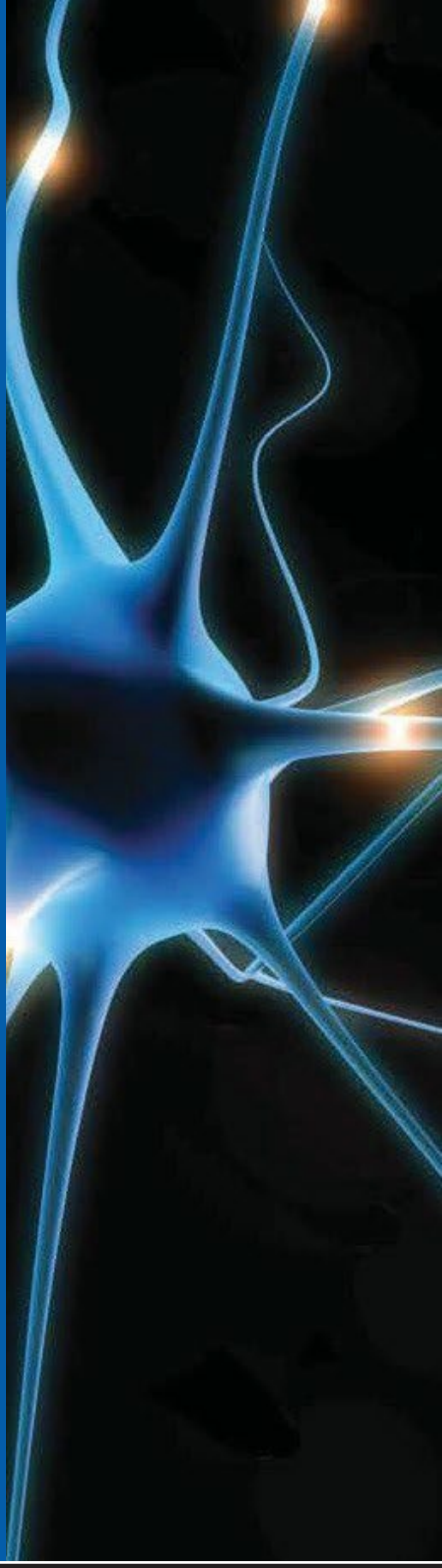
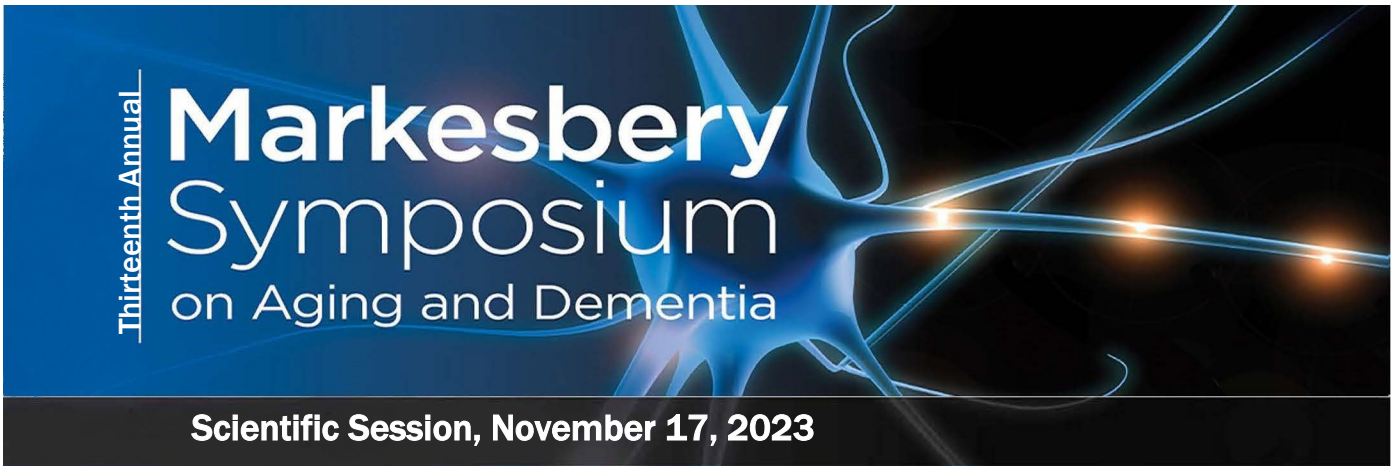
Markesbery Symposium on Aging and Dementia

Scientific Session
November 17, 2023
11:00am – 4:30pm



Sanders-Brown Center on Aging



On behalf of the Sanders-Brown Center on Aging, our philanthropy council, and the symposium planning committee, I am pleased to welcome you to the 13th annual "Markesbery Symposium on Aging and Dementia."

The symposium is named in honor and memory of the late William R. Markesbery, MD, founding director of the Sanders-Brown Center on Aging and Alzheimer's Disease Center at the University of Kentucky. Dr. Markesbery's legacy of groundbreaking research at the Center on Aging has formed the bedrock for our quest to understand and treat Alzheimer's disease and to improve the quality of life of the elderly. We have no doubt that Dr. Bill Markesbery's work will live on for generations to come as we continue the work he started here four decades ago.

In the scientific session today you will have the opportunity to hear clinicians and researchers from the University of Kentucky and other institutions share current findings, trends, and latest updates on dementia and aging disorders, particularly as related to Alzheimer's disease.

We are honored that so many of you have chosen to join us in seeking to expand our knowledge and friendships. I hope the symposium will be both scientifically rewarding and enjoyable.

Sincerely,

Linda Jo Van Eldik

Linda J. Van Eldik, Ph.D.
Director, Sanders-Brown Center on Aging & Alzheimer's Disease Research Center

Symposium Planning Committee:

Linda Van Eldik, PhD	Frederick Schmitt, PhD	Charlotte Wood	Derrick Hord
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2023 Markesbery Scientific Symposium Agenda
Friday, November 17, 2023

10:45 am	**Display posters by 11am**	*Lee T. Todd Atrium*
11:00 am	**Registration**	*Lee T. Todd 152*
11:15 am 11:20 am	**Symposium Welcome** Linda Van Eldik, PhD, Director, Sanders-Brown Center on Aging and University of Kentucky ADRC	
	Tribute to William R. Markesbery, MD Peter Nelson, MD, PhD, Director, Neuropathology Core ADRC and Professor, Pathology and Laboratory Medicine	
11:30 am	***Targeting Chronic Inflammation and the Gut-Brain Axis to Reduce Risk for Neurodegeneration*** Malu Tansey, PhD, Professor, Neuroscience and Neurology and Director, Center for Translational Research in Neurodegenerative Disease, University of Florida	
12:30 pm 2:00 pm	**Lunch break and poster session** Boxed lunches provided	*Lee T. Todd Atrium*
3:00 pm	***Immune Activation in Models of Tauopathy*** David Morgan, PhD, Director Alzheimer's Alliance, MSU Foundation Professor, Translational Neuroscience, Michigan State University	*Lee T. Todd 152* *Lee T. Todd 152*
3:30pm 4:00 PM	***Blood-Brain Barrier Repair to Improve Cognition in Alzheimer's Disease*** Anika Hartz, PhD, Professor, Pharmacology and Nutritional Sciences	
	A Genome-wide Association Study of Neuropathological Endophenotypes David Fardo, PhD, Professor, Biostatistics	
	Poster Award Presentations	

KEYNOTE SPEAKERS

"Targeting Chronic Inflammation and the Gut-Brain Axis to Reduce Risk for Neurodegeneration"

Dr. Malu Tansey
Director of the Center for Translational Research in Neurodegenerative Disease at the University of Florida

In 2019, she was recruited to the University of Florida to be Director of the Center for Translational Research in Neurodegenerative Disease (CTRND) and the first endowed Norman and Susan Fixel Chair in Neuroscience and Neurology at the University of Florida.

Dr. Tansey's lab employs multi-disciplinary approaches to investigate the role of inflammation and immune system responses in brain health and the development of neurodegenerative diseases with particular focus on the gut-brain axis. Her long-term goal is to train the next generation of scientists who can/and to develop better therapies to prevent and/or delay these disorders.

As a Hispanic American, Dr. Tansey has served as a role model to numerous undergraduate, graduate and post-graduate trainees, many of them women from under-represented groups in STEM. She served as Co-Director of Emory's R25 Initiative for Maximizing Student Development (IMSD) whose mission is to strengthen institutional efforts to enhance recruitment and retention of diverse student and faculty bodies at Emory, by providing research training and mentoring opportunities to both. Dr. Tansey is a fierce advocate for women and other under-represented groups in STEM and has earned several mentoring awards from students and faculty for her efforts in this area.

KEYNOTE SPEAKERS

"Immune Activation in Models of Tauopathy"

Dr. David Morgan
Director Alzheimer's Alliance, MSU Foundation Professor, Translational Neuroscience, Michigan State University

Dr. Morgan's research interests are aging and brain function, focusing on developing and testing treatments for Alzheimer's dementia. A major therapeutic approach for his team is modifying innate immune system activity to slow or prevent neurodegeneration. Towards this end the Morgan lab has used immunotherapy and gene therapy approaches.

In the community, Morgan has started a clinical research program through the Alzheimer's Alliance. Community Based Memory Screening is training volunteers to provide free memory evaluations for older adults in their neighborhoods. The Alliance is also recruiting older adults into the NIH supported clinical trial, "Preventing Alzheimer's with Cognitive Training (PACT)". This study is testing the hypothesis that some types of computer games may delay or prevent cognitive impairment in normal older adults.

He is presently an MSU Research Foundation Professor and Director of the College of Human Medicine Alzheimer's Alliance. Dr. Morgan has over 200 peer-reviewed publications and has been continuously funded through NIH since 1989. Morgan served for 6 years on the Program Committee for the Alzheimer's Association International Conference, the last 3 years as Chairperson.

With apologies: Sanders-Brown is unable to share speaker PowerPoint presentations.

"Blood-Brain Barrier Repair to Improve Cognition in Alzheimer's Disease"

Anika Hartz, PhD, Professor, Pharmacology and Nutritional Sciences
University of Kentucky, Lexington, KY

Dr. Hartz started her academic career in 2002 as a Ph.D. student at the University of Heidelberg. After receiving her Ph.D. in 2005, she continued as a postdoctoral researcher at NIH/NIEHS and later as a Research Associate at the University of Minnesota. In 2010, she was appointed Assistant Professor in the College of Pharmacy at the University of Minnesota. In 2014, Dr. Hartz joined the Sanders-Brown Center on Aging at the University of Kentucky as Associate Professor and was appointed Full Professor in 2023.

Her research program is focused on developing novel therapeutic strategies to repair blood-brain barrier dysfunction to lower amyloid-β brain burden with the ultimate goal of improving memory loss and delaying the onset and slowing the progression of Alzheimer's disease.

"A Genome-wide Association Study of Neuropathological Endophenotypes"

David Fardo, PhD, Professor, Biostatistics
University of Kentucky, Lexington, KY

Dr. David Fardo is a professor, and the inaugural Stephen W. Wyatt Endowed Professor of Public Health. He serves as Affiliate Faculty in the Sanders-Brown Center on Aging and as co-Investigator in the National Institute on Aging (NIA)-funded UK Alzheimer's Disease Research Center (ADRC).

Dr. Fardo is currently the principal investigator of two awards from the NIA, researching genetic risk factors contributing to various neuropathological endophenotypes and multiple neurodegenerative diseases. His currently funded collaborative work ranges from therapeutic targeting of the genes TREM2 and SHIP1 for AD to investigating novel pathogenetic mechanisms for hippocampal sclerosis and risk factors for conversion to mixed dementias.

He has developed several courses across the spectrum of CPH degree programs and offers graduate courses in statistical genetics. He has served in various roles including as an Academic Leadership Academy Fellow and Chair of CPH Faculty Council and the Appointment, Promotion and Tenure Committee at the University of Kentucky.

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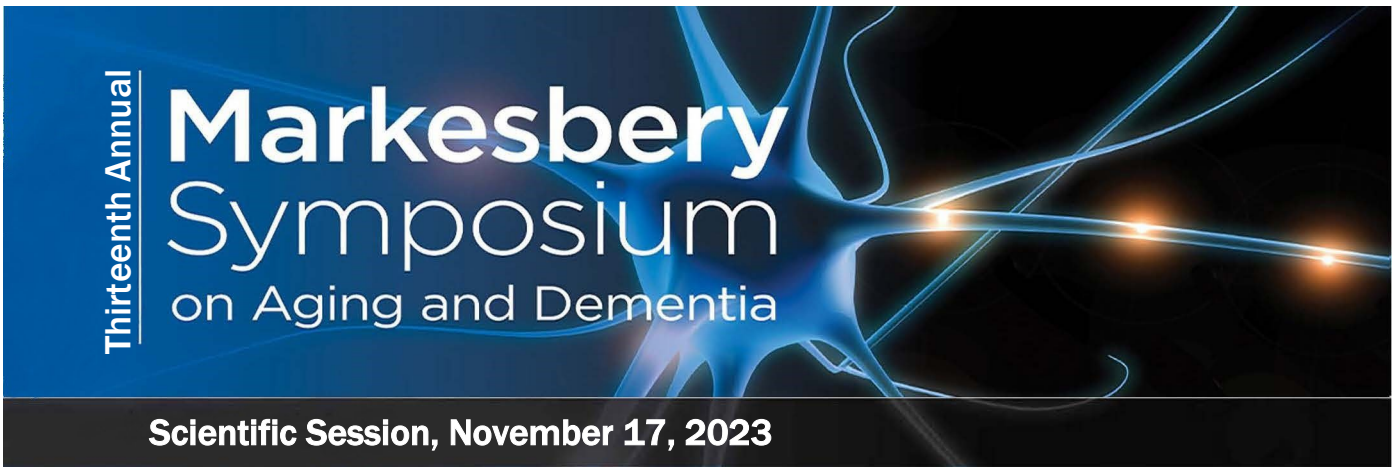

Please take an opportunity to review the posters from our emerging scientists, that are located in the Lee T. Todd Building Atrium.

Poster Abstracts

Sanders-Brown
Center on Aging

Poster #	Last Name	First Name	Status
1	Aguzzoli Heberle	Bernardo	Graduate Student
2	Vincent	Jon	Graduate Student
3	Alcorn	Jayden	Undergraduate Student
4	Littlejohn	Erica	Faculty
5	Zhu	Zhihui	Staff
6	Roberts	Taylor	High School Student
7	Doyle	Patricia	Graduate Student
8	Palacio	Sara	Graduate Student
9	Shahidehpour	Ryan	Graduate Student
10	Fox	Grant	Graduate Student
11	Gordon	Lacey	Graduate Student
12	Tripathi	Priyanka	Staff
13	Page	Madeline	Staff
14	Siano	Dahlia	Undergraduate Student
15	Ontawong	Atcharaporn	Postdoctoral Fellow
16	Turner	Andrew	Graduate Student
17	Nehra	Geetika	Postdoctoral Fellow
18	Chumboatong	Wijitra	Postdoctoral Fellow
19	Campbell	Kelsey	Graduate Student
20	Venkatesan	Tharunika	Staff
21	Sutton	Abigail	Staff
22	Gazula	Meghana	Undergraduate Student
23	Bello	Tara	Graduate Student
24	Golden	Lesley	Graduate Student
25	Satish	Diksha	Undergraduate Student
26	Gant	Chris	Staff
27	Gollihue	Jenna	Staff
28	Wu	Xian	Postdoctoral Fellow
29	MacLean	Steve	Graduate Student
30	Aung	Khine Zin	Postdoctoral Fellow
31	Desai	Rohan	Undergraduate Student
32	Constantino	Nick	Graduate Student
33	Irmen	Riley	Graduate Student
34	Promkan	Moltira	Staff
35	Ruei-Lung Lin	Lin	Staff
36	Dimas	Sophia	Graduate Student
37	Sabra	Hady	Graduate Student
38	Gebhardt	Jessica	Graduate Student
39	Johnson	Carrie	Graduate Student
40	Weiss	Blaine	Graduate Student
41	Thomas	Matt	Postdoctoral Fellow
42	Wright	Nicholas	Staff
43	Geleta	Urim	Staff
44	Drummond	Kristin	Undergraduate Student
45	Arizaca Maquera	Karol	Graduate Student
46	Sims	Sophiya	Graduate Student
47	Fister	Cooper	High School Student
48	Bailey	Caleb	Postdoctoral Fellow
49	Frazier	Hilaree	Postdoctoral Fellow
50	Saunders	Chris	Graduate Student

ABSTRACT TITLE: Using deep long-read RNAseq in human postmortem Alzheimer's disease brains to assess clinical relevance of RNA isoform diversity.

Category: Graduate student

Authors: Bernardo Aguzzoli Heberle[1,2], J. Anthony Brandon[1,2], Madeline Page[1,2], Kayla A. Nations[1,2], Mark E. Wadsworth [1,2], Ketsile I. Dikobe[1,2], Sara Goodwin[5], Elena Ghiban[5], Robert Wappel[5], Senem Mavruk Eskipehlivan[5], Peter T. Nelson, Justin B. Miller[1,3], John D. Fryer[4], Mark T.W. Ebbert[1,2,3]

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[2]Department of Neuroscience, College of Medicine, University of Kentucky, Lexington, KY.

[3]Division of Biomedical Informatics, College of Medicine, University of Kentucky, Lexington, KY.

[4] Department of Neuroscience, Mayo Clinic, Scottsdale, Arizona.

[5] Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, United States.

