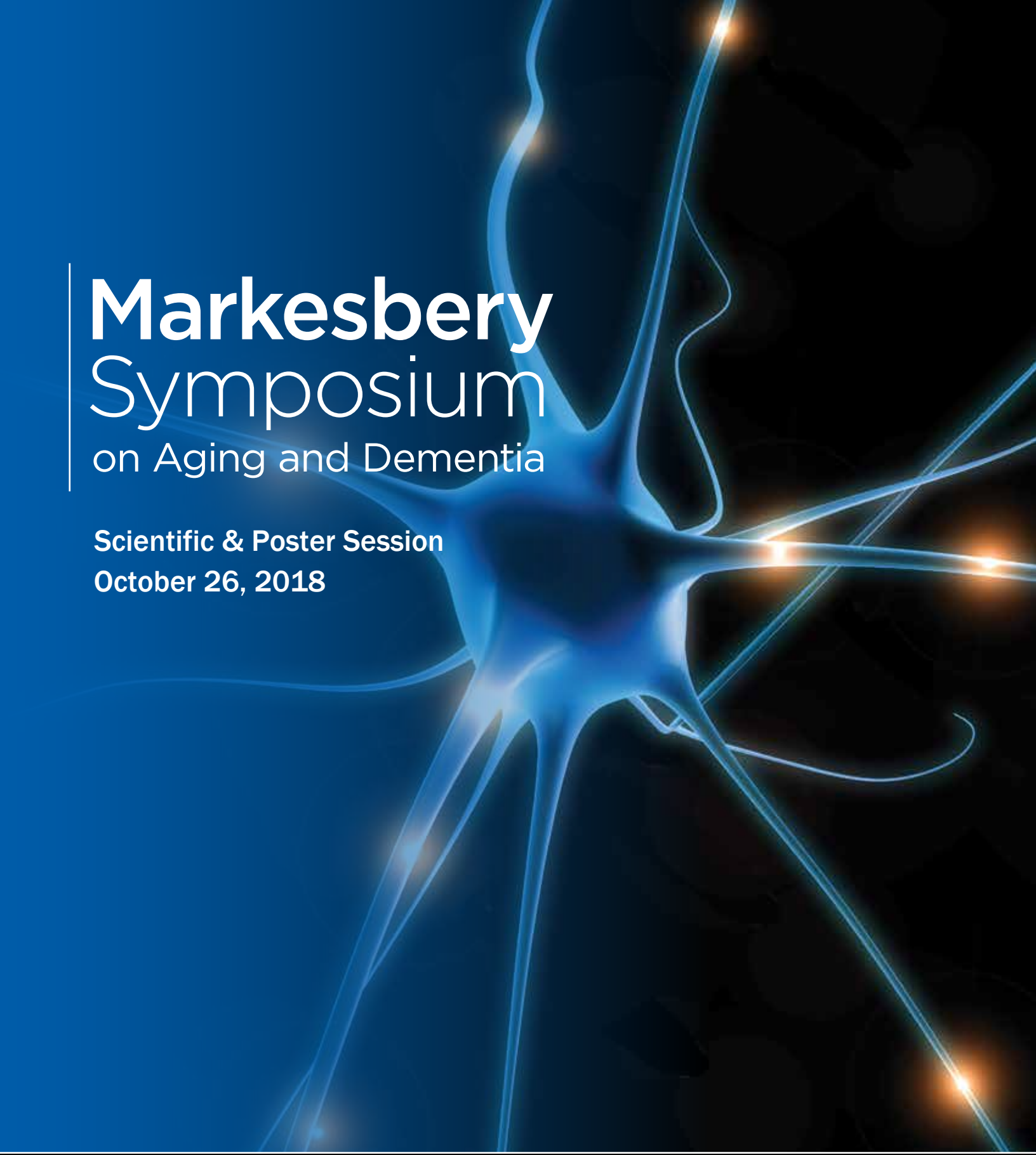
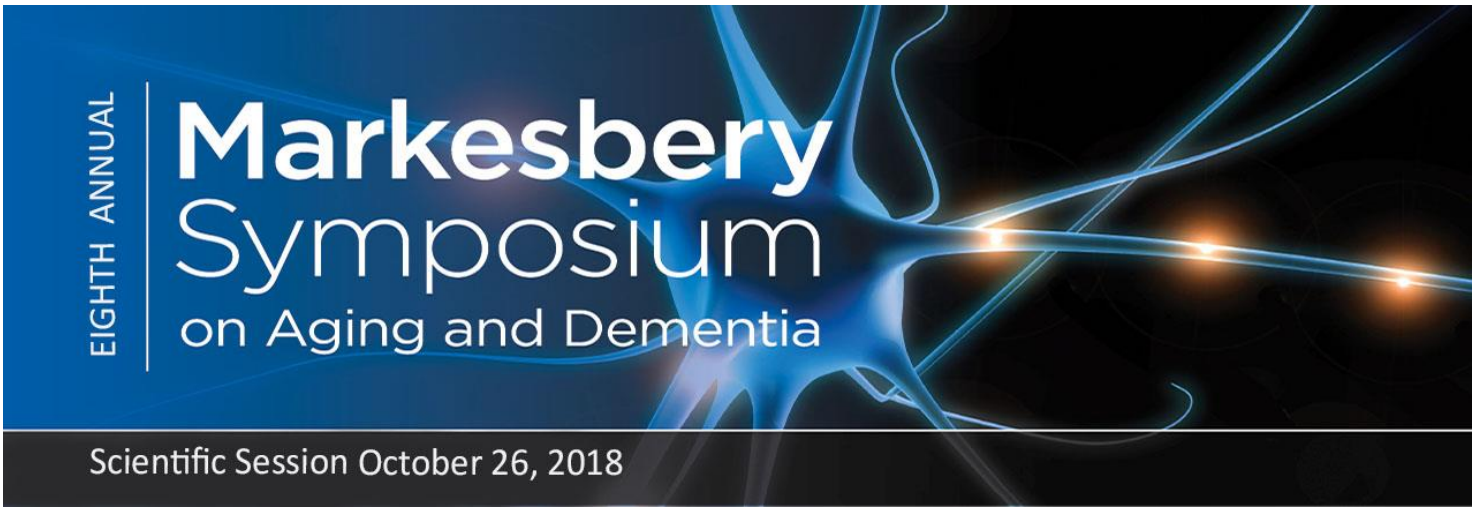


