dyes will be used to measure ABC activity. ABC Stimulation & Culture: *Ex vivo* stimulation of ABCs in culture with post-stroke blood plasma will take place to ascertain binding specificities of BCR, TLR7, & TLR9 as well as the secretion of various antibodies & pro- & anti-inflammatory cytokines. Receptors will be blocked using antagonists. Cytokine & antibody secretion will be measured via sandwich ELISA. Behavior: Motor & cognitive tests such as Catwalk & operant chambers will be used to measure functional recovery over a 6wk time period following tMCAo.

Conclusions: These experiments will help characterize ABCs in a never-before studied post-stroke environment in aged mice. It is expected that aged females will contain higher numbers of autoreactive ABCs.

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Poster #13

Novel TREM2 isoforms highly expressed in human brain and their potential impact on Alzheimer's disease

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Multiple variants in TREM2 which decrease protein function act as risk factors for Alzheimer's Disease (AD). In the brain, TREM2 is primarily expressed in microglia. The five exons of the TREM2 gene encode a cell surface receptor with exon 2 encoding the ligand binding domain, while exon 4 encodes the transmembrane domain. In this study, we have found alternative splicing within TREM2 mRNA from human brain tissue which results in skipping of exons 2, 3, and 4 (D2-, D3-, and D4-TREM2, respectively) with some isoforms having multiple exons skipped. We also show that these isoforms are not brain-specific as they are detected in multiple tissues. Quantitative PCR data indicate exon 2 is skipped at a frequency of approximately 11.4%. We are evaluating whether there is a functional genetic variant which increases exon 2 skipping. In transiently transfected HMC3 human microglial cells, we show using confocal microscopy that the novel D2-TREM2 mRNA isoform is translated into protein and are currently investigating subcellular localization. Hence, we propose that these novel mRNA isoforms are likely translated to protein and represent alternative splicing-derived mimetics of the soluble TREM2 (sTREM2) and C-terminal fragment TREM2 which result from ADAM10 and gamma secretase proteolytic cleavage. Overall, modulating TREM2 splicing represents a potential avenue for AD treatment strategies.

Poster #14

Th17 dysregulation in females with mild cognitive impairment

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Fellow

Background: Dementia is a neurodegenerative process that leads to progressive cognitive decline and interferes with the ability to function independently. Inflammation and/or systemic immune cell dysfunction is a common etiologic factor associated with dementia. Numerousstudies indicate that inflammation precedes and may accelerates cognitive decline. However, the source of inflammation still remains to be elucidated. Recently, T helper 17 (Th17) cells have been identified as a major contributor to inflammation. Th17 cells are a subset of effector T helper cells. They play an important role in adaptive immunity and protect against invading pathogens. In healthy individuals and in the absence of pathogens, Th17-mediated inflammation is caused by aging and age-associated oxidative stress and high level ofTh17 cytokines is indicative of healthy aging. Clinical studies and animal models of Alzheimer's disease have identified a higher level of Th17 cells number and related cytokines in the peripheral blood. However, the function of Th17 cells were not evaluated. For this project, we ought to determine the role that Th17 plays in the development of Alzheimer's disease and related dementia.

Methods/Results: In collaboration with the University of Kentucky Alzheimer's Disease Research Center, we were able to identify participants that fit our criteria: normal or clinical dementia rating (CDR=0) and with mild cognitive impairment (MCI) or CDR= 0.5-1. Purified peripheral blood mononuclear cells (PBMC) isolated from whole blood were stimulated with T cell- or myeloid- targeted stimuli, CD3/CD28 Dynabeads (1bead/cell for 40 hrs) or purified *E. coli* LPS (25 ng/ml, 20 hrs), respectively. We measured supernatant cytokines with a multiplex protein (Luminex-based) assay. We identify Th17 associated cytokines (IL-17A, IL-17F, IL-21, CCL-20) were lower only in female with CDR=0.5-1 after CD3/CD28 stimulation.