Background: We often discuss genes as if they have a single function, yet human genes average seven RNA isoforms, potentially resulting in seven distinct proteins/functions. Due to limitations in short-read RNAseq, researchers are forced to collapse all isoforms into a single gene measurement—a major oversimplification. Long reads, however, span entire RNA molecules in a single read, allowing accurate quantification of all isoforms, including de novo isoforms. Accurately quantifying isoformsfurther enables differential isoform expression analysis and reveals isoform diversity within a single tissue/cell—a major advantage over short reads, enabling researchers to begin discerning specific functions for each isoform within a "single" gene (e.g., two BCL-X isoforms: XL = anti-apoptotic, XS = pro-apoptotic). Here, we perform deep long-read RNAseq in human brain to: (1) discover new isoforms (including de novo gene body discovery); and (2) begin exploring functions for individual RNA isoforms in human health and disease.

Methods: We sequenced 12 frontal cortex postmortem aged human brain samples—six Alzheimer's (AD) cases & six controls, (50% female)—using one Oxford Nanopore PromethION flow cell per sample (~40M mapped reads/sample). Analysis included pychopper, minimap2, bambu, and DESeq2. We only report high-confidence isoforms/genes.

Results: We discovered 267 new high-confidence spliced gene bodies expressed with median Counts Per Million (CPM) > 1 and 433 new high-confidence RNA isoforms in annotated genes, where 53 are from medically relevant genes, including MAOB and POLB. We also found five new spliced mitochondrial isoforms and confirmed two by PCR. We identified 7042 genes expressing multiple RNA isoforms in one tissue, including key AD genes: MAPT (4), TARDBP (4), APP (5), PSEN1 (5), and BIN1 (8), demonstrating the diversity of a “single” gene and the need to determine individual isoform function. As proof of concept, we identified 23 differentially expressed isoforms (FDR $p < 0.01$ & $|log2FC| > 1$) between AD cases and controls, where the gene (all isoforms collapsed as a single measurement) was not differentially expressed.

Conclusion: We identified hundreds of new high-confidence RNA isoforms and gene bodies, demonstrating significant gaps remain in our understanding of RNA isoform diversity. More importantly, we began to explore RNA isoform function for every gene by quantifying individual isoform expression levels in human brain tissue. We demonstrate that performing differential gene-level expression is important, but insufficient, and suggest that deep long-read RNAseq is necessary to understand the full complexity of transcriptional changes during disease.

Acknowledgments:

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NIH/NIA: R01AG068331

NIH/NIGMS: GM138636

We appreciate the contributions of the Sanders-Brown Center on Aging at the University of Kentucky. We are deeply grateful to the research participants and their families who make this research possible. We would like to thank the University of Kentucky Center for Computational Sciences and Information Technology Services Research Computing for their support and use of the Morgan Compute Cluster and associated research computing resources. We would like to thank Singularity Sylabs for providing support and extra cloud storage for our software containers. We are grateful for the support from the Goeke lab members who quickly and thoroughly answered our numerous questions about bambu on GitHub. We would like to thank Dr. Thiago Wendt Viola, Dr. Rodrigo Grassi-Oliveira, and Dr. Consuelo Walss-Bass for guidance and help in the early stages of the proteomics analysis.

ABSTRACT TITLE: IL-1R1 signaling in TBI: Assessing chronic impacts and neuroinflammatory dynamics in a mouse model of mild closed-head injury.

Category: Graduate student

Authors: Jonathan C. Vincent[1,2,3,4], Colleen N. Garnett[1,2,3,5], James B. Watson[1,2], Emma K. Higgins[1,2], Teresa Macheda[1,2], Lydia Sanders[1,2], Kelly N. Roberts[1,2], Ryan K. Shahidehpour[1,2,3], Eric M. Blalock[6], Ning Quan[7], Adam D. Bachstetter[1,2,3]

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[4]MD/PhD Program, University of Kentucky, Lexington, KY, United States

[5]Department of Cell, Developmental, and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, United States

[6]Department of Pharmacology and Nutritional Sciences, University of Kentucky, Lexington, KY, United States

[7]Department of Biomedical Science, Charles E. Schmidt College of Medicine and Brain Institute, Florida Atlantic University, Jupiter, FL, United States

Background: Neuroinflammation contributes to secondary injury cascades following traumatic brain injury (TBI), with alternating waves of inflammation and resolution. Interleukin-1 (IL-1), a critical neuroinflammatory mediator originating from brain endothelial cells, microglia, astrocytes, and peripheral immune cells, is acutely overexpressed after TBI, propagating secondary injury and tissue damage. IL-1 affects blood-brain barrier permeability, immune cell activation, and neural plasticity. Despite the complexity of cytokine signaling post-TBI, we hypothesize that IL-1 signaling specifically regulates neuroinflammatory response components. Using a closed-head injury (CHI) TBI model, we investigated IL-1's role in the neuroinflammatory cascade with a new global knockout (gKO) mouse model of the IL-1 receptor (IL-1R1), which efficiently eliminates all IL-1 signaling.

Methods: Both WT and IL-1R1 gKO mice underwent either a CHI or sham procedure. To analyze changes in microglia (IBA1) and astrocyte (GFAP) activity in the neocortex, corpus callosum, and hippocampus, immunohistochemistry was performed at 15 weeks post-injury. Behavioral function was assessed using the active avoidance test at 14 weeks post-injury. Additionally, we evaluated changes in RNA expression at 3, 9, 24, and 72 hours after the injury using the NanoString Neuroinflammatory panel, which includes

757 genes.

Results: We found that IL-1R1 gKO attenuated behavioral impairments 14 weeks post-injury and reduced reactive microglia and astrocyte staining in the neocortex, corpus callosum, and hippocampus. We then examined whether IL-1R1 loss altered acute neuroinflammatory dynamics, measuring gene expression changes in the neocortex at 3, 9, 24, and 72 hr post-CHI using the NanoString Neuroinflammatory panel. Of 757 analyzed genes, IL-1R1 signaling showed temporal specificity in neuroinflammatory gene regulation, with major effects at 9 hr post-CHI. IL-1R1 signaling specifically affected astrocyte-related genes, selectively upregulating chemokines like Ccl2, Ccl3, and Ccl4, while having limited impact on cytokine regulation, such as Tnfα.

Conclusion: This study provides further insight into how IL-1R1 amplifies brain inflammation after a CHI in mice, revealing that suppressing IL-1R1 can protect brain health long-term, beyond its short-term influence on inflammation genes. IL-1R1 seems to quickly but temporarily increase inflammation genes, especially within 9 hours post-injury. To understand how reducing IL-1R1 helps improve behavior and lessen microglia and astrocyte activity 15 weeks after CHI, further research is needed. Future studies should explore IL-1R1's specific roles during prolonged recovery and how early inflammation changes result in lasting effects on chronic inflammation and brain cell function, shedding light on managing long-term impacts of TBI.

Acknowledgments:
This research was partially supported by NIH Grants R01 R01NS103785, R01NS120882, RF1NS119165, F31NS116912, T32NS077889, T32AG078110, and a Kentucky Spinal and Head Injury Trust trainee fellowship.

Age- and Sex-Dependent Changes in Sleep and Nesting Behavior: An APP^{SAA} Alzheimer's Mouse Model

Category: Undergraduate Student

Authors: Jayden P Alcorn[1], Marilyn J Duncan[1], Haleigh R Whitlock[1], Will M Briones[1], Michael P Murphy[2], Sunderam Sridhar[3], Bruce F O'Hara[4], and Adam D Bachstetter[1,5]

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[3]Department of Biomedical Engineering, University of Kentucky, Lexington, KY

[4]Department of Biology, University of Kentucky, Lexington, KY

[5]Spinal Cord and Brain Injury Research Center, University of Kentucky, Lexington, KY

[6]University of Kentucky, College of Arts & Sciences, Lexington KY

Background: Adequate sleep is crucial for maintaining optimal brain health and supporting essential cognitive functions like learning, memory consolidation, and the completion of daily tasks. Disrupted sleep patterns, compromised cognitive function and impaired ability to conduct activities of daily living are often observed in individuals with Alzheimer's disease (AD). While aging is the most significant risk factor for AD, female sex is also a strong risk factor, and the reasons are not well elucidated. In rodents, nesting behavior, defined as the ability to construct a nest is often measured as an activity of daily living. Additionally, nesting is a behavior that precedes and enhances the onset and consolidation of sleep. Here, we investigated the age- and sex-dependent changes in sleep and nesting behavior in APP^{SAA} mice.

Methods: Using a longitudinal design, sleep patterns and nesting behavior were monitored in the APP^{SAA} and wild type (WT) mice (N=8/genotype/sex) from 4-17 months of age. Previous studies on APP^{SAA} mice suggest that amyloid buildup and running behavior alterations develop in this range. At intervals of 2-3 months, mice were transferred from group housing to individual cages for one week of piezoelectric sleep recording. Nesting behaviors were evaluated on a 5-point scale and analyzed using a Kruskal-Wallis test. Sleep percentages during the light, dark, and 24-hour periods were analyzed based on a 2-way ANOVA in SPSS 28.0 that considered the effects of sex and genotype.

Results: Female mice at all ages and of both genotypes slept less than males. At 17 months, females had 6.18% less total sleep and 10.56% less dark-phase sleep compared to males. In addition to sex differences, genotype differences in sleep were observed. APP^{SAA} mice had 3.38% less total sleep and 5.49% less light-phase sleep compared to WT controls. As mice of both genotypes aged, nesting scores decreased. Genotype differences in nesting were exhibited as early as 10 months and by 17 months, the mean nesting score was significantly ($p<.001$) lower in APP^{SAA} (mean=1.688) compared to WT (mean=3.080). No significant difference in nesting scores was observed between males and females.

Conclusion: This study highlights the age- and sex-dependent alterations in sleep patterns and nesting behavior in APP^{SAA} mice compared to WT controls. Notably, females slept less than males as they aged, consistent with human studies in older populations. APP^{SAA} mice demonstrated reduced overall sleep as they aged, mirroring what is seen in individuals with AD. Both genotypes showed a decline in nesting scores (an activity of daily living), with APP^{SAA} mice consistently scoring lower compared to WT. These findings suggest that APP^{SAA} mice exhibit distinct changes in sleep patterns and nesting behavior, consistent with aspects of AD-related sleep disruptions and cognitive impairment. Findings also emphasize the importance of considering age and sex in preclinical AD research.

Acknowledgments:
Funded by the National Institutes of Health (NIH-1R01AG068215); B.F.O. is a co-owner of Signal Solutions, LLC.

ABSTRACT TITLE: ACE exposure predicts early dementia phenotype in racial minorities living in the Stroke Belt: an analysis of the Behavioral Risk Factor Surveillance System (2019-2020)

Category: Faculty

Authors: Erica L Littlejohn[123], Sarah E Cprek[4], Corrine M Williams[4], and Erin L Abner[35]

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[3]Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY

[4]Department of Health, Behavior, and Society, College of Public Health, University of Kentucky, Lexington, KY

[5]Department of Epidemiology and Environmental Health, College of Public Health, University of Kentucky, Lexington, KY

Background: Adverse childhood experiences (ACEs) and racism independently increase risk for poor brain health for Black and African American adults, but little research is published on their joint effects. Here we examined the relation between ACE and cognitive decline among adult African Americans, in a sample enriched in persons experiencing low socioeconomic status, racial minoritization, and poor cerebrovascular health.

Methods: Data were obtained from the 2019-20 Behavioral Risk Factor Surveillance System (BRFSS; restricted to respondents ≥ age 45 and reporting Black/African American race), including data collected from optional modules administered by 5 Stroke Belt states (Alabama, Kentucky, Mississippi, South Carolina, Tennessee): the ACE optional module, which assesses 11 distinct ACEs; and the Cognitive Decline optional module, which assesses subjective cognitive decline (SCD) and associated impairments in instrumental activities of daily living (IADLs). The Chronic Health Conditions core section was used to assess history of stroke and other chronic conditions. Prevalence of ACE exposure was investigated across demographic and health characteristics, as well as SCD. We estimated the association between report of high ACE burden

(≥4 reported) vs lower (1-3 ACE) and no ACE burden with SCD and SCD-associated IADL-impairment via logistic regression models.

Results: Among these adult African American BRFSS respondents (N=3756), a majority (55%) reported ≥1 ACE, and 16% reported high ACE burden (≥4 ACEs). About two-thirds of respondents were women, which was similar across ACE levels. While respondents with high ACE burden tended to be younger than those with 0-3 ACEs, they were the most likely to report SCD (30%) as well as to report SCD+IADL decline (23%). A large percentage of respondents with high ACE burden reported ever having been diagnosed with a depressive disorder (43%). Additionally, in the overall sample, vascular risk factors for dementia were common: hypertension (70%), diabetes (32%), and stroke (10%). While those with high ACE burden were most likely to report fair/poor overall health (46%), they were not more likely to report hypertension (68%), diabetes (29%), or stroke (9%). In analyses adjusted for state, age, sex, education, stroke, smoking, and depressive disorder, persons with high ACE burden had higher odds of SCD (OR=4.1; 95% CI 2.9-5.8), and those with 1-3 ACEs had OR=2.3 (1.7-2.9), compared to no ACE. Similarly, when only SCD+IADL decline was considered as the event of interest (treating SCD without IADL as a non-event), high ACE burden was associated (OR=3.6; 2.5-5.4) with cognitive decline, as was 1-3 ACEs (OR=2.2; 1.6-2.9).

Conclusion: ACE exposure may dramatically increase odds of cognitive decline among African Americans. ACE are, by definition, exposures that occur well before onset of later life cognitive decline; ACE screening could lead to early identification of populations at significant risk for developing cognitive impairment and dementia, as well offer potential for intervention.

Acknowledgments:
This work was supported in part by NIH/NIA grant P30 AG072946.

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

Category: Staff

Authors: Zhihui Zhu[1], Liping Zhang[1], Xiaojia Ren[1], Priyanka Tripathi[1], Zainuddin Quadri[1], Stefka D. Spassieva[1], and Erhard Bieberich[1,2]

Affiliations:

[1]Department of Physiology, College of Medicine, University of Kentucky, Lexington, KY

[2]Veterans Affair Medical Center, Lexington, KY

Background: Previously, we showed that the sphingosine-1-phosphate (S1P) transporter spinster 2 (Spns2) mediates activation of microglia in response to amyloid peptide Aβ. Here, we investigated if Ponesimod, a functional antagonist specific for the S1P receptor 1 (S1PR1), could prevent Aβ-induced activation of microglia and Alzheimer's disease (AD) pathology

Methods: We used primary cultures of mixed glia and pure microglia as well as the 5XFAD mouse model to determine the effect of Aβ and Ponesimod on glial activation, Aβ phagocytosis, cytokine levels and activation of proinflammatory cell signaling pathways, AD pathology and cognitive performance.

Results: In astrocytes and microglia, oligomeric Aβ42 increased the levels of TLR4 and S1PR1 and induced the formation of a complex between the two receptors as shown by proximity ligation assays (PLAs) and co-immunoprecipitation experiments. Ponesimod prevented the Aβ-induced increase of TLR4 and S1PR1 as well as reduced the number of PLA signals and the amount of (co-) immunoprecipitated TLR4 and S1PR1. Aβ42 activated the pro-inflammatory signaling pathways Stat1 and p38 MAPK, which was prevented by Ponesimod, while Stat6 was activated by Ponesimod. Consistent with Stat6 activation, Ponesimod increased phagocytosis of Aβ42 in microglia in vitro. In comparison, FTY720, a functional antagonist of several S1P receptors, did not enhance phagocytosis of Aβ42. In 5XFAD mice, Ponesimod decreased TNF-α and CXCL10, two pro-inflammatory cytokines activating TLR4 and Stat1, while it increased the level of IL-33, an anti-inflammatory cytokine that activates Stat6 and induces Aβ phagocytosis in microglia. Consistent with reduced neuroinflammation and increased phagocytosis, Ponesimod decreased the number of IBA-1 (+) microglia and GFAP (+) astrocytes, and the size and number of amyloid plaques, while it improved spatial memory measured by a Y-maze test.

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

Acknowledgments:
The authors acknowledge funding by NIHR01AG064234, RF1AG078338, R21AG078601, VAI01BX003643. We thank Dr. Nelson Peter (Grant: P30 AG072946) from UK Sanders-Brown Center on Aging for providing the human samples. We also thank the Department of Physiology (Chair Dr. Alan Daugherty) at the University of Kentucky, Lexington, KY for institutional support.

Poster Number 6

ABSTRACT TITLE: Serial Immunohistochemistry Stains to Examine Glial Cells in Mice with Amyloid Precursor Protein

Category: Highschool Student

Authors: **Taylor K. Roberts**, Ryan K. Shahidepour[1,2], Adam D. Bachstetter[1,2,3]

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[3]Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY

Background: Microglia respond to abnormal pathological characteristics to maintain homeostasis within the central nervous system mice with the APP-SAA knock in mimic plaques similar to the ones in human brains using 3 genetic markers found in Alzheimer's Disease patients. This model shows the development and physiological presentation of AD.

Methods: Through a process of staining, chromogen removal, and restaining as well as using the HALO system to analyze different sections of the brain; specifically, cortex, hippocampus, and corpus collosum, we can look at different microglia in tandem.

Results: The images of the mouse model obtained during our serial staining illustrate that plaques increased as the mice aged. The study also showed elevated levels of macrophages, endothelial cells, and astrocytes surrounding the plaques.

Conclusion: This technique demonstrated a way to show robust staining of multiple resident microglia on a single piece of tissue, which gives us a novel technique to visualize interaction of multiple phenotypes of microglia. In addition, an increase in microglial activation and a decrease in homeostatic markers were seen in APP-SAA knock in mice, thus demonstrating that this model is reliable for displaying the mechanics of AD pathology.

Acknowledgments:

Special thanks to the Bachstetter Laboratory

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

Category: Graduate student

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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 demonstrate 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 in the future to distinguish gene and isoform expression signatures between anatomical 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 in different cell populations and between regions. We adapted Particle-templated Instant Partition Sequencing (PIP-seq) for long-read sequencing 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.

Acknowledgments:
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ABSTRACT TITLE: Extracellular Vesicles Derived from Glioblastoma Promote Microglia-Mediated Neurotoxicity

Category: Graduate student

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Background: 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.