Markesbery Symposium on Aging and Dementia

Scientific & Poster Session
October 26, 2018

Sanders-Brown Center on Aging

Table of Contents



On behalf of the Sanders-Brown Center on Aging, UK HealthCare, and the symposium planning committee, I am pleased to welcome you to the 8th 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 Bill Markesbery's work will live on for generations to come as we continue the work he started here four decades ago.

Over the next two days, in sessions for both the scientific and community audience, 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.

In addition to the presentations conducted by some of the world's leading scientists, we have invited investigators to display posters of their current research on aging and dementia. Please take some time to visit the research poster gallery on display in the atrium and discuss these ongoing studies with the researchers.

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

Symposium Planning Committee:

Elizabeth Head, PhD
Linda Van Eldik, PhD
Steven Estus, PhD
Donna Wilcock, PhD
Fredrick Schmitt, PhD
Ai-Ling Lin, PhD
Paula Thomason
Laura Wright
Beverly Baesler
Hardin Stevens
Jessie Collins
Lisa Greer
Lindsey Clem
Derrick Hord

University of Kentucky

An Equal Opportunity University

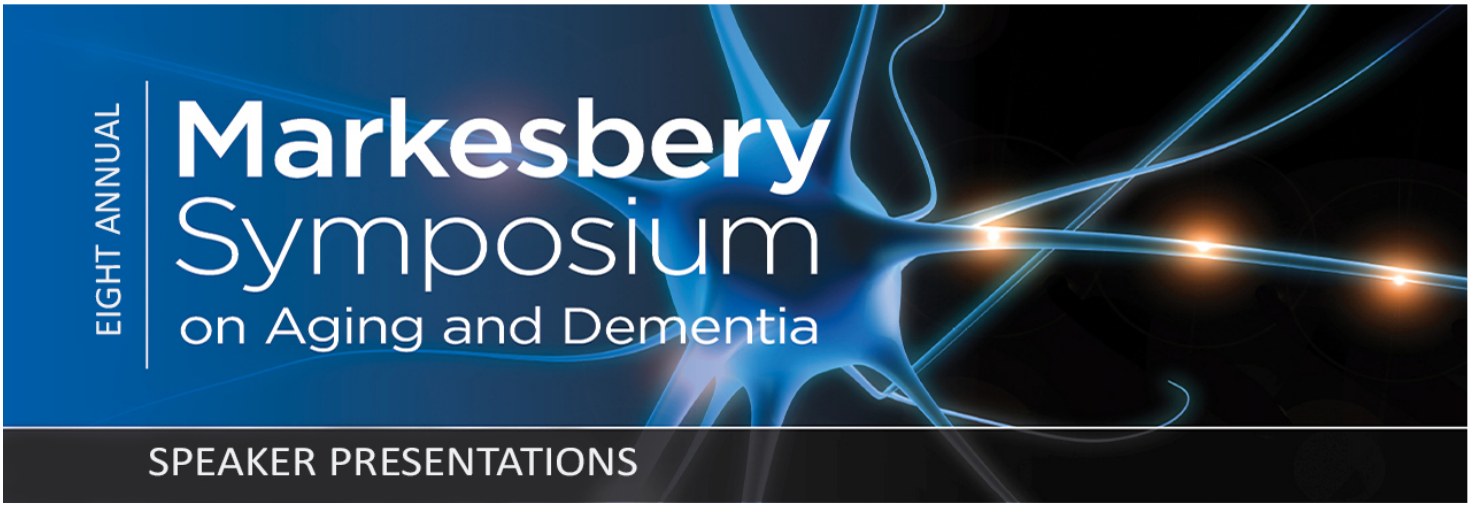
We are grateful to Sanders-Brown Center on Aging
Philanthropy Council
for their continued support and sponsorship