Conclusions: The low levels of Th17 cytokines in female with mild cognitive impairments suggest a dysregulation in Th17 function only in females not in males at early stage of dementia.

Funding: This work is supported by TRIAD "Training in Translational Research in Alzheimer's and Related Dementia" T32 AG057461 to JL and Alzheimer's Disease Center pilot grant P30 AG028383 to BN and AB.

Poster #15

Progesterone pretreatment decreases acute stress effect on cognition and impacts downstream expression

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

Poster #16

Apolipoprotein E 4/4 genotype limits response to dietary induction of hyperhomocysteinemia and resulting inflammatory signaling

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Vascular contributions to cognitive impairment and dementia (VCID) are the second leading cause of dementia behind Alzheimer's disease. Apolipoprotein E (ApoE) is a lipid transporting lipoprotein found within the brain and periphery. The *APOE* e4 allele is the strongest genetic risk factor for late onset Alzheimer's disease and is a risk factor for VCID. Our lab has previously utilized a dietary model of hyperhomocysteinemia (HHcy) to induce VCID pathology and cognitive deficits in mice. This diet induces perivascular inflammation through cumulative oxidative damage leading to glial mediated inflammation and blood brain barrier breakdown. Here, we examine the impact of ApoE e4 compared to e3 alleles on the progression of VCID pathology and inflammation in our dietary model of HHcy. We report a significant resistance to HHcy induction in e4 mice, accompanied by a number of related differences related to homocysteine (Hcy) metabolism and methylation cycle, or 1-C, metabolites. There were also significant differences in inflammatory profiles between e3 and e4 mice, as well as significant reduction in Serpina3n, a serine protease inhibitor associated with ApoE e4, expression in e4 HHcy mice relative to e4 controls. Finally, we find evidence of pervasive sex differences within both genotypes in response to HHcy induction.

Poster #17

Gut microbial dysbiosis is correlated with stroke severity markers in aged rats following stroke

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Background: An imbalanced gut microbial community, or dysbiosis, has been shown to occur following stroke. It is possible that this dysbiosis negatively impacts stroke recovery and rehabilitation. Species level resolution measurements of the gut microbiome following stroke are needed to develop and test precision interventions such as probiotic or fecal microbiota transplant therapies that target the gut microbiome following stroke. Previous studies have used 16S rRNA amplicon sequencing in young male mice to obtain broad profiling of the gut microbiome at the genus level following stroke, but further investigations will be needed with whole genome shotgun sequencing in aged rats of both sexes to obtain species level resolution in a model which will better translate to the demographics of human stroke patients.

Results: 39 aged male and female rats underwent middle cerebral artery occlusion. Fecal samples were collected before stroke and three days post stroke to measure gut microbiome. Machine learning was used to identify the top ranked bacteria which were changed following stroke. MRI imaging was used to obtain infarct and edema size and cerebral blood flow (CBF). ELISA was used to obtain inflammatory markers.
Dysbiosis was demonstrated by an increase in pathogenic bacteria such as *Butyricimonas virosa* (15.52 fold change, $p<0.0001$), *Bacteroides vulgatus* (7.36 fold change, $p<0.0001$), and *Escherichia coli* (47.67 fold change, $p<0.0001$). These bacteria were positively associated with infarct and edema size and with the inflammatory markers Ccl19, Ccl24, IL17a, IL3, and complement C5; they were negatively correlated with CBF. Conversely, beneficial bacteria such as *Ruminococcus flavefaciens* (0.14 fold change, $p<0.0001$), *Akkermansia muciniphila* (0.78 fold change, $p<0.0001$), and *Lactobacillus murinus* (0.40 fold change, $p<0.0001$) were decreased following stroke and associated with all the previous parameters in the opposite direction of the pathogenic species. There were not significant microbiome differences between the sexes.

Conclusion: The species level resolution measurements found here can be used as a foundation to develop and test precision interventions targeting the gut microbiome following stroke. Probiotics that include *Ruminococcus flavefaciens, Akkermansia muciniphila, and Lactobacillus murinus* should be developed to target the deficit following stroke to measure the impact on strokeseverity.