Methods: EVs isolated from cell lines and patient plasma were used to analyze their effect in microglia-mediated neurotoxicity. EVs were isolated with commercial kits and counted utilizing ZetaView(Particle Metrix). Confocal

microscopy was used to evaluate EVs uptake and cell morphology. Protein expression was measured with Jess (automated western blot by ProteinSimple). H_2O_2 production was evaluated using Amplex Red(Thermo Scientific) and MitoPY1(Tocris). TNFα release was quantified by ELISA(Biolegend).

Results: First, 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, 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. TNFα was measured as a marker of microglia activation and inflammatory response

Conclusion: 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.

Acknowledgments: Work supported by Startup fund to L.C., Department of Radiation Medicine and Markey Cancer Center 2023 Collaborative Bench to Bedside Pilot Grant Award and R01 CA217934 to D.S.

ABSTRACT TITLE: The Effects of TAR DNA-Binding Protein 43 Microvasculopathy in increased Gliosis in Neurodegenerative Disease.

Category: Graduate Student

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Background:
The aberrant formation of intracellular inclusions are a major facet to several neurodegenerative diseases including Limbic-predominant age-related TDP-43 encephalopathy (LATE-NC), and frontal temporal lobar degeneration with TDP (FTLD-TDP), both of which may lead to focal degeneration of the hippocampus / mid temporal gyrus, known as Hippocampal Sclerosis (HS) leading to cognitive decline. Furthermore, the presence of TDP-43 can also be observed in cases of Alzheimer's Disease where it may have a synergistic role in degeneration.

Recent studies have reported the presence of perivascular pTDP-43-positive micro structures in the brains of patients with FTLD and DLB, known as “Lin bodies.” Immunohistochemical staining and immunoelectron microscopy suggests that while these micro-structures showed variable reactivity for B-crystallin or glial fibrillary acidic protein (GFAP), they appeared near GFAP-positive astrocytes, leading to the assumption that they are indeed associated with astrocytic end feet, and therefore may affect the integrity of the blood brain barrier. However, little else is known about these micro-structures. Our goal was to characterize the glial changes occurring in the presence of Lin bodies and to identify which cells Lin bodies can be most commonly found within.

Method:
Using our method of high-volume multiplex staining and analysis (QUIVER), we began characterizing capillaries with and without presence of Lin bodies in AD+LATE-NC cases and compared the focal glial changes in TDP- vessels in cases of pure AD-NC. Our results suggest that while there is a clear difference between

diseases, there is a slight glial response within the local microenvironment of TDP +/- vessels. We further investigated whether Lin Bodies were present in astrocytes using GFAP staining as well as IBA1 for microglia. We found that these inclusions displayed variable immunoreactive for GFAP staining, however, many of these inclusions were immunoreactive IBA1, suggesting that there may be several phenotypes present within Lin Body populations.

Results:
Recent studies from our lab have also shown that in addition to changes in IBA1 reactivity and microglial phenotypes, there are marked changes in iron processing in diseased brain. In particular, we have shown that increases ferritin light chain protein is associated with a dystrophic microglial sub-population that is believed to be heavily associated with neurodegenerative diseases such as Alzheimer's disease (AD). We therefore used our QUIVER method of multiplexed staining to identify and evaluate the location and density of pTDP-43 inclusions that are colocalized with ferritin and IBA1 or GFAP.

Conclusion:
These findings suggest that the presence of pTDP-43 Lin bodies may play a role in increased gliosis observed in patients with AD+LATE-NC brains, however, further investigation is required to fully understand the role that Lin Bodies play in disease.

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Probing the Epi-Transcriptomic Landscape: Profiling PseudoU Modification Sites in the Human Brain Transcriptome Using Long-Read Direct RNA Sequencing

Category: Graduate student

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Background:

The identification & characterization of covalent RNA modifications have enhanced our understanding of the epi-transcriptomic landscape pivotal to human biological intricacies. Over 170 distinct RNA modifications permeate the four nucleotides. These modifications exhibit remarkable variation in type, abundance, location, & function across different species, tissues, & RNA types. Their intricate nature suggests an emerging role in post-transcriptional gene expression regulation. This diversity has become more discernible with advanced detection methods notably, high-throughput long-read RNA sequencing (LRS) of native RNA molecules via Oxford Nanopore Technologies (ONT).

In this study, we aimed to delineate the prevalence of Ψ sites within the human brain's transcriptome. We harnessed ONT, the sole platform currently capable of sequencing native RNA molecules. Utilizing LRS of native RNA, we can achieve precise & comprehensive examination of the roles of RNA modifications have in neurological disorders.

Methods:

We sourced postmortem human brain tissue from the University of Kentucky Alzheimer's Disease Research Center. One biological sample (n=1) from the postmortem human brain tissue was analyzed. RNA extraction was performed. Our sample exhibited nanodrop ratios >1.8 & an RNA quality number exceeding 9 collected from Agilent Fragment Analyzer 5200. Long read RNA sequencing was performed with ONT Direct RNA sequencing kit, following the

manufacturer's protocol. Sequencing was conducted on the PromethION platform using R9.4.1 flow cell & sequenced for ~72 hours.

Fast5 files were obtained from ONT's built-in Guppy algorithm with its high-accuracy model for base calling. A custom NextFlow pipeline aligned the filtered reads to the GRCh38 human reference genome using minimap2. To assess the quality, we utilized PycoQC, to detail metrics on mapping rate, read length, & other sequencing statistics.

Processed reads were subjected to Ψ modification site detection using Nanopore psU program. Providing Nanopore psU with the sample's fastq files & the GRCh38 human reference genome, initiates an alignment & pileup process, facilitating feature extraction, & prediction of Ψ sites. The output from Nanopore psU includes detailing the reference strand, base type, coverage, probability of being a U, & the probability of being a Ψ.

Results/Conclusion:
The analysis utilizing Nanopore psU technology yielded a profile of Ψ sites with a notable probability of occurring on medically relevant genes associated with transcriptional regulation (HIRA & CABIN1), ion transport (ATP2B3), & neurological functions (ATXN10, ADARB1, SYNGR1, SMS, & EPHA6), to cite a few. It is imperative to acknowledge, that a limitation of this experiment lies in its preliminary nature, as its objective is to demonstrate a procedural framework. This study showcases the potential of experimental procedure for profiling Ψ but also paves the way for researchers to explore the context of RNA modifications in various diseases.

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ABSTRACT TITLE: RNA isoform consistency from long-read RNA sequencing between different tissues and laboratories.

Category: Graduate Student

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Background: The traditional sequencing of RNA has been short read sequencing which collapses all RNA isoforms of a singular gene to one, hiding the diversity and variation of the existing individual RNA isoforms due to technical limitations. Long-read sequencing allows for processing of an entire mRNA molecule, allowing for display of overlap between different fragments of mRNA and the quantification of different isoforms of RNA. Discovery of new RNA-isoforms of known genes and new gene bodies can occur from long read sequencing. As long read sequencing is not the traditional sequencing method, there has not been many assessments to determine if its reliable across different runs and laboratory conditions. Here we are trying to determine whether consistent results will be given for the same tissue across different samples and laboratories.

Methods: We sequenced 12 post-mortem aged human brain samples from the dorsolateral frontal cortex (Brodman area 9/46)—50% female—with long-read Nanopore PCR amplified cDNA sequencing. We quantified expression of these new transcripts in 9 tissue types from GTEx data, one tissue of which was from the same brain region as our samples. These GTEx samples were also sequenced with long-read Nanopore PCR amplified cDNA sequencing. Analysis included pychopper, mimimap2, Bambu, and GRCh38.

Results: From our sequencing data, we discovered a total of 1568 transcripts from

1271 known genes and 1861 transcripts from 1677 new gene bodies, with 30% and 14% of those transcripts being expressed at a median CPM (counts per million) > 1, respectively. Limiting to just medically relevant genes, we found 149

new transcripts from 116 medically relevant genes, with 56% expressed with a median CPM > 1. The percentage of new transcripts from known genes that were found in the different GTEx tissue samples with a medium CPM > 1 ranged between 11% and 33% depending on tissue type. The percentage of new transcripts from new gene bodies with a CPM > 1 ranged between 0.9% and 6%. For new transcripts from medically relevant genes, the percentages increased to between 26% and 52%. Additional statistics are in the works and will be completed before the conference.

Conclusions: We identified that some of the new RNA isoforms from known genes and new genes are validated across human tissues and multiple areas of the brain. We demonstrate that long-read sequencing gives consistent results for the same tissue across different samples and laboratories, while suggesting that long-read sequencing can reliably discover new transcripts that are reproducible. It is encouraging that the percentage of transcripts expressed at a median CPM > 1 is comparable between our brain samples and GTEx brain samples. The discovery and reproducibility of the new transcripts helps us fill in our gaps in the understanding of RNA isoform diversity in human tissues.

ABSTRACT TITLE: Shear stress induces the secretion of extracellular vesicles from primary cilia in astrocytes

Category: Staff

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Background:
In contrast to static tissue culture, cells experience various levels of shear stress due to fluid flow in tissues. While numerous studies show activation of SMases and ceramide elevation by oxidative stress, the effect of shear stress on the lipid composition of the plasma membrane is vastly understudied.

Methods: We tested the response of primary cultured mouse astrocytes to laminar shear stress using an orbital shaker, a method well established to impose shear stress on endothelial cells and remove microglia from primary glial cell culture.

Results: We discovered that shear stress of 3 dyn/cm2 for 24 h, a value reached at capillaries under physiological conditions, reduced the number of primary cilia in astrocytes. In the residual primary cilia, shear stress increased the proportion of glutamylated tubulin, a modification characteristic of cilia that secrete ectosomes, a type of extracellular vesicles (EVs) budding from the ciliary membrane. Shear stress-induced ectosome secretion was confirmed by a 3-fold increase of EVs that were enriched with Arl13b, a ciliary membrane protein and ectosome marker, and ceramide, a sphingolipid involved in EV secretion. The increase of ectosomes was directly correlated with the level of shear stress imposed on the astrocyte culture. Ectosome secretion was prevented by ARC39 and fluoxetine, two inhibitors of acid sphingomyelinase (ASM), but not by GW4869, an inhibitor of neutral sphingomyelinase 2 (nSMase2), or genetic nSMase2 deficiency.

Conclusion: Shear stress led to the translocation of ASM to primary cilia consistent with the secretion of ceramide enriched ectosomes from astrocytes. These data indicate that intermittent shear stress leads to remodeling of primary cilia to secretory cilia, which generate ectosomes when ceramide is elevated by translocation of ASM

to cilia in astrocytes.

Acknowledgments:
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ABSTRACT TITLE: HG38 to T2T-CHM13 comparison sheds light on the need for new methods of annotation: non-syntenic genes, additional gene matches, and WASHC1 gone wild? #itscomplicated

Category: Staff

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Background: In 2022, the T2T consortium released the first truly complete human genome, CHM13, that is already impacting genomics research—including resolving errors in hg38. For most genomics work however, a reference is only as insightful as its annotation. As CHM13 grows in popularity, understanding both structure and annotation differences between CHM13 and hg38 is critical to truly understanding what we have lost, gained, and changed with CHM13. Here, we highlight important differences between the two (including mistakes and oversights) not to criticize the excellent work already accomplished, but to demonstrate the need for improved methods and importance of accurate annotations. Here's the truth: Genomics. #itscomplicated, and we aim to help improve the human reference genomes and their annotations.

Methods: We compared annotations between Ensembl hg38 v101 & CHM13 UCSC GENCODEv35 CAT/Liftoff v2, identifying genes that were not strictly syntenic and chose one category to interrogate: non-syntenic genes (NSG). We BLAT'ed NSG sequences from hg38 to both the full CHM13 reference and back to hg38. We also compared to the newer CHM13 JHU RefSeqv110 + Liftoff v4 annotation after manually adding Ensembl IDs. Finally, we compared with long-read RNASeq.

Results: We identified 1,193 genes that were not strictly syntenic between hg38 & CHM13, including 68 NSGs (i.e., changed chromosomes). Of the 68 NSGs we verified only 39 matched CHM13 v2 annotations (requiring ≥90% identity); using liftover,

however, only 9 matched. We also identified 229 additional unannotated BLAT matches from 9 NSGs, representing potentially 229 additional copies of these gene bodies. 6 NSGs were annotated ≥10kb larger in hg38 than CHM13. Comparing v2 with v4 annotations, 23,093 gene Ensembl IDs were missing in v4—likely because of compatibility challenges. Of the 68 NSGs, only 18 were found in the v4 annotation with gene ids. Some NSGs remained as annotated in CHM13 v2, some reverted to their original hg38 locus, some changed chromosome again, while others reverted to their original chromosome but were also duplicated elsewhere. *WASHC1* is an interesting example, where based on long-read RNASeq, none of the annotations appear accurate.

Conclusion: As the first complete genome, CHM13 brings powerful insights, but also highlights we still have much to learn. Specifically, our results suggest that there remain many inaccurate annotations across both references and liftovers can be wildly inaccurate, highlighting the need for better annotations and methods. Genomics requires more than just a reference—accurate annotations are vital to interpretation. In short, Genomics: #itscomplicated

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ABSTRACT TITLE: Isoform-Specific ApoE Antibodies: Validation and Optimization

Category: Undergraduate student

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Background: Apolipoprotein E (APOE) is the strongest genetic risk factor for Alzheimer's disease (AD). APOE exists in three polymorphic alleles: ε2, ε3, and ε4, which differ from each other by only one or two amino acids. These small differences have a profound effect on protein structure and function, allowing each allele to carry a different risk for AD. Relative to the most common ε3 allele, the APOE ε4 allele increases risk of developing AD, while the APOE ε2 allele confers to a decreased risk. Unfortunately, the close similarity between protein isoforms makes it difficult to develop isoform-specific antibodies that are both reliable and selective. Here, we aim to validate and optimize commercially available isoform-specific ApoE antibodies to test isoform specificity.

Methods: Plasma and brain were collected from homozygous E2, E3 and E4 humanized APOE (hAPOE) mice. Human plasma from all 6 possible APOE genotypes was also collected. Western blotting was used on plasma and brain homogenate to determine isoform-specificity of ApoE2, ApoE3, or ApoE4 antibodies. Furthermore, hAPOE mouse brain was cryosectioned for immunohistochemical analysis of isoform-specific antibody specificity. For both western blotting and immunohistochemistry, protocols were optimized to determine optimal antibody dilutions and incubation conditions.

Results: We have identified multiple antibodies thus far that are both reliable and selective when binding to the intended ApoE isoform in western blotting. Two ApoE2 antibodies have shown promise in western blotting, albeit with higher efficiency in mouse plasma samples than brain homogenate or human

plasma. Three ApoE4 antibodies have also shown potential, with one of those antibodies consistently showing specificity across all western blot control samples. No ApoE3 antibodies have been identified that are isoform-specific. Several of the described antibodies have been tested in immunohistochemistry, but there has been no specificity observed in tissue sections.

Conclusion: Together, these data suggest that several commercially available isoform-specific ApoE antibodies show selectivity, but further testing and optimization is required before it can be determined whether they will work reliably across different sample types. Future directions include further optimization of IHC protocols, and applying these antibodies to a wider range of uses, such as determining regionality and cell-specificity of different APOE isoforms. Overall, by identifying efficacious isoform-specific ApoE antibodies, we hope to provide AD researchers with important resources that are crucial for visualizing and quantifying ApoE protein isoform distribution in heterozygotes.

Acknowledgments:
The project described was supported by funding from the Alzheimer's Association.

ABSTRACT TITLE: Antioxidant Treatment Attenuates Oxidative-Induced Blood-Brain Barrier Leakage and Cognitive Impairment in an Alzheimer's Disease Mouse Model.

Category: Postdoctoral Fellow

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Background: Oxidative stress is a major factor in Alzheimer's disease (AD) that contributes to disease progression and cognitive decline. Evidence shows that oxidative stress is associated with the accumulation of amyloid β (Aβ) and neurofibrillary tangles in patients. Recent data indicate that oxidative stress can lead to blood-brain barrier dysfunction and memory decline. In the present study, we test the hypothesis that reducing oxidative stress by scavenging reactive oxygen species or by inhibiting NOX-2 repairs barrier dysfunction, increases Aβ clearance, and slows cognitive decline.

Methods: To test our hypothesis, transgenic 5xFAD mice received purified diet containing the ROS scavenger *N*-acetyl-L-cysteine (NAC) or the NOX2 inhibitor, Phox-I2. We determined oxidative stress levels (MDA and 4-Hydroxynonenal (4-HNE)), capillary leakage (TR leakage assay), Aβ brain and plasma levels (ELISA), renal function (creatinine clearance) and cognition (Y-maze, radial arm water maze).

Results: Treatment of 5xFAD mice with NAC for 4 weeks reduced brain MDA and brain and renal 4-HNE levels, prevented capillary leakage, and improved renal function and cognition. Moreover, treating Phox-I2 for 9 months reduced capillary leakage and ameliorated cognitive impairment in 5xFAD mice.

Conclusion: Our findings indicate that antioxidant treatment could be a potential strategy to reduce oxidative stress-induced damage and barrier dysfunction and may help slow cognitive decline in AD.

Acknowledgments:

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ABSTRACT TITLE: AD Protective Polymorphism Implicates T-cells in AD Risk through *HAVCR1* and *HAVCR2* Expression and Splicing.

Category: Graduate student

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Background: Alzheimer's Disease (AD) risk is approximately 60 to 70 percent genetic. A recent Genome-Wide Association Study (GWAS) identified rs6891966, a single nucleotide polymorphism (SNP), as being protective for AD risk. This SNP is near the genes *HAVCR1* and *HAVCR2*. Both genes are members of the same family and encode the T-cell Immunoglobulin and Mucin domain containing (TIM-) proteins, TIM1 and TIM3, respectively. These receptors have opposing effects; TIM1 increases immune cell activation and TIM3 decreases activation. Primarily, these genes have been associated with T-cells, although the RNA is robustly expressed in microglia as well. While the influence of microglia on AD risk is starting to be better understood, a role for T-cells in AD has only recently emerged as they were found to infiltrate and modulate inflammation in the AD brain.