The mission of the Sanders-Brown Center on Aging is to improve the health of the elderly through research, education, outreach and clinical programs.

University of Kentucky College of Pharmacy Todd Building 789 S. Limestone St.

9:00 am	**Check-in begins:** Receive poster assignment number, ID badge, and program	*Atrium*
10:30 am	**Poster Session begins**	*Atrium*
11:30 am	**Boxed Lunch**	*Atrium*
12:45 pm	**Symposium Welcome** Linda Van Eldik, PhD, Director, Sanders-Brown Center on Aging	*Room 152*
	Tribute to William R. Markesbery, MD Mark Lovell, PhD, Professor of Chemistry & Sanders-Brown Center on Aging	*Room 152*
1:00 pm	**Engaging the underserved in brain health and research** Monica Parker, MD Assistant Professor in Neurology; Education Core Member of the Alzheimer's Disease Research Center, Emory University	*Room 152*
2:00 pm	**Identification of novel fluid biomarkers for vascular cognitive impairment and dementia (VCID).** Donna Wilcock, PhD, Professor of Physiology & Sanders-Brown Center on Aging	*Room 152*
2:20 pm	**Metabolic reprogramming and Alzheimer's disease risk: the role of ApoE4"** Lance Johnson, PhD, Assistant Professor of Physiology	*Room 152*
2:45 pm	Break	*Atrium*
3:00 pm	**Preclinical biomarkers of Alzheimer's disease: emerging concepts & clinical utility** Sanjay Asthana, MD FACP, Associate Dean for Gerontology, University of Wisconsin School of Medicine and Public Health; Director, University of Wisconsin Alzheimer's Disease Research Center, Madison, WI	*Room 152*
4:00 pm	**Changes in cerebrovascular pathology as a function of age and Alzheimer disease in Down syndrome.** Elizabeth Head, PhD, Professor of Pharmacology & Sanders-Brown Center on Aging	*Room 152*
4:20 pm	**Modulation of micro-RNA pathways by gemfibrozil in predementia Alzheimer disease: clinical translational research at Sanders-Brown Center on Aging** Gregory Jicha, MD, PhD Professor of Neurology & Sanders-Brown Center on Aging	*Room 152*
4:40 pm	**Poster award presentations and closing remarks** Linda Van Eldik, PhD	*Room 152*

Please complete and return evaluation form; your feedback helps us determine next year's program