Poster #18

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

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Faculty

Background: The p38α MAPK signaling pathway is a well-established regulator of neuroinflammation that is aberrantly activated in Alzheimer's disease (AD). However, there is a lack of insight into the p38α MAPK signaling pathway involvement in non-AD comorbidities found in roughly 80% of probable AD patients. The most common of these co-morbidities is vascular pathology. Therefore, we explored the potential pharmacodynamic effects of MW150, a p38α MAPK isoform selective inhibitor currently in clinical trials, on neuroinflammation in a co-morbid environment with both AD and vascular pathology. To this end, we used a mouse mixed dementia (MD) model that combines amyloid pathology with vascular pathology induced by hyperhomocysteinemia (HHcy).

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

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

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

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Poster #19

Proteins translated from tau circular RNAs as drivers for Alzheimer's disease and Frontotemporal dementia

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Fellow

Background: Neurofibrillary tangles formed by the microtubule protein tau (*MAPT*) are the pathologic hallmark of tauopathies, including Alzheimer's disease and ~1/2 of frontotemporal lobar degeneration (FTLD-Tau) cases. We reported that *MAPT* forms circular RNAs through back-splicing of exon 12 to either exon 10 or exon 7 (BBA Dis 1864:2753; PMID: 29729314). The MAPT circRNAs require primate-specific Alu elements to form, which might explain their absence in mice. Both *MAPT* circRNAs lack stop codons and could form multimers of microtubule-binding repeats upon translation.

Methods: In cell culture studies, we used transfected reporter genes that express tau circRNAs with a 3xFlag tag. We tested the effect ofFTLD-Tau mutants (K317M and V337M), junction-specific siRNAs, and adenosine deaminase acting on RNA (ADAR) enzymes on circRNA translation, evaluated by RNAse protection, Western blots, and validated by mass spectrometry.

Results: Circular *MAPT* constructs were readily translated and regulated by endogenous factors including ADAR and customized siRNAs. The circ12 7 RNA contained a single natural occurring start codon. The circRNA is formed and translated when exons 7 and12 were flanked by authentic tau or heterologous Alu elements. The circ12 10 RNA lacks a natural start codon, but the introduction ofthe FTLD-Tau mutations K317M and V337M induced translation of the circular RNA. The presence of ADAR activity strongly promoted translation of both circRNAs, even in the absence of start codons, possibly by changing an AUA to an AUI start codon (I-inosine). Despite the lack of a stop codon, translation of the circular *MAPT* RNA products stopped at defined sites, indicating that circular RNAs use stop signals distinct from canonical ones.

Conclusion: Our data indicate that *MAPT* circular RNAs are translated into proteins that could cause tau aggregations relevant to tauopathies. As most FTLD-tau causing mutations are in the regions near the circularizing part of the gene, they could act through regulating circular RNA formation and/or translation. ADAR-dependent upregulation of circTau translations caused by inflammation and/or brain injury could trigger tau pathology *via* this mechanism. Finally, tau circRNA can be selectively removed using siRNAs. Weare assessing the impact of *MAPT* circRNAs on proteinaceous aggregations.

Poster #20

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

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

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Poster #21

Harmony at HOME: Proof-of-concept clinical trial of an innovative telehealth intervention for adults with Alzheimer's diseasewith behavioral symptoms

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Fellow

Background: Nearly 90% of adults diagnosed with Alzheimer’s disease and related dementias experience behavioral symptoms, such as agitation, depression, anxiety, or apathy. Current treatment options, both pharmacological and non-pharmacological, lack high efficacy and pose dangerous side effects. Innovative approaches are needed to enhance care for this population. The objective of this study was to assess feasibility of the Harmony at HOME (Help Online Modifying the Environment) intervention as a means to educate care partners in the use of sensory stimulation to promote behavioral regulation and functional performance in the home of adults with Alzheimer’s disease.