Given this, we sought to test the hypotheses that 1) rs6891966 influences splicing of *HAVCR1* and *HAVCR2*, 2) rs6891966 decreases *HAVCR1* expression and increases *HAVCR2* expression, and 3) microglia are the brain cells expressing TIM1 and TIM3.

Methods: PCR and qPCR to visualize and quantify expression and splicing of *HAVCR1* and *HAVCR2* in genotyped brain and buffy coat cDNAs; immunofluorescence and visualization with confocal microscopy to identify the cells in human and PS19 mice brain expressing TIM1 and TIM3.

Results: We identified a novel isoform of *HAVCR2* lacking exon 3 (referred to as D3) in both AD and non-AD brain. We found that carriers of the rs6891966 minor A allele had significantly decreased levels of *D3* expression relative to total *HAVCR2* in brain ($p=0.003$). In addition, the rs6891966 minor A allele was significantly associated with reduced *HAVCR1* expression ($p=0.03$) such that, relative to *HAVCR2* expression, there was a significant ratio decrease ($p=0.002$). Neither splicing nor expression was affected by AD neuropathology. Through immunolabeling, we found that TIM1 and TIM3 are not co-expressed with the microglia marker IBA1, but were co-expressed with the T-cell marker CD3e in human brain. A similar immunolabeling pattern was observed in PS19 mice brains. To evaluate genetics of TIM expression in samples with robust numbers of T cells but no microglia, we repeated the expression and splicing experiments on blood buffy coat samples. This revealed a rs6891966 minor A allele dose-dependent decrease in expression of *D3* relative to total *HAVCR2* ($p=6x10^{-8}$). Moreover, the ratio of *HAVCR1*:*HAVCR2* decreased significantly with rs6891966 in buffy coat ($p=1x10^{-5}$).

Conclusion: This study found that rs6891966 was associated with a significant decrease in D3 relative to total *HAVCR2* and as well as the ratio of *HAVCR1* to *HAVCR2* expression in both brain and buffy coat. Both TIM1 and TIM3 expression was limited to T-cells but not microglia in the brain.

Acknowledgments:
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ABSTRACT TITLE: Aβ Pathology-Driven Barrier Dysfunction in Alzheimer's Disease

Category: Postdoc

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Background: Blood-brain barrier dysfunction is one of the earliest pathological hallmarks of Alzheimer's disease (AD) but the extent of Aβ-driven barrier dysfunction is unclear. In this study, we investigated barrier dysfunction in postmortem samples of AD patients and cognitive normal individuals (CNI) and in 5xFAD mice that overexpress human Aβ proteins.

Methods: *Postmortem Study:* Plasma samples from AD patients and CNI were assayed for S100β protein levels by ELISA. Brain tissue sections were cleared and immunostained for COL-IV, GLUT-1, vWF, PDGFRβ, and fibrinogen. Sections were imaged by confocal microscopy and analyzed for vascular diameters. Vessel diameters were assessed for correlations by generalized linear modeling. *5xFAD Study:* Aged wild-type (WT) and 5xFAD mice were tested for spatial memory deficits by Morris Water Maze testing. Mice were implanted with cranial windows, injected with 3 kDa and 70 kDa fluorescent-labeled dextrans, and imaged *in vivo* by two-photon microscopy. Images were evaluated for intravascular and extravascular intensities of fluorescent-labeled dextrans.

Results: *Postmortem Analysis:* AD patients had 2.7-fold higher S100β levels (p = 0.073) in the plasma compared to CNI. Cortical vessels from AD and CNI brain samples showed similar diameters for COL-IV, PDGFRβ, GLUT-1, vWF, and Fibrinogen. *5xFAD Study:* Extravascular intensities and microvascular diameters were similar in 5xFAD and age-matched WT mice for fluorescent-labeled dextrans.

Conclusion: Our findings show increased plasma S100β levels but similar microvascular diameters in AD patients and CNI. Generalized linear modeling did not reveal any significant associations between AD pathology and microvascular diameters. Aged 5xFAD and WT mice showed similar microvascular diameters and intra and extravascular intensities. These findings suggest that the extent of Aβ pathology-driven barrier dysfunction is too subtle to be captured by current imaging methods. Further investigation with smaller markers combined with advanced imaging techniques is needed to reliably assess barrier integrity in AD models and postmortem tissue.

Acknowledgments:
2R01AG039621; PI: Hartz

ABSTRACT TITLE: Effects of Cannabidiol on oxidative stress and cognition in 5xFAD mice

Category: Postdoctoral Scholar

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Background: Cannabidiol (CBD), a non-psychoactive compound found in Cannabis sativa, has the potential to have a beneficial effect in CNS disorders due to its antioxidative and anti-inflammatory properties. In this study, we assessed the effect of CBD on brain oxidative stress and cognitive function in a mouse model of AD

Methods: We used 3-4-month-old male wild type (WT) and 5xFAD mice and treated the animals with a purified diet containing 200 and 500 mg/kg_{Diet} CBD for 3 and 8 months, respectively. Using the Morris water maze to measure cognitive impairment and MDA assay to measure MDA levels in the brain and kidney tissues.

Results: We found that 5xFAD mice displayed increased escape latencies compared to WT mice; CBD at a dose of 500 mg/kg_{Diet} was associated with significantly lower latency compared to the lower dose of 200 mg/kg_{Diet}. Furthermore, our study revealed the link between cognitive impairment and the accumulation of oxidative stress in the brain by assessing levels of malondialdehyde (MDA), an end-product of lipid peroxidation that serves as a marker for oxidative stress. We found that brain MDA levels significantly increased in 5xFAD mice at 7-8 months of age compared to corresponding WT mice ($p < 0.05$). This result indicates that 7-8-month-old 5xFAD mice have increased brain lipid peroxidation and oxidative stress. The treatment of CBD at the

dose of 200 mg/kg diet and 500mg/kg diet for three months significantly reduced brain oxidative stress, as shown by the decline in MDA levels in treated mice. Brain MDA levels were also significantly increased in 13-14 month-old 5xFAD mice compared to WT mice. The decrease in MDA levels was observed in 5xFAD after CBD20 and CBD50 administration for 3 months.

Conclusion: Our data suggest that CBD ameliorates oxidative stress in the brain which could be beneficial to slow cognitive decline.

ABSTRACT TITLE: Modulation of nutrient sensing receptor GPRC6a reduces tau neuropathology and impacts lysosomal biology in PS19 tauopathy mice

Category: Graduate student

Authors: Kelsey R. Campbell[1,2], Huimin Liang[2], Chao Ma[2], Jerry Hunt[2], Madeline Killion[2], Daniel C. Lee PhD[1,2]

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Background: Several reports indicate that arginine increases during certain neurodegenerative diseases, including Alzheimer's disease (AD) and animal models of tauopathies. The kinase complex mechanistic target of rapamycin (mTORC1) senses nutrients in eukaryotes. mTORC1 is recruited to the lysosomal surface to increase protein synthesis and cell growth during nutrient abundance. When nutrients are scarce mTORC1 fails to translocate to the lysosome and autophagy is uninhibited. During neurodegenerative diseases mTORC1 signaling can become uncoupled and precipitate proteinopathies. Arginine is a potent activator of mTORC1 and signals through various protein sensors and lysosomal transporters. GPRC6a is an amino acid receptor that binds arginine with high affinity, signals to mTORC1, and increases in AD brains and animal models of tauopathy. We hypothesize that tau pathology promotes nutrient sensing dysfunction that promotes hyper mTORC1 activation. We posit that GPRC6a suppression activates autophagy, to rebalance proteostasis and reduce tau pathology.

Methods: Tau transgenic mice (PS19) and non-transgenic littermates were bred to mice with GPRC6a hemizygous deletion to generate four genotypes: (nTg, GPRC6a +/-, PS19, PS19/GPRC6a +/-). Mice were aged to 7-9 months before brains were harvested for western blot and bulk RNA-seq. Tau biochemistry was measured in detergent soluble and urea soluble fractions from the anterior and posterior cortex. Markers for mTOR activation, lysosomal function, and autophagy were also measured. Hippocampal tissue was used for bulk RNA seq Nanostring for transcriptome pathway analysis.

Results: Hemizygous deletion of GPRC6a (PS19/GPRC6a +/-) decreased total tau (HT7) and various forms of phospho-tau (Ser199/202, PHF1, AT8) in soluble and insoluble fractions compared to PS19 mice. GPRC6a suppression in PS19 mice significantly decreased phospho mTOR2448/ total mTOR ratio suggesting

decreased mTORC1 activation compared to PS19 mice. Autophagy significantly increased in PS19/GPRC6a +/- mice, as measured by active ULK1. Both LC3-I and LC3-II increased in PS19/GPRC6a+/- mice compared to PS19 mice suggesting increased lysosomal biogenesis. PS19 mice showed increased transcripts associated with autophagy, neurogenesis, and proteotoxic stress compared to nTg littermates, and hemizygous deletion of GPRC6a (PS19/GPRC6a+/-) normalized these signatures to that of control levels.

Conclusion: These data indicate that GPRC6a expression regulates proteostasis of tau and could serve as a therapeutic target in tauopathies. Additionally, our data suggests that GPRC6a impacts lysosomal function and mTORC1 activation state.

Acknowledgments:
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ABSTRACT TITLE: Low-Frequency Oscillations During Resting and Task Differentially Associated with Working Memory Performance in Older Adults

Category: Staff

Authors: Tharunika Venkatesan[1], Ellen Fei[1], Tyler C. Hammond[1], Xiaopeng Zhao[2], Olivier Thibault[1], Pradoldej Sompol[1], Christopher M. Norris[1], Gregory A. Jicha[1], and Yang Jiang[1]

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Background:

Increased intrinsic slow delta oscillations during resting state are associated with brain aging and increased Alzheimer's disease/related dementia (ADRD) pathologies, i.e., synaptic network dysfunctions and astrocyte over-reactivity in human and animal models. Despite evidence of "slowed brain frequency in the AD brain", limited understanding of brain oscillation changes in the preclinical AD remain. Here we examined the relations of Working Memory (WM) performance with low frequency (delta-theta) electroencephalogram (EEG) oscillations during pre-task rest, task, and post-task recovery rest.

Methods:

34 cognitively normal adults from UK-ADRC participated in the study. EEG was recorded using a wireless wearable headset (EMOTIV) while participants partook in resting state eyes-closed (EC) and eyes-open (EO) pre- and post-task and performed a visual WM task. Participants first used their dominant hand (faster cognitive motor) then used their nondominant hand, each for 5 mins, to indicate a target in the WM EEG protocol. EEG data was cleaned and extracted for frequency power using two in-house programs developed by Dr. Zhao's BME Lab at UTK. Frequency results were aligned with performance measures via Excel. Outliers were removed under 2.5 standard deviations for reaction times and accuracy of WM retrievals. Pearson correlations were applied using Patheon programs developed in Dr. Jiang's ABC Lab at UKCOM.

Results:

Increased delta is associated with higher accuracy during resting state EO and EC pre- and post-task in the left and right parietal and frontal sites and the WM task in the left frontal area. It is associated with longer reaction times during

resting state EC pre-task and the WM task in the right frontal site. An exception is resting state EO post-task in left frontal site, which exhibits a shorter reaction time.

Increased theta is associated with lower accuracy during resting state EO and EC post-task in the right frontal and left parietal sites and the WM task in the left frontal site. However, resting state EO pre-task in the right parietal site shows a higher accuracy. Increased theta is also associated with a longer reaction time during resting state EC pre- and post-task in the left and right frontal sites and the WM task in the right frontal sites. Exceptions include resting state EO pre-task in the left and right parietal sites, which have a faster reaction time.

Conclusion:

Delta and theta are differentially associated with memory performance with mostly opposite accuracy correlations. Both have slow reaction times in the frontal region, but theta and delta also show faster reaction times in the bilateral posterior sites and left frontal area, respectively. Increased synchronization in the delta frequency range is also observed in aged and dementia animals models. Low frequency neural slowing during rest and task are promising indicators of pathophysiological changes during brain and cognitive aging.

Acknowledgments:
All participants are from University of Kentucky's Alzheimer's Disease Research Center (UK-ADRC). Supported by R56 AG060608, NIA P30 AG028383, P01 AG078116.

ABSTRACT TITLE: Exploring Sleep via Biometric Outcomes Among the Sanders-Brown Center on Aging Longitudinal Cohort

Category: Staff

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Background: Growing evidence suggests sleep disturbances may be an early indicator of incident and prevalent dementias, as well as accelerating cognitive decline. Those with Lewy body dementia and Alzheimer's disease commonly suffer from increased sleep fragmentation or excessive daytime sleepiness. Therefore, participants in the Sanders-Brown Center on Aging longitudinal cohort are now asked to wear an actigraph device to measure sleep for one week following their annual study visit. The actigraph is an accelerometer with an ambient light sensor optimized for activity and sleep data collection. Associations of sleep with the vast Sanders-Brown participants' health history, lab values, cognitive test performance, and other pertinent indicators can be examined cross-sectionally and longitudinally.

Methods: The UK IRB reviewed and approved the actigraphy procedures for the longitudinal study. Participants were informed of actigraphy procedures and rationale during informed consent at their annual visit. 134 consented participants wore the actigraphy on their wrist and 21 on the ankle, each provided with a daily activity log containing prompts to record sleep and activity levels. For participants with dementia, this was explained to a consenting caregiver. Participants were asked to wear the actigraph device for 7 days after

their study visit and provided prepaid mailers. Sleep data was processed using the Cole-Kripke algorithm and included: total sleep time, sleep efficiency, time awake after sleep, sleep fragmentation, and self-reported count of bathroom visits during the night. Sleep variable means were compared between categories of interest using ANOVA, and associations with continuous variables with linear regression to control for age sex, education, and nonclinical, MCI, or dementia clinical status. Associations between sleep variables and medical morbidity, depression, subjective well-being and memory appraisal, and cognitive test performance will be explored.

Results: 155 participants have consented to actigraphy and 26 have declined. Eight who consented either did not wear the device consistently or keep the daily activity log, so sleep data could not be processed. Ultimately, 129 participants have processed sleep data available as of 11 OCT 2023: 99 are nonclinical, 23 are MCI, and 7 are dementia. Background demographic data will be reported for the 129 participants.

Conclusions: Using remote wearable technology, the Sanders-Brown ADRC can explore longitudinal sleep habits and irregularities in aging adults in the Lexington community. This data may identify sleep markers that could inform diagnosis or early detection of Alzheimer's disease and related dementias. Limitations of these early data include a well-educated middle-class sample and that actigraphy sleep markers are derived from motor activity versus more formal sleep studies. The clinical and scientific significance of this ongoing work and future directions will be reported.

Acknowledgments:
This research was supported by NIH/NIA grant P30 AG072946.

ASTRACT TITLE: Investigating The Role Of TDP-43 Pathology On Astrocytic Activation And Their Impact On Neurovascular Coupling In A Transgenic Mouse Model.

Category: Undergraduate student

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Background: TDP-43 proteinopathy has been identified as one of the pathogenic factors that correlates with the early cognitive decline and severity of Alzheimer's Disease (AD) and Limbic-Predominant Age-Related TDP-43 Encephalopathy (LATE). Previously, our laboratory demonstrated that TDP-43 overexpression resulted in significant infiltration of mice IgG, CD3+, and CD4+ T cells, as well as endothelial and pericyte activation, indicating blood-brain barrier (BBB) permeability. The primary objective of this study is to examine the impact of TDP-43 pathology progression on neurovascular coupling, including astrocytic and brain vasculature functional impairments, and neuronal activity network synchronization in a transgenic mouse model.

Methods: Animal Model: TAR6 and TAR 6/6 mouse models express wild-type human TDP-43. The homozygous TAR6/6 mice manifests TDP-43 cytoplasmic inclusions and phosphorylated aggregates, impaired brain and spinal cord motor neurons, functional decline, and early mortality. The mice were divided into four groups based on age and genotype: group 1=nTg (n=7, 10-12 months), group 2=TAR6, (age-matched, n=7, 10-11 months), group 3=nTg (n=6, 4-5 months), and group 4=TAR6/6 (age-matched, n=7, 4-5 months).
Immunohistochemistry (IHC): Monoclonal Rabbit S100B (Abcam, 1:3,000) was used to see increased levels of calcium-binding protein S100B and the presence of homeostatic astrocyte population. Polyclonal GFAP (Dako, 1:3,000) antibody was used to measure reactive astrocytes and their end feet attachment to vasculature.
Multiphoton Imaging: Mice were injected with calcium flux indicator AAV9-GCAMP8f in the barrel cortex as recently described (*Sompol et al., JNI 2023)*. Imaging and analysis were done in collaboration with the Norris, Thibault and Sompol laboratories.

Results: Multiphoton imaging revealed that progression of TDP-43 pathology in TAR6/6 mice increased neuronal activity following whisker stimulation and displayed synchronized neuronal activity within networks. A significant reduction in vascular dilation was recorded, suggesting uncoupling of neurovascular signaling. Immunohistochemical analysis demonstrated the accumulation of TDP-43 positive cytoplasmic inclusions in TAR6/6 mice. GFAP image analysis demonstrated induced astrocytosis in TAR6/6 mice following TDP-43 pathology. Further analysis of GFAP + astrocytic end-feet demonstrated a significant reduction in coverage of blood vessels by astrocytic end feet. Homeostatic astrocytes measured by S100B show confirmed reactive status of this population and induced branching in S100B+ astrocytes in TAR6/6 mice.

Conclusion:
Our results indicate that TDP-43 pathology has a significant impact on neurovascular function, which is critical for metabolic processes and neuroprotection. The data also provides the rationale for future research to investigate the effects of TDP-43 pathology on other components of the Blood Brain Barrier such as blood vessels, pericytes and endothelial cells.