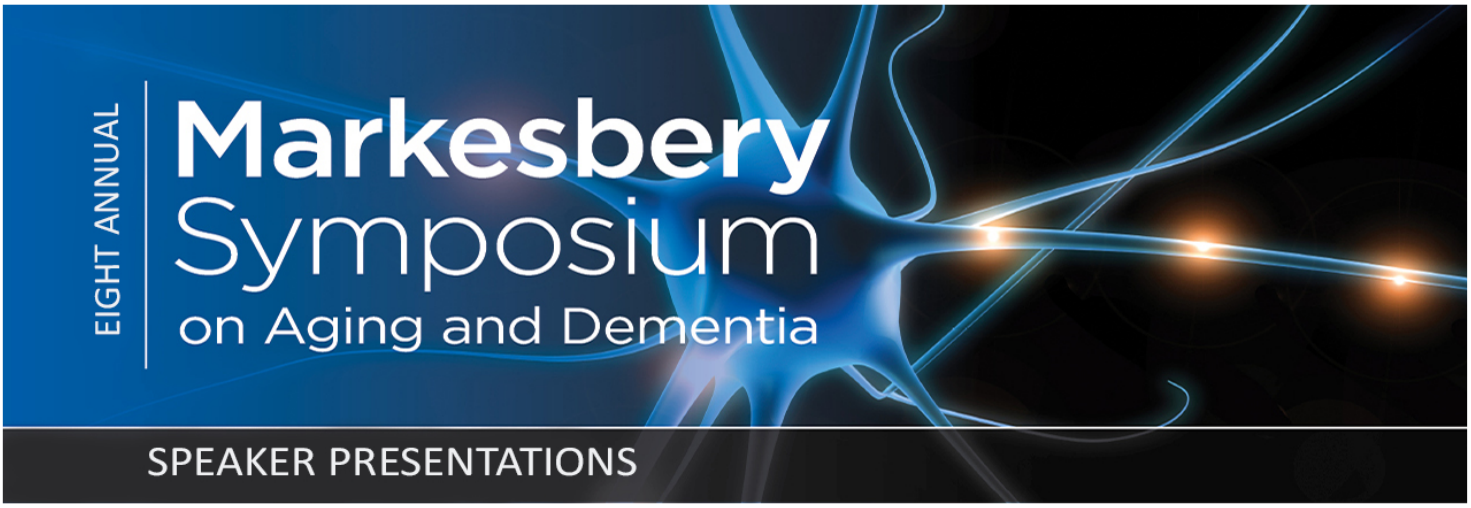
"Engaging the underserved in brain health and research"

Monica W. Parker, MD
Emory University

Assistant Professor in Neurology
Education Core Member and Director
Minority Engagement Core
Emory University Alzheimer's Disease Research Center

Monica Willis Parker, MD is an Assistant Professor in Neurology, and Education Core Member and Director of Minority Engagement Core at Emory University Alzheimer's Disease Research Center (ADRC). She is a graduate of Fisk University and The University of Nebraska Medical Center. She completed a Family Medicine residency at the University of Mississippi Medical Center followed by additional training at Stanford University's "Geriatrics in Primary Care" and Morehouse School of Medicine Faculty Development Programs.

Dr. Parker recently received a National Institute of Health (NIH) Minority Supplement award to study dementia in ethnic persons in the Emory ADRC where she plays an integral part in leading research projects to test caregiver education programs. Under her leadership, the Emory ADRC has established a community-academic partnership, the Registry for Remembrance, to educate and recruit minority persons for long term research participation with the goal to increase participation of ethnic persons in longitudinal neurologic research studies. Dr. Parker frequently speaks to civic and church groups about aging successfully and maintaining brain health.



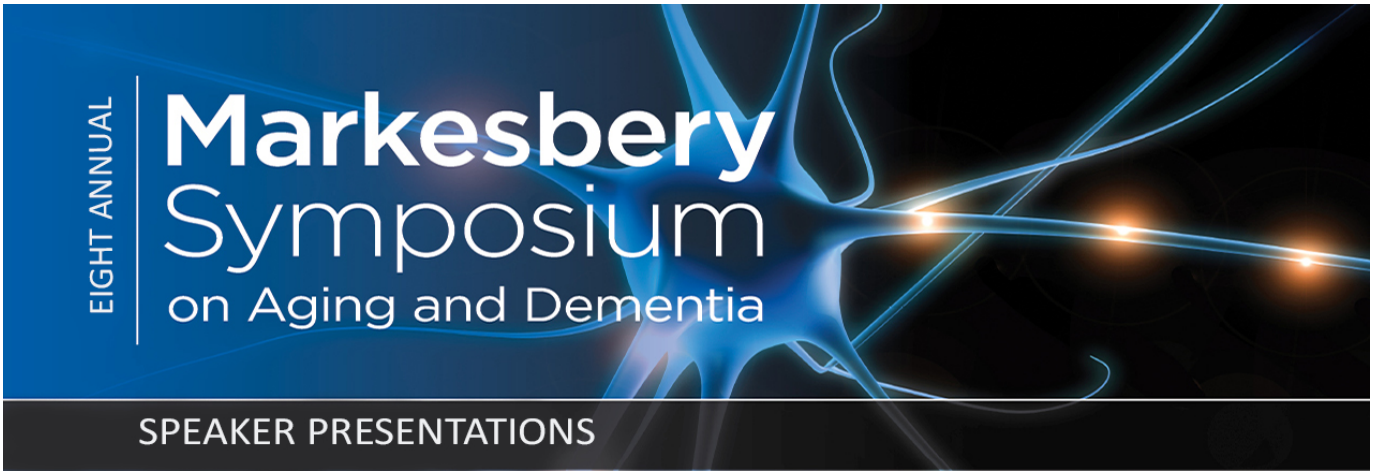
“Preclinical biomarkers of Alzheimer's disease risk: emerging concepts & clinical utility”