Methods: Using a single-blind, three-arm, randomized controlled trial design, we assessed the feasibility of a telehealth occupational therapy intervention for community-dwelling adults with Alzheimer’s disease exhibiting behavioral symptoms. Arms included: 1) individualized intervention (I) with tailored training regarding sensory stimulation; 2) standardized intervention (S) received a set curriculum for care partner training; 3) control arm (C) received standard of care. Both intervention arms received ‘sensory stimulation kits’ containing supplies to facilitate behavioral regulation. All participants completed 6 visits, one per week, with an occupational therapist and a four-week follow up. The primary outcome of feasibility was measured by attendance to weekly visits (>75% attendance over six weeks) and patient retention (maintained enrollment throughout six-week intervention window). Secondary outcomes included patient performance, caregiver satisfaction, and neuropsychiatric behaviors.

Results: Thirty participants agreed to participate and were randomized. Analyses demonstrate the intervention is feasible with high adherence to weekly visits (I=88%; S=100%; C=60%) and high participant retention in the individualized arm (I=80%; S=60%; C=50%). Preliminary analyses of secondary outcomes for the individualized arm identify significant improvements in patient performance (baseline $\bar{x}$ =4.67; post intervention $\bar{x}$ =6.67; p=0.006), caregiver satisfaction (baseline $\bar{x}$ =4.23; post intervention $\bar{x}$ =7.33; p<0.001), and improved total scores on the Neuropsychiatric Inventory with baseline mean score of 20 to post intervention mean score of 15.14.

Conclusion: Harmony at HOME was feasible as an intervention for community-dwelling older adults with Alzheimer’s disease. This pilot project refined protocolization of the intervention which is now being adapted and tested specifically for the needs of residents in rural communities. Pilot data indicate the intervention shows promise for improved patient performance, caregiver satisfaction, and behavioral regulation with a goal to prolong community residency for this population.

Poster #22

Systemic amylin dyshomeostasis leads to metabolic dysfunction and affects cognition

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

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

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

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

Poster #23

Comparison of the subjective memory assessment and cognitive function index scales for operationalizing subjective memory complaints

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Student

Objectives: Subjective memory complaints (SMCs) can be a harbinger of Mild Cognitive Impairment (MCI) or dementia. Operationalizing SMCs canbe problematic due to the large number of scales available, most of which are poorly validated. In this study, we explored two measuresof SMCs collected simultaneously: the Subjective Memory Assessment (SMA) and Cognitive Function Index (CFI) in order to assess the utility of such scales for clinical diagnostic purposes.

Methods: The SMA (range 0-12) and CFI (range 0-14) were administered to consecutive participants from March to October of 2021 at their annual visit in the longitudinal study at the University of Kentucky Alzheimer's Disease Research Center. We assessed order effects todetermine if administering one of the assessments first influenced the scores of the other. We explored the SMA and CFI within diagnostic status: cognitively intact, MCI, and dementia. We examined how the SMA and CFI were related to depressive symptoms (Geriatric Depression Scale; GDS), neuropsychological tests of memory, medical morbidity (Functional Comorbidity Index;FCI), and participant demographics. This was done to determine if either the SMA or CFI might be preferable for clinical use. The assessments were administered during the same study visit as the neuropsychological tests and medical exam. Roughly half of the participants were given the SMA first and the other half were given the CFI first.

Results: The CFI scores were statistically similar regardless of which assessment was administered first ($t(191) = 0.904$, $p = 0.37$), while the SMA was significantly lower when completed after the CFI ($t(191) = 2.55$, $p = 0.01$). MCI participants (n=54) had significantly higher SMA and CFI scores than normals (n=119), *p*-values < 0.001 and the mean score for demented participants (n=19) was lower than for MCI and higher than that seen for normals, but neither relationship was significant. For all participants the CFI-SMA correlationwas $r=0.516$ ($p<0.001$). CFI was modestly correlated with age ($r = 0.24$), FCI ($r = 0.21$), and MoCA score ($r = -0.22$), all *p*-values <0.01. SMA was modestly correlated with FCI ($r = 0.18$), story delayed recall ($r = -0.15$), and MoCA Score ($r = -0.16$), all *p*-values < 0.05,controlling for order of administration.