Acknowledgments:
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Title: Exploring the Eudaimonic Activity model of Wellbeing and Relationship Status Among Older Adults

Category: Undergraduate Student

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Background: The Eudaimonic Activity (EA) model posits that eudaimonic motives and activities lead to psychological need satisfaction which, in turn, leads to subjective well-being. The existing research on the EA model suggests that it applies to young- and middle-aged adults but not necessarily older adults. Older adults are at risk for isolation and loneliness, loss of functional abilities, and infirmity, as well as subclinical depression and anxiety. Widowhood and other relational changes can compound these factors. Also, emergent psychiatric symptoms can be a harbinger of early neurodegenerative changes in the brain, independent of psychological states. Therefore, further studying the EA model in older adults will advance clinical application and research evaluation of psychotherapies in this population.

Methods: Emails were sent to nonclinical and MCI participants in the SBCOA cohort in August 2023 (with one reminder one week later). Participants were asked to contribute additional data regarding eudaimonic wellbeing by completing a secure online survey. Measures include Scale of Positive and Negative Experiences (SPANE), Psychological Wellbeing Scale (PWBS), and Satisfaction With Life Scale (SWLS). The current relationship status (i.e., single or involved) of the participants was considered. All analyses reported control for age, sex, education, MCI or nonclinical, and having >=1 Apoe4 or not.

Results: The survey was emailed to 410 nondemented participants and responded to by 190 within two weeks; 158 nonclinical and 28 MCI. Only 9.1% of the 66 males were single, compared to 46.7% of the 124 females, $x2 = 27.4$, $p < 0.001$. The Geriatric Depression Scale 15-item was moderately related to SPANE positive ($r = -0.60$, $p < 0.001$) and negative ($r = 0.51$, $p < 0.001$) scales, PWBS ($r = -0.54$, $p < 0.001$), and SWLS ($r = -0.55$, $p < 0.001$). All measures were modestly associated with the various scales of the SF-36 ($|0.25| < |r| < |0.45|$). The single participants on average reported higher ratings on SPANE positive ($p = 0.008$) and negative ($p = 0.04$), both about 1.5 points, or roughly half the total sample SD. The relationship status groups did not statistically differ on PWBS or SWLS (ps > 0.06).

Conclusion: The measures commonly used to operationalize eudaimonic wellbeing are related to validated measures of depressive symptomatology and

subjective well-being in this sample of older adults. Limitations for the study include a predominantly white well-educated middle-class sample, a dearth of single males, and using an online only sample. These findings suggest that clinicians could use techniques that increase eudaimonic well-being to decrease depressive symptoms and foster increased subjective well-being. Future studies may examine activities or strategies that increase eudaimonic well-being, for instance, activities that increase psychological flexibility.

ABSTRACT TITLE: Mid-life *APOE4* to *APOE2* 'Switching' Alters the Cerebral Transcriptome and Decreases AD Neuropathology

Category: Graduate student

Authors: Lesley R. Golden[1,2], Steven M. MacLean[1], Isaiah O. Stephens[1], Dahlia S. Siano[1], Sangderk Lee[1,3], Callie M. Whitus[1], Danielle Goulding[2], Amy Gorman[2], Jing Chen[4], Haining Zhu[7], Kumar Pandya[6], Scott Gordon[1,5], Josh Morganti[2,3] and Lance A. Johnson[1,2]

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Background: Compared to the 'neutral' E3, the E4 allele of Apolipoprotein E (*APOE*) confers up to a 15-fold increase in Alzheimer's Disease (AD) risk. Conversely, the neuroprotective E2 allele decreases AD risk by a similar degree. Here, we aimed to assess the therapeutic potential of allelic 'switching' by investigating the physiological changes associated with an inducible, *in vivo* *APOE4* to *APOE2* transition in a novel transgenic mouse model.

Methods: The *APOE* "switch mouse" (APOE4s2) uses the Cre-loxP system to allow for inducible *APOE* allele switching from E4 to E2. These mice express a floxed human *APOE4* coding region followed by the human *APOE2* coding region. Allelic discrimination (RT-PCR) and mass spec-based proteomic analyses were employed to validate the E4 to E2 transition. Single-cell RNAseq was used to measure physiological changes following the E4 to E2 allele switch. Behavioral measures and neuropathological analyses were

applied to assess the effects of the allelic switch on AD pathology.

Results: mRNA and protein analyses confirm that APOE4s2 mice synthesize full-length human *APOE4* pre-switch, and that tamoxifen induces an efficient recombination and expression of human *APOE2* in target tissues. Single-cell RNAseq reveals that global, genetic replacement of *APOE4* with *APOE2* results in distinct alterations to glial cell transcriptomes affecting pathways involved with metabolism, inflammation, and amyloid beta. As scRNAseq implicated astrocytes as the most affected cell type post-switch, we next explored the effects of an astrocyte-selective (*Aldh1l1-Cre*) E4 to E2 transition. This astrocyte-specific E4 to E2 'switching' significantly decreases amyloid-associated astro- and micro-gliosis and improves cognition when compared to controls.

Conclusion: Together, these data suggest that a successful transition from E4 to E2 has broad impact on the cerebral transcriptome and that an astrocyte-specific E4 to E2 'switch' improves AD associated pathologies.

Acknowledgments:
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ABSTRACT TITLE: Treatment of Monocyte-Derived-Macrophages with Plasma to Determine Age and *APOE* Effects on Lipid Droplet Accumulation

Category: Undergraduate Student

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Background: Lipid droplets (LD) are cellular organelles that serve to store neutral lipids such as cholesterol esters and triacylglycerols. Apolipoprotein E (ApoE), is a lipid transport protein most notably produced in hepatocytes and astrocytes, where it aids in cholesterol metabolism by shuttling lipids between cells. There are three polymorphic alleles for *APOE (E2, E3, E4)*, with *APOE4* representing the strongest genetic risk factor for late-onset Alzhiemer's disease (AD). The three ApoE isoforms differentially affect lipid transport and lipoprotein metabolism, though their effects on lipid droplets remain unknown. The accumulation of LDs in the brain is of interest since it has been correlated with neurodegenerative diseases, including AD. Additionally, a previous study in mice has shown that transfusing aged mice with plasma samples from young mice decreased LD accumulation and a pro-inflammatory state in the brain. While there is a correlation between LD buildup in the brain and the neuropathology of Alzheimer's disease, it is unclear if *APOE* genotype plays a role in lipid droplet accumulation patterns. Here, we aimed to

determine the effects of *APOE* genotype, sex, and age on LD accumulation in monocyte-derived human macrophages, and whether treatment with sex-matched exogenous serum from various ages and genotypes will alter LD accumulation.

Methods: Peripheral Blood Mononuclear Cells (PBMCs) isolated from whole blood samples, from a single donor with an *ε3/ε3* genotype, using density-gradient centrifugation in SepMate™ tubes were cultured for 7-10 days until differentiation into macrophages. Plasma samples were treated with Calcium chloride to derive serum and prevent media coagulation due to the presence of plasma clotting factors. Serum samples were assayed for triglyceride and cholesterol concentrations to normalize for these varying levels across samples. Macrophage cell cultures were treated with a serum:media solution based on this normalization. Additionally, macrophages were

treated with ApoE2, ApoE3, and ApoE4 recombinant protein in Human Plasma-Like Medium to determine effects of exogenous protein on LD patterns.

Results*:*There is a significant positive correlation between the age of the serum samples used to treat *ε3/ε3* macrophages and LD accumulation in the cells. This pattern remained consistent regardless of whether *ε3/ε3* macrophages were treated with *ε3/ε3* or *ε3/ε4* serum samples. Macrophages treated with ApoE2, ApoE3, and ApoE4 recombinant protein had no significant differences in lipid droplet accumulation between the isoforms.

Conclusion : We currently have a database of over 200 *APOE* genotyped individuals to age and sex match for venipuncture and subsequent experimentation. Using the above-mentioned optimized protocols, we will begin culturing and treating cells from human subjects to understand how *APOE* genotype, age, and sex drive myeloid LD accumulation and transport.

Acknowledgements
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ABSTRACT TITLE: Longitudinal two photon imaging in intact mice reveals dynamic insult-related morphologic changes in astrocytes that are resolved by administration of tacrolimus (FK506)

Category: Staff

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Background: Hypertrophic changes in astrocyte morphology are associated with a number of deleterious conditions related to inflammation, neurodegeneration and brain damage. Although the effect of such insults on astrocyte morphology is commonly accepted, it is unclear if these changes are dynamic. Can the astrocytes recover from this reactive state and return to a normal (non-hypertrophic) morphology? It is hypothesized that the expression of GFAP leads to the hypertrophic state and the increase in GFAP is related to neurodegeneration. Calcineurin is an important modulator of multiple astrocyte phenotypes and synaptic transmission in AD models. However, it is unclear if modulation of calcineurin or calcineurin-dependent transcription factors (i.e. NFAT4) can rescue astrocyte vulnerability to inflammation.

Method: Mice were injected with adeno associated virus (AAV.GFA2.eGFP into the surface of the cortex) to visualize astrocytes. After 4 weeks, baseline images of astrocytes were acquired in defined field of views (FOVs) on a dual-photon microscope. Mice were then subjected to LPS or saline for 1 week for a total of 7.5 mg/kg (i.p.) followed by a post-LPS imaging session of the same FOVs (i.e. same astrocytes) within the same mice. Immediately after the post LPS imaging session, mice were implanted with peripheral miniosmotic pumps charged with saline or the calcineurin inhibitor tacrolimus (5mg/kg/day). Imaging sessions on the same FOVs were then performed once per week for the next 3 weeks. Astrocyte morphometric measures were obtained from each imaging point for statistical comparison. Additionally, 6-month-old 5XFAD mice, with advanced astrocyte reactivity, were concomitantly injected with AAV expressing eGFP or VIVIT.mCherry into each hippocampus of the same animal. Following 3 months the tissue was processed and the morphology of astrocytes in each hippocampus was assessed using a confocal microscope.

Results: Astrocytes from mice that received LPS showed significant hypertrophy compared to controls. In mice that received saline following LPS, the astrocytes

continued to become progressively hypertrophied whereas astrocytes from mice that receive tacrolimus began to revert to their original morphology. A number of interesting observations were made including the apparent death of astrocytes, the emergence of new GFAP+ astrocytes and the loss of eGFP expression in some astrocytes. This suggests that levels of GFAP changed in some astrocytes throughout the experimental manipulations. AAV.VIVIT also appeared to resolve astrocyte reactivity in 5xFAD mice.

Conclusions: These results demonstrate that astrocyte reactivity is a dynamic process that results in morphological changes. This reactivity continues, if left untreated, however, treatment with tacrolimus (an inhibitor of calcineurin) appears to resolves reactivity. In addition, treatment with a NFAT4 inhibitor is effective for the resolution of reactive astrocyte phenotypes in a 5XAD model.

Acknowledgements:
RF1AG027297, P01AG078116

ABSTRACT TITLE: Overexpression of astrocytic glutamate transporters alters hyperexcitability in mouse model of Alzheimer's Disease

Category: Staff

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Background: Astrocytes are the main cells responsible for clearing glutamate from the synapse; their glutamate transporters quickly and efficiently clear glutamate within the synaptic cleft, preventing neuronal excitotoxicity. In mouse models and human cases of Alzheimer's Disease (AD), the astrocyte-specific glutamate transporter (Glt1 in rodents and EAAT2 in humans) is decreased. Our goal is to study the effects of decreased glutamate transporters on synaptic measures of function and plasticity.

Methods: Using a 5XFAD mouse model of amyloid pathology, we utilized an adeno-associated virus (AAV) to over-express Glt1 in the hippocampus. Electrophysiological field recordings examined the role of astrocyte-specific glutamate transporters in synaptic plasticity and hyperexcitability. Animals received injections of AAV-Luciferase (control) in one hippocampus, and AAV-Glt1a in the contralateral hippocampus. Field recordings were performed to assess excitatory postsynaptic potentials in the hippocampal CA1 stratum radiatum elicited by stimulation of CA3 axons.

Results: Compared to control (AAV-Luciferase), AAV-Glt1 significantly increased the expression of glutamate transporters in hippocampal tissue. We then compared measures of neuronal hyperexcitability, synaptic strength, and long-term synaptic potentiation. Results indicate that there is more hyperexcitability in control tissues compared to AAV-Glt injections, as evident by decreased population spike thresholds.

Conclusion: Others have reported hippocampal hyperexcitability may lead to memory impairment; in human cases of AD it is thought to encourage epileptic episodes. By using our AAV-directed technique to increase glutamate transporters, and thus reduce network hyperexcitability, we will be able to

examine how reactive astrocytes and their loss of glutamate transporters contribute to memory or cognition loss. Ongoing studies will do exactly this- use a mouse model of AD with and without AAV Glt1 overexpression to determine changes in behavioral cognitive outcome measures that may be attributed to hyperexcitability in neuronal networks.

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ABSTRACT TITLE: Genetic Factors of Longitudinal Cognitive Changes Related to Alzheimer's Disease: A Systematic Review and Meta-Analysis

Category: Postdoctoral Fellow

Authors: Xian Wu[1, 3], Jing Zhang[4], Khine Zin Aung[1, 3], David W. Fardo[1, 3], Erin L. Abner[2, 3], Yuriko Katsumata[1, 3]

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Background: 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 and 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.

Methods: 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 inclusion criteria are 1) published from 2013 through 2023; 2) analyzed original data of human subjects; 3) measured at least one cognitive function related to LOAD; 4) conducted genetic analysis with SNPs; 5) included sufficient data to calculate study effect sizes. The exclusion criteria are 1) empirical study/qualitative study/case study/systematic review/meta-analysis; 2) studied on early-onset AD or other types of dementia; 3) subjective (i.e., self-reported or caregiver-reported) cognitive decline/changes; 4) studied associations of polygenetic risk scores (PRS) rather than individual SNPs; 5) written in non-English languages.

Results: Through systematic literature searches, 1,499 references were found and included into title/abstract screening after duplicates were removed. Of the references, 719 were included in full-text screening. As a polit study for this project, two reviewers have independently coded 20 studies and a summary table has been generated to demonstrate study design, databases/cohorts included, genetic ancestry, sample size, cognitive domains measured, and statistical modeling approaches. Pooling effect sizes and 95% confidence interval (CI) across studies will be estimated and forest plots will be created. Publication bias will be estimated as well.

Conclusion: The current systematic review and meta-analysis 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.

Acknowledgments:
CCTS DREAM scholar program and P01AG078116

ABSTRACT TITLE: Mitigation of APOE4 Associated Alzheimer's Disease Pathology by Selective APOE2 Expression in Hepatocytes

Category: Graduate student

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Background: Apolipoprotein E (*APOE*) is the strongest genetic risk factor for late-onset Alzheimer's disease (LOAD). Of the three common *APOE* alleles, *APOE4* dramatically increases risk for LOAD, while *APOE2* reduces risk relative to the most common *APOE3* allele. The cell type that produces the highest concentration of ApoE in the body are the hepatocytes of the liver. Although hepatocyte-derived ApoE does not cross the blood brain barrier (BBB), several recent studies have shown evidence that it still contributes to LOAD pathology, including amyloid accumulation, BBB dysfunction, and cognitive impairments. However, no study has been conducted to test whether the beneficial effects of peripheral ApoE2 can help mitigate the harmful effects of cerebral ApoE4.

Methods: Our lab is employing a novel *APOE*4-Switch-*APOE*2 (*APOE*4s2) mouse model which uses the Cre-LoxP system. At birth, these mice express the high-risk *APOE4* allele in all cell types. Following activation of Cre recombinase, affected cells will stop producing ApoE4 and will instead produce ApoE2. By delivering Cre recombinase via an AAV with the hepatocyte-specific TBG promoter, we have generated mice that express ApoE2 in the periphery while retaining normal ApoE4 expression in the brain. To verify this strategy, we used Western blot with antibodies specific to the ApoE2 and ApoE4 proteins. Off target organs were imaged to check expression of GFP, a component of the AAV and the Ai9 Cre reporter, which is expressed when Cre recombinase is

activated. To assess BBB integrity, we are using Two-photon microscopy to visualize the leakage of dextran into the BBB in real time. To assess cognition and memory, we will use two behavioral tasks: The Y-maze and the contextual and cued fear conditioning tasks.

Results: Western blots of plasma from AAV-injected animals show expression of ApoE2 but not ApoE4, whereas PBS-injected animals express ApoE4 and not ApoE2. Imaging of off-target organs shows little to no GFP or Ai9 expression in the brain, kidney, heart, spleen, lung, or intestine. Together, these results confirm a liver-specific E4→E2 replacement in our model system. Feasibility data using two photon microscopy shows significant leakage of 10kDa, but not 40kDa dextran, in aged ApoE4 animals.

Conclusion: Western blot and fluorescent imaging show that the AAV strategy in *APOE*4s2 mice is both highly efficient and highly specific to the liver. Preliminary two-photon imaging of dextran leakage demonstrates feasibility for measuring BBB integrity in this model. A follow-up experiment will test whether hepatic ApoE2 expression is sufficient to mitigate dextran leakage in the presence cerebral ApoE4. Future studies will test cognition and memory in these animals with the Y-maze and the contextual and cued fear conditioning tasks.

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

Category: Postdoctoral Fellow

Authors: Khine Zin Aung [1,2], Xian Wu [1,2], Erin L. Abner [2,3], Peter T. Nelson[2,4], David W. Fardo [1,2], Yuriko Katsumata [1,2]

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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 hypothesized as important contributing factors. However, the exact mechanisms are still unknown. Research suggests that cancer patients receiving chemotherapy are more 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 may be useful for cancer screening. To date, there have been no published findings connecting cancer with AD and related dementia disorders, through PRS. Here, we investigated the genetic connection between various types of cancer and dementia using PRS. Our research framework is (a) to examine associations of cancer PRS with dementia-related phenotypes including clinically diagnosed AD, cognitive test scores, and disease-specific neuropathologies; (b) to identify which types of cancer (and chemotherapies) are more likely to be linked to the inverse association by examining correlation between each PRS of cancer types and AD; and (c) to explore which underlying and interconnected mechanisms are contributing to these associations.