Sanjay Asthana, MD, FACP
University of Wisconsin

Associate Dean for Gerontology
Professor, Department of Medicine
Duncan G. and Lottie H. Ballantine Endowed Chair in Geriatrics
Director, NIH-funded Wisconsin Alzheimer’s Disease Research Center (ADRC)
Head, Division of Geriatrics and Gerontology
Director, Madison VA Geriatric Research, Education and Clinical Center (GRECC)
University of Wisconsin School of Medicine & Public Health, Madison, WI

Dr. Asthana received his medical degree at the University College of Medical Sciences, University of Delhi in New Delhi, India and completed his residency training in internal medicine at the University of Saskatchewan School of Medicine in Canada. He obtained his Geriatric Fellowship training at the Johns Hopkins University School of Medicine and completed an additional Senior Staff Fellowship in Alzheimer’s disease research at the Laboratory of Neurosciences of the National Institute on Aging (NIA), National Institutes of Health (NIH) in Bethesda, Maryland.

Dr. Asthana is internationally recognized for his research on the neurobiology of estrogen and related gonadal hormones and their effects on cognition in healthy postmenopausal women and those with Alzheimer’s disease. His additional area of research interest includes preclinical biomarkers of AD. For over 23 years, Dr. Asthana’s research program has been supported by multiple peer-reviewed grants from NIH, the US Department of Veterans Affairs, and various philanthropic organizations. He has published over 250 peer-reviewed papers to date, with several published in high-profile journals.



Research at the Sanders-Brown Center on Aging: An Update

"Identification of novel fluid biomarkers for vascular cognitive impairment and dementia (VCID)"

Donna Wilcock, PhD
University of Kentucky

Donna M. Wilcock, PhD is the Sweeney-Nelms Professor in Alzheimer's Disease Research at the Sanders-Brown Center on Aging and Professor in the Department of Physiology at the University of Kentucky. Dr. Wilcock received her Bachelor's degree in Pharmacology from Cardiff University. She obtained her Ph.D. at the University of South Florida, and completed postdoctoral training at Albert Einstein College of Medicine and Duke University.
Dr. Wilcock's research is focused on vascular cognitive impairment and dementia (VCID); the second most common cause of dementia behind Alzheimer's disease. In addition to being a major cause of dementia, Alzheimer's disease patients commonly have VCID as a co-morbidity. She is performing translational research on VCID, ranging from studying molecular mechanisms through identification of novel biomarkers in patients. She is primarily focused on inflammatory processes, as well as studying the influence VCID has on the progression and severity of Alzheimer's disease. She is funded by the National Institute on Aging and the National Institute on Neurological Disorders and Stroke.

"Metabolic reprogramming and Alzheimer's disease risk: the role of ApoE4"

Lance Johnson, PhD
University of Kentucky

Lance grew up in Asheville, NC and attended the University of North Carolina for both his undergraduate and graduate studies. He completed his PhD in the prestigious joint laboratory of Excellence Professor Nobuyo Maeda and Nobel Laureate Oliver Smithies. Under their exceptional guidance, Lance studied how the various isoforms of apolipoprotein E (apoE) affected atherosclerotic plaque development. Fascinated by advances in neuroscience and intrigued by the role of apoE in the brain, Lance secured a position as a NIH-funded Postdoctoral Fellow at Oregon Health & Science University, in beautiful Portland, OR. Under the mentorship of Professor Jacob Raber, he investigated the role of apoE in metabolism and cognitive function, and explored the mechanisms underlying insulin resistance-associated cerebrovascular dysfunction and recovery. Lance joined the Department of Physiology at the University of Kentucky in December 2016. In addition to tinkering around in the lab, Lance loves spending time with his wife and kids, being outdoors, cooking, and playing basketball (poorly).

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

"Changes in cerebrovascular pathology as a function of age and Alzheimer disease in Down syndrome"

Elizabeth Head, PhD
University of Kentucky

Dr. Head received a Masters in Psychology and a Ph.D. in Neuroscience from the University of Toronto, Canada. She received postdoctoral training at the Institute for Brain Aging & Dementia at the University of California – Irvine. She was co-leader of the Neuropathology Core of the University of California Irvine Alzheimer's Disease Research Center and was Director of the Institute's Brain Bank. Dr. Head moved to the University of Kentucky in January of 2009 and is currently a Professor and Associate Director of Education at the Sanders-Brown Center on Aging.