Conclusions: While the SMA and CFI scores had an appreciable correlation, their correlations with age, FCI, and GDS varied. The CFI was moresensitive to age and FCI, which could be due to the CFI having more concrete items. Some of these items, such as questions abouttroubles with driving and hobbies, could be affected more by normal age-related changes, such as loss of eyesight or physical strength, as opposed to the SMA which contains more general memory questions. Further work examining the relationships between the CFI and SMA and longitudinal diagnostic outcomes is underway in order to further assess the comparative predictive validity of these SMC scales.

Poster #24

Deciphering neuronal and astrocytic multiphoton data to address synchrony, connectivity, density and NVU function

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Objectives: Multiphoton data presented here was obtained on our Scientifica Hyperscope using different animal models of aging and amyloidosis. To investigate *in vivo* neurovascular unit (NVU) function in the somatosensory cortex (S1FL & S1HL), we monitored cerebral blood flow (CBF) proxies from rhodamine dextran-injected animals, as well as neuronal and astrocytic Ca^{2+} signals using GCaMP6 expression. The activity in CBF and GCaMP6 signal was monitored before, during, and after peripheral tactile stimulation (3-Hz, 10v for 5 sec).

Methods: To infer on CBF *in vivo* using movement of non-fluorescent blood cells in rhodamine-labeled plasma, two types of line-scan images were captured: 1) along the vessel and 2) across the vessel. High precision iterative Radon transform routines (with increasing precision) were used on images obtained along the vessel, to derive measures of velocity and blood flow. Images obtained with laser paths across the vessel were used to determine the transient diameter changes across time.
The Ca^{2+} transient records were extracted and analyzed using MATLAB codes developed in-house, including automatic thresholding to determine the region of interest, trace extraction and binarization of the signal for each cell. The continuous wavelet transform (CWT, Morse wavelet), was used for signal binarization to determine the power of responses during tactile activation across several frequencies (0.3-14 Hz). This approach was based on clinically-relevant techniques often employed in EEG data extraction. The CWT helped extract variables associated with network connectivity, connection length, synchronicity and overall activity. We also used periods of baseline activity before tactile activation to extract dominant frequency of the network. Here we provide several methods to investigate network properties, using binarized signals for calculating correlation coefficient between tens of thousands of neurons.

Results and Conclusion: Using the approaches described above, we are able to evaluate NVU function and highlight differences between groups from young and aged F344 rats, to different mouse models of amyloidosis and aging. Taken together, these 2 signal extraction methods are robust and powerful tools for studying dynamic relationships between individual cells and to infer on neuronal network properties as well as blood flow characteristics *in vivo* in the brain. Although the animals were anesthetized, the data using local drug application shows the network is responsive and sensitive to local influences. New protocols for awake animals are currently being investigated.

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Poster #25

Impact of the purinergic P2X4 receptor on neuronal and astrocytic Ca2+ kinetics *in vitro* and *in vivo* in the amyloidogenic 5xFAD mouse model

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Objectives: P2XRs are purinergic ATP-gated cation channels permeable to Ca2+ that have been implicated in several neurological disorders. Most P2XR studies have focused on the P2X7 subtype; however, P2X4 not only has a higher permeability to Ca2+ but is also known to be reduced in the Alzheimer's disease (AD) brain. Dysregulated Ca2+ in the brain contributes to reduced neuronal plasticity and altered excitability. Further, recent evidence from our group indicates that altered network communication in regions associated with sensorimotor processing, such as the somatosensory cortex (S1), correlates with impairments in locomotor stability. Thus, we tested the effects of a P2X4 allosteric modulator (moxidectin [MOX]) and antagonist (5-BDBD [BD]) on intracellular Ca2+ transients *in vitro* using cultured neurons and astrocytes and also characterized the role of these receptors in S1 neuronal Ca2+ network communication in 5xFAD mice using *in vivo* imaging techniques.