Methods: We obtained phenotype data from the National Alzheimer's Coordinating Center (NACC), genotype data from the Alzheimer's Disease Genetics Consortium (ADGC), and summary statistics for each cancer type from the United Kingdom (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 diagnostic criteria were based on clinical scores and neuropathology scores (Thal Ab phase, Braak NFT stage, CERAD score, and NIA-AA Alzheimer's disease neuropathologic change). The analyses were conducted both cross-sectionally and longitudinally.

Results: Preliminary results indicated that individuals having higher cancer PRS were relatively likely to have a lower risk of AD/dementia-related phenotypes. Underlying shared mechanisms will be explored.

Conclusion: Our results will gain insights into genetic risks for both AD and cancers. These insights will be useful for early detection and prevention and may contribute to precision medicine for treatment while lowering the side effects encountered during anti-cancer treatment.

Acknowledgments:
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ABSTRACT TITLE: Increased eIF5A Hypusination Drives Neuroinflammation and TDP-43 Pathology in a Transgenic Mouse Model**.**

Category: Undergraduate student

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Background: The cytoplasmic accumulation and inclusions of TAR DNA-binding protein (TDP-43) are described as hallmarks of Alzheimer's Disease and Limbic-Predominant Age-Related TDP-43 Encephalopathy (LATE). Eukaryotic Translation Initiation Factor 5A (eIF5A) is the only known eukaryotic protein that undergoes a post-translation modification: hypusination. This modification of lysine (K) to hypusine (hypk50) is catalyzed by the 2-step enzymatic activity of deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). Our laboratory has previously shown that this modification affects TDP-43 phosphorylation and aggregation. Recent literature, including ours, also suggests that inflammatory pathways, driven by the activation of microglia and astrocytes, can directly contribute to neurodegenerative disease pathogenesis. These findings, coupled with literature demonstrating that hypusination is crucial to the macrophage activation state, provided the premise of the current study. Therefore, we investigated the effects of induced eIF5A^{hypK50} via AAV-directed DHS/DOHH overexpression on TDP-43 pathology and

neuroinflammatory profile in a TDP-43 transgenic (TAR) mouse model.

Methods: Animal Surgery: Non transgenic (Non-Tg, n=5) and heterozygous TAR (TAR4, n=4-5) mice received cortical and hippocampal injections of AAV9-empty capsids, eIF5A, or DHS/DOHH. **Immunohistochemistry (IHC):** Total TDP-43 expression was analyzed with rabbit (Proteintech, 1:50,000) antibody. Microglial activation (IBA-1) was measured with goat (Abcam, 1:10,000) antibody. Homeostatic microglia levels (TMEM119) were analyzed with rabbit (Abcam, 1:10,000) antibody. eIF5A^{hypK50} levels were analyzed with (21st Century, 1:5,000) hypusine specific antibody. Reactive astrocytes (GFAP) were measured with rabbit (Dako, 1:3,000) antibody. Images were analyzed with NearCyte, Zeiss, and Morpheus/ImageJ software.

Results: Our analysis revealed increases in eIF5A^{hypK50} following both AAV-DHS/DOHH & AAV-eIF5A+DHS/DOHH injections ($p<0.001$). Considering the relationship between eIF5A^{hypK50} and macrophage function, we investigated the effects of this modification on glial activation. Analysis of microglial morphology showed increased numbers of ameboid profiles following both AAV-DHS/DOHH & eIF5A+DHS/DOHH injections ($p<0.0001$). We also identified a significant reduction in homeostatic microglia levels following the induction of hypusination ($p<0.01$). An increase in reactive astrocyte levels following AAV-DHS/DOHH & AAV-eIF5A+DHS/DOHH injections was also observed ($p<0.001$). Notably, we identified TDP-43 cytoplasmic inclusions comparable to FTD/ALS/LATE human disorders following increased hypusination.

Conclusion: This study provides evidence for the regulatory role of hypusinated eIF5A on neuroinflammation and reinforces the relationship between eIF5A and TDP-43 pathology. Further investigation is required to examine the exact mechanism of eIF5A in TDP-43 proteinopathies.

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Poster Number 32

ABSTRACT TITLE: Kir6.2-K_{ATP} channels couple metabolism and neuronal activity

Category: Graduate student

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Background: Metabolism is intrinsically linked to sleep-wake states. Neuronal excitability is coupled with metabolism and modulates sleep-wake activity. ATP sensitive potassium (K_{ATP}) channels act as metabolic sensors to couple metabolism with cellular excitability. We hypothesized that K_{ATP} channels act as metabolic sensors to regulate sleep. We found that K_{ATP} channels composed of Kir6.2 subunits are expressed on excitatory and inhibitory neurons and are differentially expressed in the Alzheimer's brain. Additionally, Kir6.2-K_{ATP} channels couple changes in cerebral metabolism with neuronal activity and amyloid-beta release, suggesting a role for Kir6.2-K_{ATP} channels in Alzheimer's disease. Here we explored how Kir6.2-K_{ATP} channel deletion in mice affected brain metabolism, neuronal activity, and sleep.

Methods: Cerebral glycolytic flux in Kir6.2-/- mice was determined via stable isotope-resolved metabolomics. Neuronal activity and sleep-wake staging was assessed via diurnal recordings of cortical activity using skull screw electroencephalography (EEG). Intracranial biosensors measured sub-second fluctuations of hippocampal interstitial fluid (ISF) glucose and lactate in concert with EEG recordings. Kir6.2-/- mice were injected with glucose to determine brain responses to peripheral metabolic challenges. Lastly, behavioral testing explored changes in arousal and learning/memory in Kir6.2-/- and WT mice.

Results: Kir6.2-/- mice shunt brain glucose towards glycolysis and away from neurotransmitter synthesis. EEG absolute power was dampened in Kir6.2-/- mice, suggesting alterations in neuronal activity due to reductions in neurotransmitter synthesis. Kir6.2-/- mice spent more time awake in the light period, primarily around light/dark (LD) transitions. Diurnal rhythms of brain ISF lactate were altered in Kir6.2-/- mice, with lower peak amplitude that correlated with a 2.5-hour phase delay in this rhythm, around LD transitions. ISF glucose peak amplitude was higher in Kir6.2-/- mice, peaking at ZT19. Given that changes in metabolim and neuronal activity clustered around LD transitions, we explored whether Kir6.2 channel subunits (*Kcnj11* and *Abcc8*), had circadian oscillations. Both *Kcnj11* and *Abcc8* are rhythmic. Kir6.2-/- mice lose fluctuations in ISF lactate during sleep-wake transitions. Kir6.2-/- mice are unresponsive to glucose challenges, lacking changes in time spent in wake or fluctuations in ISF lactate, as seen in WT mice. Together, we hypothesized that Kir6.2-K_{ATP} channels play a role in arousal. We found that Kir6.2-/- mice have decreased startle response and anxiety-like behaviors, and slight alterations in memory on the MWM task.

Conclusion: Kir6.2-K_{ATP} channels act as both a metabolic and circadian sensor. Their absence is sufficient to disrupt sleep and arousal. Given the changes in KATP channel expression in the human AD brain, this suggests a possible role for KATP channel activity in sleep and circadian dysfunction in AD.

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ABSTRACT TITLE: Alzheimer's disease related tau pathology alters sleep, metabolism, and neuronal activity in the P301S PS19 mouse model.

Category: Graduate student

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Background: Alzheimer's disease is defined by the pathological development of amyloid-beta (Aβ) and hyperphosphorylated tau (ptau) aggregation. While the presence of both biomarkers is necessary for diagnosis, tau pathology develops later in disease coinciding with cognitive impairment and neurodegeneration. AD patients often exhibit other symptoms like metabolic and sleep impairments. Currently, it is unclear if changes in metabolism and sleep are a cause or consequence of Aβ or tau aggregation. First, we demonstrated that Aβ aggregation causes metabolic and sleep dysfunction in mouse models overexpressing Aβ (e.g. APP/PS1). Conversely, we demonstrated that cerebral and peripheral changes in glucose metabolism cause elevations in brain lactate levels to stimulate wakefulness. This bidirectional relationship is well described relative to Aβ, sleep, and metabolism; however, it is unclear if tau aggregation similarly alters metabolism and sleep. Therefore, we investigated how tau pathology alters sleep, metabolism, and excitability in mice.

Methods: To explore changes in peripheral metabolism, body weights were recorded and glucose tolerance tests were conducted on P301S PS19 and wild type (WT) mice. Mice were dosed with 2g/kg of glucose followed by blood glucose measures every 15-minutes for two hours. The TSE Phenomaster metabolic platform was used to explore peripheral metabolism over the diurnal cycle via indirect calorimetry, food intake, and locomotion. Paired glucose and lactate biosensors placed in the mouse hippocampi tracked second by second fluctuations in the interstitial fluid (ISF) to examine how cerebral metabolism changes with age or tau pathology. EEG and EMG electrodes recorded sleep-wake cycles over a 3-day period. Methods done in 3, 6, and 9 month old P301S and WT female mice (n=6-12).

Results: Tau pathology increases whole body utilization of carbohydrates shown by GTT and respiratory exchange ratio; yet, this is not accompanied by an increase in total energy expenditure. In the brain, diurnal fluctuations of ISF glucose and lactate are exaggerated with tau pathology and early neurodegeneration, suggesting increased cerebral glucose utilization with tau

aggregation. Disruption in sleep-wake cycles occurs at earlier stages of pathology, with increased time in wake and decreased sleep, specific to the light phase. Changes in both NREM and REM were observed, but effects on total sleep depend heavily on REM with decreased bout number and duration. Further, power spectral analysis revealed delta, theta, and beta bands were altered during various sleep/wake states with tau aggregation, suggesting alterations in excitatory/inhibitory balance.

Conclusion: Results from this study suggest that early tau aggregation and neurodegeneration are associated with altered neuronal excitatory/inhibitory balance. These effects ultimately cause alterations in sleep-wake cycles, potentially due to increased peripheral and cerebral glucose metabolism.

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ABSTRACT TITLE: The Role Of Oxidative Stress In Cerebrovascular Pathology Of Alzheimer's Disease.

Category: Staff

Authors: Moltira Promkan[1,2], Susan D. Kraner[1], Irina A. Artuishan[1], Peter T. Nelson[1], Pradoldej Sompol[1]

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Background: Oxidative stress is a critical mechanism in the pathogenesis and pathophysiology of neuronal disorders such as Alzheimer's disease (AD). It also contributes to vascular injury. Cerebrovascular lesions are highly comorbid with AD pathology and can exacerbate disease progression while reducing the efficacy of treatment. In this study, our objective is to examine the nitration status of fibronectin, a multifunctional extracellular matrix protein found in both the bloodstream and brain parenchyma, where it plays a role in maintaining vascular and perivascular integrity. Additionally, study of mitochondrial antioxidant enzyme, manganese superoxide dismutase (MnSOD), is ongoing.

Methods: We conducted immunolabeling to assess the levels of fibronectin, nitrotyrosine and MnSOD in postmortem brain specimens from individuals with Alzheimer's disease, which were confirmed to exhibit vascular pathology. These specimens were obtained from the UK-ADC brain bank.

Results: We found various lesions associated with various stages of vascular and brain pathology. The immunoreactivity of fibronectin and nitrotyrosine around multiple arterioles and venules indicates acute vascular leakage. Moreover, increased levels of fibronectin and nitrotyrosine in reactive astrocytes surrounding these vessels suggest that oxidative stress is involved in astrocyte activation.

Conclusion: Our findings provide evidence that oxidative damage to fibronectin may serve as a novel biomarker and further reinforce the connection between oxidative stress and vascular complications in Alzheimer's disease. This could be advantageous for future research exploring the combined pathology of AD.

Acknowledgments:
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ABSTRACT TITLE: *In vigilo* age- and sex-dependent alterations in S1 neuronal network dynamics may contribute to gait dysregulation

Category: Staff

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Background: 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.

Methods: Here, we used two-photon imaging of awake, ambulating mice, to acquire GCaMP8f signal from the hindlimb somatosensory cortex (S1HL) region, during ambulation and while stationary. In order to investigate age- and sex-related changes in the neuronal network, a continuous wavelet transform-based binarization and pair-wise correlation coefficient analysis was introduced in MATLAB to extract measures of network communication at single-cell across hundreds of neurons. Following imaging, gait behavior was characterized to test for changes in locomotor stability.

Results: During ambulation, in both young (~4 months) and aged mice (~22 months), an increase in network connectivity and synchronicity was noted. An age-dependent increase in network synchronicity was observed in ambulating 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 S1HL Ca^{2+} dynamics and network synchronicity are potential contributors of locomotor stability.

Conclusion: We believe this work raises awareness of central elements at play in S1, where neuronal network dysregulation is seen with aging, perhaps highlighting potential therapeutic targets that may help offset age-dependent increases in falls.

Acknowledgments: P01AG078116 & R01AG033649

ABSTRACT TITLE: Examining the neuroinflammatory roles of astrocyte-specific RelA in a 5xFAD mouse model of cerebral amyloidosis

Category: Graduate Student

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Background: Despite consistent overlapping evidence suggesting astrocyte involvement in microglial responses linked with Alzheimer's disease pathology, little work has been accomplished to examine potential mechanisms driving feed-forward signaling between these two critical cell populations. In order to determine whether canonical NFkB signaling is involved in astrocyte:microglia crosstalk, we created a novel mouse model that combines astrocyte-specific conditional removal of the NFkB RelA subunit in tandem with harboring the 5xFAD familial amyloid mutations.

Methods: four genotypes of mice were created: 1) *WT-RelA$^{FL/FL}$*, 2.) *WT-Aldh1l1CreERT2^{+} RelA$^{FL/FL}$,* 3.) *5xFAD^{+}RelA$^{FL/FL}$*, and 4.) *5xFAD^{+} Aldh1l1CreERT2^{+} RelA$^{FL/FL}$*. At 7 months of age, all mice received IP (5 daily injections) of tamoxifen. 3 weeks following tamoxifen, mice were examined for cognitive function using Morris Water Maze. Following completion of MWM, mice were euthanized for tissue biochemistry, histology, and single cell RNA sequencing.

Results: MWM results showed improvement of working memory in the 5XFAD-conditional knockout (cKO) group compared to the 5XFAD-wt groups. scRNAseq demonstrates reductions in the accumulation of "ARM"-like disease associated microglia populations, as well in the 5xFAD-cKO, compared to mice with RelA intact in their astrocytes.

Conclusion:
Our findings may suggest that canonical NFkB signaling in astrocytes is associated with driving poorer cognitive performance in the 5xFAD mouse model and this could be linked with alterations in specific subsets of microglia.

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

Category: Graduate student

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Background: 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.

Methods: We experimentally validated the predicted effects of *rs2405442:T>C* with quantitative polymerase chain reactions (qPCR), and enzyme-linked immunosorbent assays (ELISA). We used Chinese hamster ovary (CHO) cells harboring the synonymous variant and compared it to wildtype CHO cells lacking the variant.

Results: We showed 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.

Conclusion: 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.

Acknowledgments:
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ABSTRACT TITLE: Aging drives chronic exacerbated interactions of disease associated microglia and CD8+ T cells following traumatic brain injury

Category: Graduate student

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Background: Aged individuals are highly susceptible to adverse outcomes following traumatic brain injury(TBI). Despite worsened correlates related to functional outcomes following injury, as well as increased likelihood to acquire a TBI, our understanding of the cellular mechanisms governing the poorer outcomes associated with aged individuals post-TBI are not well-characterized.

Methods: In the current study, we examined microglial heterogeneity following TBI, in our mouse model of focal TBI across both acute (3d) and chronic (28d) intervals in both young (4m) and aged (18m) maleC57B6J mice. At the appropriate interval, brains were collected and processed for single cell RNAseq(scRNAseq) and histology. A follow up study examining the interaction of TBI and aging upon T cell receptor (TCR) sequences was utilized to examine clonal expansion and lymphocyte heterogeneity in young and aged mice chronically after TBI.

Results: Our findings demonstrate that TBI drives diverse transcriptional heterogeneity of microglia in both the young and aged condition. Acutely at 3 days post injury an inflammatory response is seen both in the young and aged mice. However, chronically at 28 days post injury, the microglia from young animals more closely resemble their pre-injury (i.e. Sham) phenotypes, in comparison to the aged brain where there is a persistence of APOE-linked ‘DAM-like’ microglial response. Inferential analyses point toward these aging-related chronic populations of microglia as having distinct transcription factor utilization and metabolic pathway enrichment, including Hif1α upregulation and interferon gamma (INFγ) signaling. Furthermore, linkage with chronically accumulated CD8+ T cells and interferon signaling may underlie these phenotypes. Using single cell TCR sequencing, we demonstrate stark contrasts in the responses due to aging and TBI in the clonal expansion of infiltrated CD8+ T cells chronically following TBI.

Conclusion: Our studies demonstrate that *aging* is a centrally associated with the persistence of chronically reactive microglia in the brain and that these responses are linked, in part, with the protracted accumulation of CD8+ T cells following TBI.

Acknowledgments:
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ABSTRACT TITLE: Breeding History Affects Alzheimer's Related Pathology, Cognition, and Sleep Patterns in Female Mice

Category: Graduate student

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Background: It is well known that fluctuations in hormones can affect sleep quality, cognition, and Alzheimer's Disease (AD) pathology. Drastic increases in sleep disruptions and hormonal changes throughout menopause have been linked with increased AD risk. Other substantial hormonal fluctuations in women, i.e. pregnancy, have been studied very little in regards to sleep and AD. Studies show that in reproductive years, the menstrual cycle affects sleep quality. There is some evidence that pregnancy history may contribute to AD risk and neuropathology, however it is rarely, if ever, accounted for in rodent or human studies and outcomes have been conflicting. In rodents and humans, pregnancy history increased amyloid-beta (Aβ) levels, decreased cognition, and increased AD risk. To our knowledge, no studies have been done to specifically investigate the influence of pregnancy history on both sleep and AD. As our studies focus on sleep disruption and AD, and use a mixture of female mice with varying breeding histories, we tested a subset of females to investigate potential influences of breeding history on AD-related pathology, cognition and sleep.