Dr. Head has published over 150 peer reviewed papers, over 30 review papers and book chapters and serves as a grant reviewer for the National Institutes on Health. Dr. Head has dedicated over 20 years to the study of aging and Alzheimer's disease with a focus on people with Down syndrome.

"Modulation of micro-RNA pathways by gemfibrozil in predementia Alzheimer disease: clinical translational research at Sanders-Brown Center on Aging"

Gregory Jicha, MD, PhD
University of Kentucky

Dr. Jicha is a Professor and Vice Chair for Academic Affairs in the Department of Neurology and an Associate Director of Sanders-Brown Center on Aging. Dr. Jicha holds the Robert T & Nyles Y McCowan Endowed Chair in Alzheimer's Research and leads the Clinical Core of the UK NIA-funded Alzheimer's Disease Center. He also serves as the Medical Director of KY Telecare and directs the Telemedicine Cognitive Clinic at UK, designed to reach out to rural populations across KY for both clinical and research-related activities in the area of AD and related disorders.

He is the principal investigator at UK for the National Alzheimer's Disease Cooperative Study Group, the Alzheimer's Clinical Trial Consortium, and serves on the Clinical Task Force and Steering Committee for the National Institute of Aging Alzheimer's Disease Center Program. He is Immediate Past-Chair of the Geriatric Section of the American Academy of Neurology. His current research interests are preclinical disease states of dementia, mild cognitive impairment, vascular contributions to dementia, and clinical trials of disease modifying therapies for degenerative dementias

1

Inducing elevated insulin receptor signaling via a constitutively active human insulin receptor leads to alterations in glucose metabolism

Hilaree Frazier, MS [1] • Katie Anderson [1] • Adam Ghoweri [1] • Susan Kraner, PhD [2] • Gabriel Popa, PhD [3] • Michael Mendenhall, PhD [3] • Christopher Norris, PhD [2] • Olivier Thibault, PhD [1]

[1]Pharmacology & Nutritional Sciences, University of Kentucky • [2]Sanders-Brown Center on Aging, University of Kentucky • [3]Molecular & Cellular Biochemistry, University of Kentucky

Graduate

Recent studies indicate that insulin signaling diminishes with aging as evidenced by decreased signaling markers, reduced insulin mRNA, and lower insulin receptor (IR) density. Current evidence highlights the role of insulin in normal brain function, with early stage clinical trials reporting a positive impact of intranasal insulin on memory recall in patients with mild cognitive decline or Alzheimer's disease (AD). Yet, the mechanisms behind this effect remain unclear. To address this, we conducted a series of experiments exploring the relationship between insulin signaling, cellular metabolism, and calcium homeostasis in neurons and astrocytes by using a molecular approach to constitutively increase insulin signaling, thus bypassing the need for exogenous insulin delivery.

Mixed, primary hippocampal cultures were infected with plasmids encoding a red fluorescent protein (mCherry), with or without a truncated, constitutively active form of the human insulin receptor (IRβ), using a lentiviral system. To address cell selectivity, a synapsin or GFAP promoter was included to limit expression to either neurons or astrocytes, respectively. Immunocytochemistry against HA-tagged IRβ was used to confirm IRβ expression. Western immunoblots measuring pAkt/Akt ratio were performed to obtain IR signaling levels. To assess the effect of increased IR signaling on glucose metabolism, 2-NBDG imaging experiments were performed. Glucose uptake was obtained by measuring initial 2-NBDG fluorescence. Fluorescent signal decay over time was recorded as an indirect measure of glucose utilization (Pancani et al., 2011). To test if changes in glucose were related to GLUT4 receptor density, immunocytochemistry using GLUT4 antibody was performed.

Lentiviral infection was successful for all constructs. Immunocytochemistry showed the presence of IRβ in 80% of cells. Western blots provided evidence that IRβ expression confers elevated IR signaling compared to controls. 2-NBDG imaging indicated IRβ expression was associated with increased glucose uptake and utilization in hippocampal neurons. Results were corroborated by evidence of changes in GLUT4 in IRβ-expressing cells.

This characterization provides insights into potential mechanisms governing insulin's effect on memory and learning and highlights the validity of exploring molecular approaches to enhance insulin signaling to combat cognitive decline associated with AD and aging.