Methods/Results: Intracellular Ca2+ measures were obtained in mixed, primary hippocampal cultures using FURA-2 imaging. Cells were first exposed to a solution containing only DMSO, MOX, or BD, then perfused with a DMSO, MOX, or BD solution supplemented with 0.5 μM ATP and 5 μM bradykinin. Fluorescent images were used to derive intracellular Ca2+ changes. To investigate P2X4 expression in the brain, cytosolic fractions of tissue samples from WT and 5xFAD+ mice were probed for P2X4 using Western blot techniques. *In vivo* characterization of the S1 neuronal Ca2+ network in response to tactile stimulation was accomplished in anesthetized 5xFAD- or 5xFAD+ mice expressing GCaMP6 using two-photon imaging. Images were acquired at a rate of 30 Hz before, during, and after (5 s each) stimulation (10 V, 10 ms pulse duration). Recordings were then analyzed to extract measures of overall activity, connectivity, connection lengths, and synchronicity. Results from cell cultures showed that both MOX and BD were associated with increased intracellular Ca2+ compared to DMSO, but only in neurons. Western blots revealed that P2X4 levels trended toward a reduction in the 5xFAD mice. *In vivo* 2P imaging results highlighted a significant difference between 5xFAD- and 5xFAD+ mice, with FAD+ animals having reduced S1 overall activity, connectivity, and connection lengths, suggesting that greater AD pathology is associated with impaired network communication.

Conclusions: Together, our findings show that P2X4 ligands can alter neuronal Ca2+ kinetics and that AD pathology can significantly impact aspects of the S1 network. Future work will characterize similar *in vivo* network measures in 5xFAD mice following local delivery of MOX or BD to S1, as well as investigate P2X4 expression in brain tissue samples from AD patients and cognitively normal subjects in order to address the translatability of this work to the clinic.

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Poster #26

Reactive astrocyte signaling contributes to cerebrovascular dysfunction in a mouse model of small cerebral vessel disease

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We've shown previously that inhibition of NFAT transcription factors in reactive astrocytes preserves synaptic function in mouse models of Alzheimer's disease (AD) and related dementias (ADRD). Here, we used two photon imaging and other in vivo approaches to assess the impact of astrocytic NFAT signaling on cerebrovascular dysfunction in a hyperhomocysteinemia (HHcy) diet model of small cerebrovascular disease. C57BL/6J mice were fed with control diet (CT) or diet that was deficient in B6 and B9 vitamins (and enriched in methionine) to induce HHcy and cerebrovascular pathology. Some mice received intracortical or intrahippocampal injections of AAV-Gfa2 to drive expression of VIVIT (an NFAT inhibitor) or EGFP (control) specifically in astrocytes. Other mice did not receive AAV-Gfa2-VIVIT or EGFP, but instead were injected three weeks before endpoint with AAV-Gfa104-GCaMP6f to visualize Ca2+ fluctuations in astrocytes. 2 photon imaging was used to assess astrocyte reactivity, astrocyte Ca2+ fluctuations, and neurovascular coupling (NVC) in barrel cortex (Ca2+ and NVC were assessed in fully awake mice). In another cohort of CT and HHcy diet mice, the effect of AAV-Gfa2-VIVIT or EGFP was assessed on open field behavior and Y maze performance. Treatment with HHcy diet led to progressive astrocyte reactivity and an increased number of spontaneous astrocytic Ca2+ transients in the barrel cortex of awake mice. HHcy diet also caused deficits in NVC (elicited by whisker stimulation) and Y maze performance, which were attenuated by inhibition of astrocytic NFATs. The results suggest that reactive astrocyte signaling contributes to cerebrovascular dysfunction in ADRD.

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