Methods: We used APPxPS1 female knock-in, and wild type (WT) control mice (n=72) aged 9-13 months with retired breeder and non-breeder status. Mice were tested for spatial memory using the Radial Arm Water Maze (RAWM) and brain tissue was analyzed for amyloid-beta Aβ levels via an ELISA. Previous data from a sleep disruption study of 127 mice (aged 7-12 months) was filtered to examine sleep patterns and Aβ levels of female breeders vs non-breeders. Sleep disruption was implemented by way of sleep fragmentation (SF). For 3-4 weeks, mice were exposed to SF or undisturbed sleep. SF consisted of 4 daily

1hr sessions of enforced wakefulness evenly interspersed throughout the light phase. PiezoSleep cages were used to record sleep in study weeks 1 and 3.

Results: Female mice with a history of breeding (1+ litters) had increased levels of Aβ ($p=0.031$). Additionally, number of litters affected Aβ levels with 1 litter being associated with significantly higher levels of Aβ ($p<0.001$). RAWM showed that APPxPS1 breeders were the only tested group with impaired spatial memory. Female breeders that underwent SF showed a greater redistribution of sleep over the 24-hour day, with an increase in sleep in the dark phase as compared to non-breeders.

Conclusion: The increased levels of Aβ, impaired learning abilities, and increased

susceptibility to sleep disruption in female APPxPS1 breeders indicates a relationship between pregnancy history, sleep, and AD-related pathology and behavior. As two-thirds of those with AD are women, determining the underlying mechanisms between hormonal changes and increases in sleep disruption with age in women is critical. Understanding this complicated relationship may aid in developing preventative therapeutics and lifestyle strategies that mitigate AD risk, especially for women.

Acknowledgments:
NIH (AG068215 and NS116824) T32 (AG078110)

Acknowledgments: Add grants later

ABSTRACT TITLE: Ca2+ signaling alterations from the perivascular space in the 5XFAD model of amyloid induced astrocyte reactivity

Category: Graduate student

Authors: Blaine E Weiss, B.S [1,2] John C Gant, PhD [1,2], Pradoldej Sompol, PhD [1,2], Olivier Thibault, PhD [1], Susan D. Kraner, PhD [2], and Christopher M. Norris PhD [1,2]

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Background: Amyloid deposits (Aβ) in the brain are a primary diagnostic marker of Alzheimer's disease, and are associated with the degeneration of synapses and cognitive decline. However, recent advances in cell specific approaches have revealed that glial processes may also contribute to the progression of disease pathology. Astrocytes are a glial cell in the brain responsible for a plethora of essential functions in the brain, such as the removal of synaptic glutamate, and control over cerebrovascular function. Astrocyte Ca2+ signaling is closely coupled to these functions which are impaired with the onset of disease. Our group showed previously in a diet model of VCID, that changes in astrocyte calcium signaling and network synchronicity were associated with deficits in cerebrovascular function. Here we examined the spatial and temporal relationship of astrocyte Ca2+ signals to neurovascular coupling in the 5XFAD mouse model to determine the relationship between amyloid reactive astrocytes and cerebrovascular function.

Methods: 5XFAD and littermate control mice were aged to 6 months before they were injected with AAV2/5-Gfa104-jGCaMP8f into barrel cortex with cranial window installation. Mice were imaged 3 weeks later using two-photon microscopy while awake. Cerebral architecture was illuminated using 500kD rhodamine dextran. Experiments on neurovascular coupling induced functional hyperemia were conducted by timed air puff stimulation of whiskers. Calcium signals were recorded from activated astrocytes at rest and after whisker stimulation. Transients were analyzed by change in fluorescence ΔF/F over different cellular compartments by custom Matlab algorithms. Using a modeling algorithm, vascular tone in response to stimulation was measured, along with attached endfoot calcium levels.

Results: Astrocytes from 5XFAD mice showed a significant reduction in endfoot calcium signaling amplitudes ($p < 0.01$) and delayed rise time compared to wild type controls ($p = 0.012$). Signaling properties such as amplitude, kinetics, and connectivity within networks were also characterized between the 5XFAD and

control mice. Astrocyte calcium signaling was initiated after the onset of neurovascular coupling, and 5XFAD mice exhibited a significant increase in latency between neurovascular tone changes, and peak endfoot calcium transience. ($p = 0.016$)

Conclusion: Amyloid induced pathology induces changes in brain activity at the neurovascular unit. The results support prior studies in lower models connecting astrocyte calcium signaling parameters with the onset of vasodilation. However, with this study we characterize a deficit in paracrine communication evident by the increase in latency between vascular activity, and astrocyte calcium signals.

Acknowledgments:
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ABSTRACT TITLE: Sleep and Eating Rhythms are Associated with Metabolic Risk in Postmenopausal Women

Category: Postdoctoral Fellow

Authors: J. Matthew Thomas[1,2], Philip A. Kern[3], Dorothy D. Sears[4], Samuel E. Armstrong[5], Cody Bumgardner[5], Aaron Mullen[5], Jean L. Fry[6], Courtney Murray[1], Jasmine Coatley-Thomas[1], Julie S. Pendergast[1]

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Background: Postmenopausal women are vulnerable to metabolic dysfunction. Compelling evidence suggests that this is because they lack the protective effect of estrogens. Furthermore, midlife metabolic risk is associated with cognitive decline. Therefore, identifying behaviors associated with metabolic health in postmenopausal women is critical to prevention of late-life cognitive decline and dementia. Few studies have investigated whether postmenopausal women have disrupted eating and sleep-activity rhythms that could contribute to their metabolic dysfunction. The purpose of this study was to investigate the relationship between eating rhythms, sleep, and metabolic risk in postmenopausal women.

Methods: We studied 7 days of sleep and food intake behaviors in overweight postmenopausal women without diabetes, who were not taking hormones (estrogens ± progestin). Sleep timing was assessed by actigraphy and sleep logs. First and last calorie intake times each day were collected from participants with a texting system. Body composition (DXA), BMI, and waist circumference were collected as markers of obesity. Lipid metabolism and glycemic control were assessed by fasting lipid panel

and HbA1c as well as oral glucose tolerance test. Insulin sensitivity was estimated using the Matsuda Index and HOMA-IR.
Results: Forty-five postmenopausal women (age mean ± SEM; 57.6 ± 0.6 years) participated. Later timing of sleep onset was associated with later calorie window and greater BMI, waist circumference, and body fat percentage.
Longer daily calorie window and later time of last calories were associated with decreased insulin sensitivity. Later time of last calorie was also associated with greater waist circumference and BMI.

Conclusion: These data suggest that interventions that shorten the daily calorie intake window and advance the timing of last calorie intake and sleep onset may improve metabolic risk in postmenopausal women.

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ABSTRACT TITLE: Age- and sex-dependent alterations in neuronal calcium network dynamics in S1: relationship to gait

Category: Staff

Authors: Nicholas A. Wright, Leopoldine B. Galopin, Sami L. Case, Jacquelyn Rhinehart, Ruei-Lung Lin, Sophiya Sims, and Olivier Thibault

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Background: 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 non-ambulating 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.

Methods: Here we tested the hypothesis that several calcium-fluxing proteins might be able to reflect alterations seen in individual transients using 2P imaging in S1. We used S1 samples in combination with Western blot analyses to quantify L-VGCC (Cav1.2), NMDAR (NR2B), and ryanodine receptor (RYR2) across 43 samples from young-adult (4 months) and aged (22 months) C57BL/6J males and females. Standard SDS-PAGE protocols in combination with primary and secondary antibodies were used to report on age- and sex-dependent changes in S1 samples.

Results: While an age-dependent reduction in NR2B and Cav1.2 proteins in both sexes was seen, a main effect of sex was noted in NR2B proteins showing increases in females compared to males. RYR2 did not show significant changes across sex or age of the animals, and Cav1.2 levels were unchanged across sex. 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.

Conclusions: These results may highlight a mechanism where increases in NR2B receptors in females mediate the increase in calcium transients seen during ambulation on our 2P microscope. We hope our work also raises awareness about

the central components of gait dysregulation with age and sex, focusing on network alteration in S1 and the influence of neuronal calcium-centric processes.

Acknowledgments:
P01AG078116 & R01AG033649

ABSTRACT TITLE: Behavioral characterization of aged miR-223-3p KO mouse model following traumatic brain injury

Category: Staff

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Background: Alzheimer's disease (AD) is the most common form of dementia and among the top 5 leading causes of death in populations over sixty-five. AD has multifaceted components that comprise both genetic and environmental risks. Traumatic brain injury (TBI) is not only one of the major causes of disability and death in the world, but also a potent risk factor of AD pathophysiology. Population based studies show that TBI incidence peaks in early childhood, early adulthood, and in the elderly. With the growing older demography and increasing lifespan, there has been a rise of interest to understand the interplay between TBI and dementia. Evidences demonstrate that age at the time of injury is crucial and the subsequent recovery is associated with several factors including injury induced neuroinflammation. MicroRNAs (miRNA) are a class of small non-coding RNAs that are involved in posttranscriptional gene regulation. MicroRNA-223 (miR-223) plays an effective role in inflammatory regulation and have been implicated in both TBI and dementia. Our experiments revealed that miR-223 knockout (KO) mice had a significantly increased levels of inflammatory markers such as *IL-1β*, *TNFα*, and *NFκB* in naïve mice. Here, we present the behavioral assessment of aged miR-223 KO mice after sustaining TBI.

Methods: We performed neurobehavioral studies comparing miR-223 KO and WT mice. Our experiment proceeded by establishing two separate aged mice cohort: one cohort that sustained controlled cortical injury (CCI) at the age of 8

months and then aged to 18 months (CCI-Aging cohort) prior to neurobehavioral tests; and the second cohort were aged to 18 months and then sustained CCI (Aged-CCI cohort) two weeks before neurobehavioral tests. A total of five behavioral tests (frailty, nesting, marble burying, open field and Y-maze) were conducted.

Results: Our data showed that there is no significant difference in frailty state, marble burying, and nesting behavior between KO and WT mice regardless of injury or time of injury. Nevertheless, miR-223 KO cohort subjected to CCI at 8 months of age explored less distance and time in the open field tests compared to age and treatment matched WT mice. In Y-maze tests, aged naïve KO mice had a notable reduced novel arm visits compared to age-matched WT mice. However, no statistical difference was seen between sham-surgery of KO and WT mice. Remarkably, both cohorts of KO mice that sustained CCI had a significant less novel arm visits and shorter total distance travels compared to the CCI WT mice.

Conclusion: Overall, these data suggest that deficiency of miR-223 resulted in a cognitive deficit in mice particularly following a brain injury, which may associate with the higher neuroinflammatory state observed in the KO mice.

Acknowledgments:
This project is supported by National Institute on Aging (R01AG082142).

ABSTRACT TITLE: Rose Bengal Photoinduction Mediates Capillary And Astrocyte Injury

Category: Undergraduate student

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Background: 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.

Methods: 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.

Results: 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.

Conclusion: Rose Bengal photoinduction provides an effective model of oxidative stress-induced microvascular pathology. Cerebrovascular damage induced by Rose Bengal photoinduction reduces astrocyte activity. Rose Bengal photoinduction causes a reduction in the networking abilities of the surrounding astrocytes.

Acknowledgments:
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ABSTRACT TITLE: Endogenous circular MAN2A1 RNA expresses a protein

Category: Graduate Student

Authors: Karol Andrea Arizaca Maquera[1], Justin R. Welden[1], Giorgi Margvelani[1], Noémie Robil[4], Pierre de la Grange[4], Álvaro Gonzalo Hernandez[5], Peter T. Nelson[2,3] and Stefan Stamm[1]

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Background: We previously identified numerous circRNAs from human brains at different Alzheimer stages. 276 circRNAs correlates with Alzheimer's disease severity, expressed as Braak stages. For all circRNAs, there is a statistically significant increase and correlation of adenosine to inosine RNA editing with Braak stages. Importantly, this correlation cannot be detected with mRNAs.

Methods: Total RNA was isolated from human brain (entorhinal cortex and frontotemporal) We used polyA+ RNA Dynabeads mRNA purification to select the mRNA for Nanopore sequencing. The samples were also prepped for Illumina sequencing and the results were analyzed through bioinformatics. Circular MAN2A1 was one of the highest circRNAs expressed in the latest Braak stages, we cloned it into constructs with a 3X flag and those were cotransfected with ADAR enzymes in HEK293 cells to evaluate protein expression through Western Blot and the new peptides were detected in Braak human brain by immunohistochemistry.

Results: We found that A>I editing is increased in the latest Braak stages with circular RNAs when compared with linear RNAs. Several circRNAs as CircTau CircMAN2A, circHOMER and circNOGO; are translated after cotransfection with ADAR1. The nanopore sequencing shows regulated retained introns in AD brain, which suggest that AD progression affects the alternative splicing. The circMAN2A1-400 isoform creates a circRNA-specific protein of 18kDa. We raised antisera against the circRNA-specific part of this protein and detected strong expression in human brain. Immunohistochemistry shows cytosolic staining is that concentrated in dots in advanced Alzheimer stages.

Conclusion: Our data indicate that endogenous circMAN2A1 is translated into a protein after its RNA undergoes Adenosine to inosine RNA editing. There is a general increase in A-to-I editing of circRNAs in Alzheimer's disease, suggesting that

an increase of translation of circRNAs occurs during Alzheimer's disease development. Finally, the increase of circRNAs expression and translation generates a new specific proteome for Alzheimer's Disease

Acknowledgments:
NIH-R21 AG064626-01, DoD AZ180075.

ABSTRACT TITLE: Characterizing ATP:ADP using the PercevalHR nanosensor in mixed hippocampal cultures

Category: Graduate student

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Background: 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 (E18) were extracted and maintained for 12-16 days *in vitro* (DIV). Cultures were treated with lentivirus (Human Ubiquitin C promoter) containing the PercevalHR nanosensor.

Methods: 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).

Results: Glutamate and KCl resulted in rapid decreases in ATP:ADP ratios. Insulin did not demonstrate any changes 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.

Conclusion: 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).

Acknowledgments:
Funding support was granted to OT by via the National Institute of Aging (P01AG078116)

ABSTRACT TITLE: Comparing detection sensitivity of CCAAT-Enhancer Binding Protein-β antibodies in human brain tissues

Category: High school student (Junior)

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Background: Alzheimer's Disease (AD) is a major type of dementia that primarily affects the elderly. Currently, there are more than 6 million people living with AD and as life expectancy increases, the number of people affected by AD may grow to more than 12 million in the US by 2050. AD is caused by many genetic and environmental factors. Apolipoprotein E (*ApoE*) is one of the genetic risk genes strongly associated with AD. Furthermore, neuroinflammation plays a critical role in the development of AD. CCAAT-Enhancer Binding Protein-β (C/EBPβ) is a transcription factor that regulates inflammatory processes and ApoE. To better understand the role of C/EBPβ in regulation of neuroinflammation and ApoE, it is necessary to effectively detect it. In this project, we tested several C/EBPβ antibodies and compared their immunoreactivity in human brain tissues.

Methods: Sections of formalin-fixed paraffin-embedded (FFPE) human superior middle temporal gyrus (SMTG) were obtained from SBCoA. Following antibodies were purchased and tested: CEPB-C-terminal antibody (ab32358, Abcam), Phospho-C/EBPβ antibody (3084, Cell Signaling) , CEBPB Polyclonal antibody (23431-1-AP, Proteintech), C/EBP beta Antibody (H-7) (sc-7962, Santa Cruz), and C/EBPβ-1H7 antibody (606202, Biolegend). VECTASTAIN® Elite® ABC-HRP Kit, Peroxidase (Standard) (PK-6100) and ImmPACT NovaRED® Substrate Kit, Peroxidase (HRP) (SK-4805) were from Vector Laboratories. Standard immunohistochemistry (IHC) procedures were carried out in this project. Briefly, slides were incubated at 40°C overnight prior to deparaffinize in a series of xylene and alcohol. The tissues then underwent antigen retrieval using selected buffers. Endogenous peroxidase activity was blocked by treating sections in 3% H_2O_2 in methanol. The slides were blocked in 5% goat or horse serum prepared in TBS + 0.1% Triton-X (TBST) before incubating in primary antibodies at desired dilutions overnight. Following washes, slides were incubated with secondary antibodies, then ABC solution, and signals were developed with NovaRED or DAB, counterstained with hematoxylin. Finally, slides were dehydrated, covered with coverslip, imaged, and analyzed.

Results: The tested antibodies returned various results. Santa Cruz C/EBP beta antibody returned strong signals resembling plaque-like structures and glia-like cells. Cell Signaling Phospho-C/EBPβ antibody also returned strong signals resembling plaques and cells. Proteintech CEBPB antibody seemed to return positive signals, but was very weak. Antibodies from Abcam and Biolegend did not return visible positive signals.

Conclusion: Of the five antibodies tested, Santa Cruz C/EBP beta and Cell Signaling Phospho-C/EBPβ antibodies both returned strong signals and similar detection patterns, which are good candidates for further experiments. Nevertheless, we will continue to test other antibodies and IHC conditions to optimize the C/EBPβ detection in human brain tissues.

Acknowledgments:
This project is supported by National Institute on Aging (R01AG082142).