2

Cognitive and happy life expectancy in the US

Anthony Bardo, PhD [1] • *Scott Lynch, PhD* [2]

[1]Sociology, University of Kentucky • [2]Sociology, Duke University

Faculty

Objectives: We examine the number of years to be lived with and without cognitive impairment and with high self-assessed quality of life (i.e., happiness). Our key question is whether happy life expectancy exceeds cognitive life expectancy. Special attention is given to racial/ethnic differences in remaining life expected to be lived cognitively un/impaired and un/happy. Method: Data from nine waves of the Health and Retirement Study (1998-2014) were used to estimate transition probabilities into and out of cognitively un/impaired-un/happy states, as well as to death. Recently extended Bayesian multistate life table methods were used to estimate age-sex-race/ethnicity specific cognitive and happy life expectancy net of education and birth cohort. Results: Happy life expectancy is approximately one-fifth to two-thirds longer than cognitive life expectancy at age 65, and by age 85 happy life expectancy is roughly double cognitive life expectancy on average. However, there are large racial/ethnic disparities in the number of years, and the proportion of remaining life, expected to be lived in each of the four un/impaired-un/happy states. Discussion: Cognitive impairment does not equate to unhappiness. The lower proportion of unimpaired and happy years among racial/ethnic minorities is driven by reduced cognitively healthy life expectancy, and not by differences in happiness.

3

NFAT 4 is up-regulated in astrocytes in aging canine brain model

Susan Kraner, PhD [1] • Francesca Triani, PhD [1] • Chris Norris, PhD [1] • Elizabeth Head, PhD [1]

[1]Sanders Brown Center on Aging, University of Kentucky

Staff

Pathophysiological changes associated with Alzheimer's disease (AD) may be driven or exacerbated by the protein phosphatase calcineurin (CN). The most important implication of this hypothesis is that CN inhibitors could provide an attractive alternative or complimentary approach to anti-AD therapies currently under investigation in clinical trials. We are particularly interested in the role of astrocytes in this process, as our previous work implicated astrocyte activation in AD, and in particular, the CN-nuclear factor of activated T cells (NFAT) signaling pathway associated with astrocyte activation. Of the four CN-dependent NFAT isoforms, NFAT4 undergoes selective upregulation in activated astrocytes and provides a robust biomarker of injury and CN activation in astrocytes. Blocking this pathway, in astrocytes, ameliorates glutamate dyshomeostasis, neurodegeneration, synapse dysfunction, and cognitive loss in mouse models of AD.

To demonstrate the broader implications of these findings, we propose to investigate the role of the CN-NFAT pathway in a more advanced model, the aging canine brain. Aged canines (beagle) show beta-amyloid accumulation in plaques and cognitive decline similar to early signs of AD in people. We have a bank of canine brain tissue from which we can draw samples for analyses. Focusing on NFAT4, we carried out Western analyses to determine the amount of NFAT4 expressed globally in cortex, and immunostaining and confocal microscopy to look at patterns of expression as well as overall levels in samples from aged (9.67-12.74 years old, n = 5) versus young brains (0.83-5.66 years old, n = 5). Our results demonstrate that NFAT4 expression is increased in aged canine brain, but exhibits robust labeling in activated astrocytes, regardless of age.

Astrocytes surrounding and feeding into the vasculature were particularly well-labeled with NFAT4 antibody and the astrocyte marker, GFAP, while in other regions there were astrocytes that expressed high levels of NFAT4 and lower levels of GFAP. Taken together, these data suggest there is heterogeneity in the astrocyte population, but NFAT4 is up-regulated in aged canine brain, consistent with our previous observations in rodent models. These results suggest that NFAT4 inhibition may be a target for intervention to prevent cognitive decline in the canine model of human aging and AD.

Grant Support:
Work supported by awards from the NIH (AG027297), the Kentucky Spinal Cord and Head Injury Research Trust (12-10A), and The Hazel Embry Research Fund to CMN, NIH/NIA R01AG056998 to EH/CMN.

4

Alzheimer's pathology-related, but not age-related, white matter diffusivity changes predict longitudinal decline in executive function

Christopher Brown, PhD [1] • *Brian Gold, PhD* [1]

[1]Neuroscience, University of Kentucky

Other

Introduction: Alterations in microstructure of white matter (WM) pathways connecting the default mode network (DMN) mediate age- and Alzheimer's disease (AD) pathology-related changes in DMN function, which in turn contribute to poorer executive function (EF). Previous work has focused on the summary measure of fractional anisotropy, but it is unclear whether alterations in specific component diffusivity measures are impacted by age and AD pathology. Further, it is unclear how these different component diffusivity measures may predict decline in EF over time.