ABSTRACT TITLE: The functional role of astrocyte p38α in neuroinflammation and tau burden in aged mice receiving high fat diet

Category: Postdoctoral fellow

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Background: Insulin resistant type 2 diabetes (T2D) is a chronic inflammatory disease that significantly increases the risk of Alzheimer's disease (AD). While the pathological relationship remains relatively undefined, neuroinflammation is hallmark in both AD and T2D. Neuroinflammation is a transient response of activated astrocytes and a targetable feature of AD and other tauopathies. Chronic astrocyte activation in compounding diabetogenic and AD contexts is influenced by the p38α kinase, inhibitors against which are linked to the amelioration of tau burden, reduced production of neuroinflammatory cytokines, and improved memory function. Here we propose the use of high fat diet (HFD) to examine the impact of prodromal T2D in aged mice with or without conditional astrocyte p38α knockout (KO). This study will provide insight into the functional effects of astrocyte p38α and subsequent neuroinflammatory, tauopathic, and cognitive changes under a diabetogenic diet.

Methods: Mice at human-equivalent middle age (~12 months) with or without astrocyte p38α KO will receive non-invasive baseline metabolic assessments, including blood panels, MRI, and behavioral assessments. Counterbalanced groups across p38α KO conditions will then be maintained on standard diet (STD) or switched to HFD for the subsequent 6 months. At 18 months, the combined effects of p38α KO and longitudinal HFD will be measured by repeating the initial assessments. Following animal sacrifice, immunohistochemical and western blot assessments will be conducted to identify AD- and T2D-related pathological brain changes in the hippocampus and cortex. Together, these findings will determine whether astrocyte p38α KO ameliorates the effect of HFD on dysregulated metabolism, neuroinflammation, tau pathology, and behavioral performance.

Results: We anticipate baseline measures to be equivalent between the p38α KO and wildtype mice. We hypothesize that the astrocyte p38α KO will longitudinally dull metabolic and neuroinflammatory responses to chronic HFD. Specifically, compared to wildtype mice receiving chronic HFD, we expect the astrocyte p38α KO will rectify behavioral deficits and terminal

pathological brain alterations. The compounding pathological effects of late-age and HFD in the wildtype mice will be evidenced at the terminal endpoints by impaired behavioral performance, tau hyperphosphorylation, and glial overactivation – all of which we hypothesize will be rescued in the p38α KO group.

Conclusion: This study will elucidate how astrocyte p38α interacts with diabetogenic diet, sex, and age to differentially produce brain dysfunction. Findings will be clinically relevant, given that p38α inhibitors are currently in clinical trials to combat AD pathology in humans, although p38α inhibitors have yet to be used in individuals exclusively with comorbid T2D and AD.

Acknowledgments:
This work is supported by the National Institute on Aging *Translational Research in Alzheimer's and Related Dementias* T32 AG078110.

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

Category: Postdoctoral Fellow

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Background: 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 (p38α) MAPK, a regulator of neuroinflammation, may represent an effective target for AD therapies. For example, MW150, a small molecule p38α inhibitor, was shown to improve cognition and decrease cytokines in amyloidogenic mice. However, p38α 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]).

Methods: 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.

Results: 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.

Conclusion: Our findings support further investigations of p38α inhibitors in the clinic, and also suggest that neuronal p38α 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).

Acknowledgments:
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ABSTRACT TITLE: Citrullination of TDP-43: Structural effects on pathological liquid-liquid (LLPS) to liquid-solid phase separation (LSPS) transition state of the protein

Category: Graduate student

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BACKGROUND: TAR DNA-binding protein 43 kDa (TDP-43) is a nuclear DNA/RNA binding protein with pathological associations in neurodegenerative diseases. TDP-43 proteinopathy is characterized by cytoplasmic mislocalization and aggregation alongside nuclear loss of function. Our laboratory uncovered a previously unknown but significant post-translational modification (PTM) of TDP-43; citrullination by peptidyl-arginine deiminase type-4 (PAD4), resulting in an amino acid charge change from a positive arginine to neutral citrulline. This study aims to investigate the impact of this PTM on protein structure and folding properties as key factors in pathological protein kinetics and aggregation.

METHODS: Recombinant human TDP-43 and PAD4 were used to perform in vitro enzymatic reactions resulting in citrullinated TDP-43 (citR TDP-43). Citrullinated sites were confirmed by Western blot utilizing our novel site-specific citR TDP-43 antibodies. TDP-43 and citR TDP-43 were also incubated with yeast RNA. Low Complexity Glycine- regions of recombinant human TDP-43 (TDP-43^{LCD}) were citrullinated (citR TDP-43^{LCD}) and incubated with RNA. We performed turbidity, Thioflavin-T (ThT), and TEM analyses on each sample. Reactions of citR TDP-43 were conducted with logarithmic concentrations of BB-Cl-Amidine, a PAD inhibitor, and citrullination levels were analyzed by Western blot.

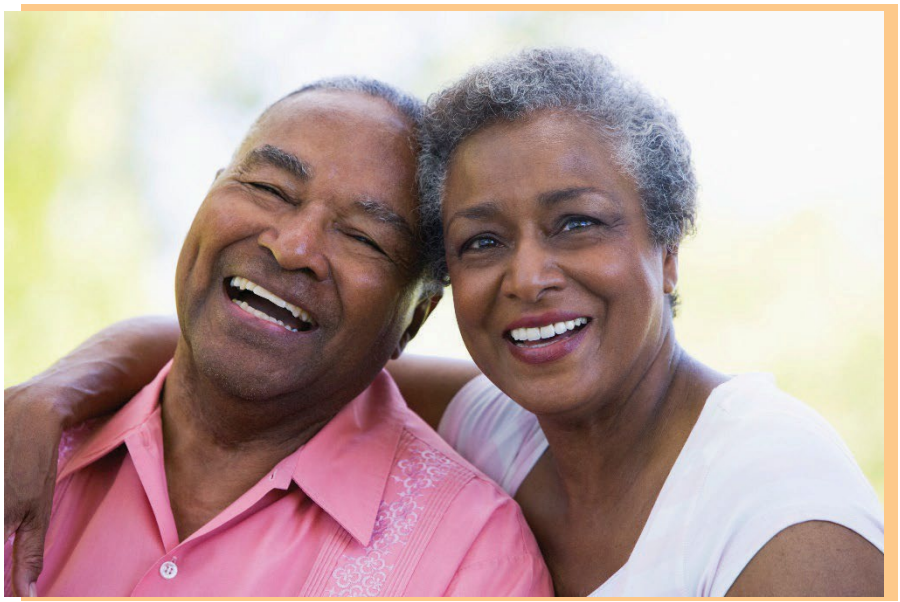
RESULTS: TDP-43 is prone to aggregation into globular/amorphous structures. Our findings from TEM imaging and 3D tomography analysis identified that citrullination significantly reduced aggregate volume and size, compared to unmodified TDP-43. Kinetic studies further revealed that citrullination delayed TDP-43 interactions with RNA, contributing to LSPS condensates. Unmodified TDP-43 remained in solution, potentially in LLPS, upon addition of RNA. This was further confirmed using TDP-

43^{LCD} and citR TDP-43^{LCD} in LLPS forming conditions, with time and RNA concentration as altering variables. ThT florescence intensity analysis confirmed that citrullination delayed TDP-43 interaction with RNA and its aggregation by 36 hrs to the advantage of solid condensate formation. The specificity of PAD4 induced citrullination was confirmed by dose-response incubation with BB-Cl-Amidine, a PAD4 inhibitor. We observed a significant band shift in citR TDP-43 compared to untreated citR-TDP-43 by Western blot. Using this metric, we conclude that inhibition of PAD4 activity and TDP-43 citrullination directly impacts protein properties.

CONCLUSION: We posit that citrullination serves as a molecular switch for LLPS to LSPS transitions, possibly through delayed RNA-protein interactions, which can lead to persistent LSPS condensate accumulation and toxicity in TDP-43 proteinopathies. Our work with BB-Cl-Amidine as a PAD4 inhibitor of TDP-43 citrullination shows a potential mechanism and future therapeutic target against TDP-43 proteinopathy.

ACKNOWLEDGMENTS:
TEM, STEM, and TEM tomography were performed with the assistance of the University of Kentucky Electron Microscopy Center; Thioflavin-T kinetic assays were performed with the help of Baylor University College of Medicine; novel citR antibodies were developed in collaboration with 21st Century Biochemicals Inc.; molecular graphics and analyses performed with UCSF ChimeraX, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from National Institutes of Health R01-GM129325 and the Office of Cyber Infrastructure and Computational Biology, National Institute of Allergy and Infectious Diseases.

Sanders-Brown Center on Aging



The Sanders-Brown Center on Aging (SBCoA) was established in 1979, and received funding as one of the original ten National Institutes of Health Alzheimer's Disease Centers in 1985. Internationally acclaimed, the SBCoA is recognized for its contributions to the fight against brain diseases that are associated with aging.

Our vision: The University of Kentucky Sanders-Brown Center on Aging will be recognized locally and nationally as a premier, vitally productive and innovative aging center that effectively translates research findings into interventions and information that will benefit older adults.

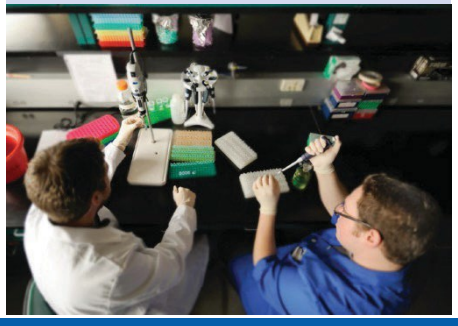
ALZHEIMER'S DISEASE FACTS

Normal Age-Related Memory Changes

- Missing a monthly payment
- Forgetting which day it is and remembering later
- Sometimes forgetting which word to use
- Losing things from time

Warning Signs of Dementia

- Poor judgment and decision making
- Inability to manage a budget
- Losing track of the date or the season
- Misplacing things and being unable to retrace steps to find



More than 100 faculty and staff pursuing the following areas of research:

- **Basic and clinical research in Alzheimer's disease**
- **Neurodegenerative disorders**
- **Risk factors for dementia**
- **Healthy brain aging**

A global pioneer in Alzheimer's disease research, the Center has over forty years of published work and 800 study volunteers (some with the disease and some without). These individuals are studied over time and will donate their brains upon death. Our cutting-edge research focuses on identifying problems as early as possible, before memory loss develops, so that Alzheimer's disease can be prevented or delayed.

The ultimate goal of the Center on Aging is to catalyze innovative and outstanding brain research, while ensuring a more rapid rate of progress toward new therapies, so that our volunteers, patients and caregivers become the beneficiaries of our advances in knowledge.

Unless science finds a way to slow the progression of this devastating disease, the United States will see a nearly 50 percent increase in the number of victims by 2030. In addition to the direct impact on the patient, Alzheimer's disease also affects the lives of family members and friends.

The Center is directed by Linda J. Van Eldik, PhD, Professor, Department of Neuroscience, Director, Alzheimer's Disease Research Center and Co-Director, Kentucky Neuroscience Institute

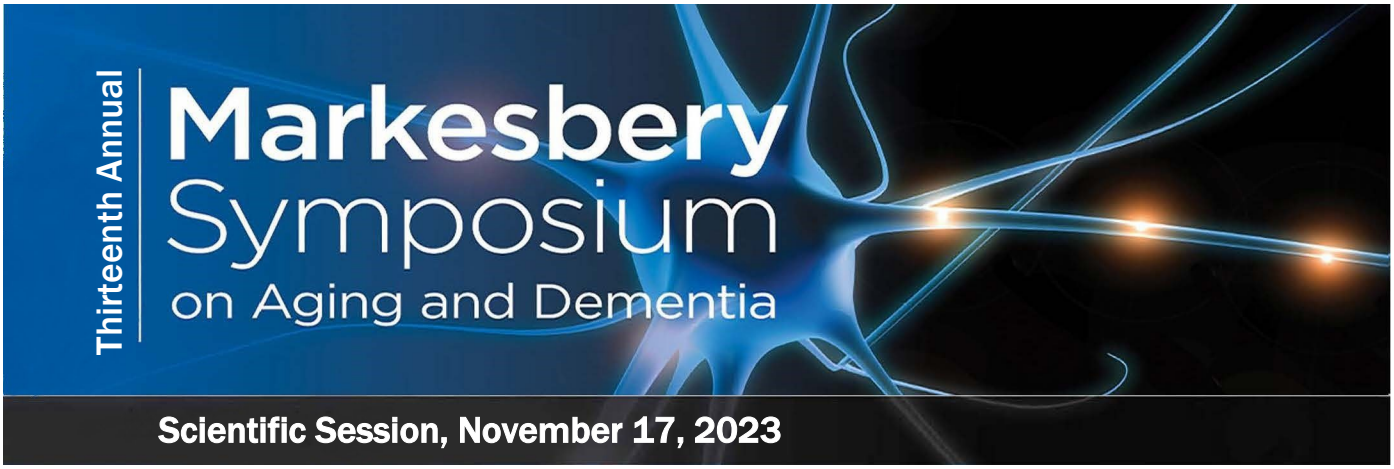
- Alzheimer's disease is the most common cause of dementia, accounting for an estimated 60 to 80 percent of cases. Recent large autopsy studies show that about half of individuals with Alzheimer's dementia have Alzheimer's disease brain changes (pathology) as well as the brain changes of one or more other causes of dementia, such as cerebrovascular disease or Lewy body disease. This is called mixed pathology and, if recognized during life, is called mixed dementia.

- The likelihood of having mixed dementia increases with age and is highest in the oldest-old (people age 85 or older).

- In 2020, approximately 75,000 persons age 65 and older in Kentucky are living with Alzheimer's disease. This number is estimated to increase to 86,000 (14.7%) by 2025.

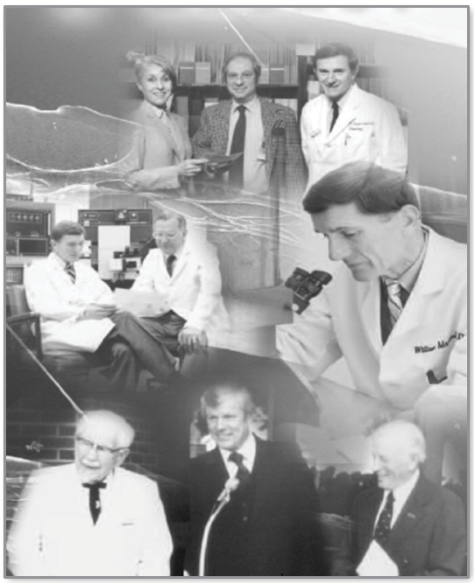
From the 2019 Alzheimer's Association Facts & Figures publication.

Please help us today in our fight against Alzheimer's disease.
For more information on research, clinical trials and ways to get involved,
contact us at 859-323-5550 or visit our website https://medicine.uky.edu/centers/sbcoa/



WILLIAM R. MARKESBERY, MD (1932-2010)

The Markesbery Symposium on Aging and Dementia is named in honor of William R. Markesbery, MD, a gifted scientist and internationally recognized neurologist and neuropathologist. Dr. Markesbery's creativity and commitment to aging research provided the impetus for the University of Kentucky to establish the Sanders-Brown Center on Aging in 1979 and name him as the first director. He held that position until his death in January 2010.



In 1985, Bill Markesbery became the director of the Alzheimer's Disease Research Center, one of the original 10 National Institute on Aging (NIA)-funded centers in the United States, with a primary focus on neuropathology. After more than 35 years, the Alzheimer's Disease Center continues to be funded by NIA, a remarkable achievement that demonstrates the strength and caliber of this program. During his academic career, Dr. Markesbery published more than 400 scientific papers and was one of the world's leading experts on Alzheimer's disease and oxidative stress. He will always be remembered as a compassionate and caring physician, a brilliant researcher, and an inspirational leader.

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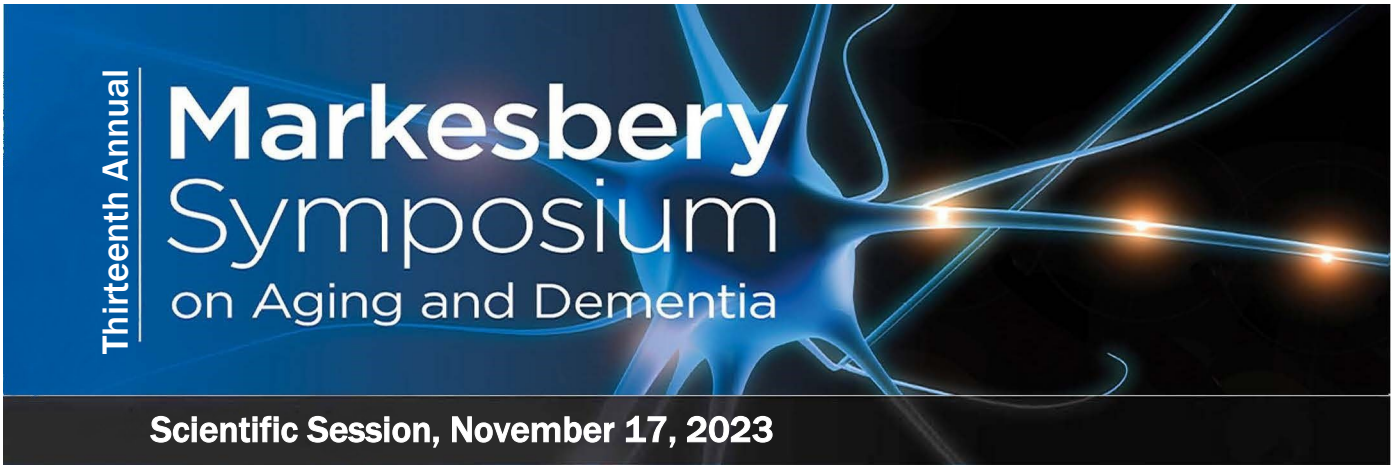

Your opinion matters.

Please take a few minutes to complete the evaluation for this program, so that we may improve future programs.

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