Methods: Thirty-two cognitively normal (CN) older adults with available diffusion tensor imaging (DTI), cerebrospinal fluid (CSF) levels of tau and β-amyloid (Aβ), and annual neuropsychological testing were included in the present study. DTI measures of DR and DA were measured within a previously developed template of DMN WM pathways. AD pathology was quantified using the CSF tau/Aβ ratio. An EF composite measure was created by averaging the standardized residuals of Trails-B and Digit Symbol tests after regressing out Trails-A as a measure of processing speed. Annual change in EF composite was calculated for each year and then averaged to calculate a single measure of average annual change in EF over the three-year follow-up period. Bivariate correlations were performed to examine relationships between DTI metrics, AD pathology, and age. Further partial correlations were performed to examine the relationship between DTI metrics and average annual change in EF after controlling for baseline EF.

Results: Bivariate correlations revealed that higher CSF tau/Aβ ratios were associated with increased DR ($r = 0.37$, $p = .040$) but not DA ($p = .369$), while increasing age was associated with increased DA ($r = 0.37$, $p = .040$) but not DR ($p = .396$). Three participants were identified as outliers in annual change in EF (>3 SD from mean) and were excluded from longitudinal analyses. Annual change in EF was associated with DR ($r = -0.48$, $df = 26$, $p = .009$) but not with DA ($r = -0.26$, $df = 26$, $p = .188$).

Conclusion: These findings suggest different mechanisms underlying age- and AD pathology-related changes in WM microstructure. Further, it appears that longitudinal change in EF is associated with AD pathology-related changes in WM microstructure but not age-related changes in this CN cohort. These findings suggest that AD-related WM change plays an important role in future cognitive performance and may be an important target for studies aiming to slow or prevent cognitive decline.

5

Role of MCP-1/CCR2 in behavior deficit induced by neonatal alcohol exposure

Kai Zhang Zhang [1] • Bin Huang, PhD [2] • Jia Luo, PhD [1]

[1]pharmacology, University of Kentucky • [2]Public health, University of Kentucky

Graduate

Alcohol exposure during development causes profound physical and neurocognitive deficits that are collectively termed "fetal alcohol spectrum disorders" (FASD). FASD is the leading cause of mental retardation in the United States. The behavioral deficits associated with FASD are due, in large part, to alcohol-induced neuronal losses in the developing brain. Alcohol-induced neuroimmune activation, such as microglial activation and production of proinflammatory molecules, may mediate the neuronal loss and are believed to play an important role in long-term neuropathological and cognitive defects observed in FASD. Monocyte chemoattractant protein 1 (MCP-1), also called chemokine (CC motif) ligand 2 (CCL2), is a key chemokine involved in neuroinflammation. Our recent studies showed that MCP-1/CCR2 signaling was involved in alcohol-induced neuroinflammation and neurodegeneration in the developing central nervous system (CNS); inhibition of MCP-1/CCR2 signaling reduced alcohol-induced neuronal death. In this study, we sought to determine whether knock out of MCP-/CCR2 ameliorates neonatal alcohol exposure-induced behavioral deficits. C57BL/6 and MCP-1/CCR2 deficient (MCP-1-/-/CCR2-/-) mice were exposed to alcohol (5 g/kg) by subcutaneously injection on postnatal days (PD) 4. A series of behavioral tests including Open Field (PD 35-36 and PD 70-71), Rotor-Rod (PD 38 and PD 73), Balance Beam (PD 40 and PD75) and Morris Water Maze (PD 42 and PD77) were performed in the adolescence and adulthood. We observed that neonatal alcohol exposure or MCP-1/CCR2 deficiency did not affect anxiety-related behavior of adolescent and adult mice in Open Field testing; MCP-1-/-/CCR2-/- mice were resistant to neonatal alcohol exposure-induced deficits in general motor function of adolescent and adult mice in Rotor-Rod testing; MCP-1 and CCR2 deficiency protected mice against neonatal Ethanol exposure induced long lasting deficits in motor coordination and balance in Balance Beam testing; MCP-1 and CCR2 deficiency also protected mice against neonatal Ethanol exposure induced long lasting deficits in learning and memory

6

Intracranial atherosclerosis and other vascular contributions to cognitive impairment and dementia in nonhuman primates

Peter Hecker [1] • Lei Cai, PhD [2] • Donna M. Wilcock, PhD [3] • Elizabeth Head, PhD [1] • Peter Nelson, MD, PhD [4] • Ryan Temel, PhD [5]

[1]Pharmacology and Nutritional Sciences, University of Kentucky • [2]Saha Cardiovascualar Research Center, University of Kentucky • [3]Department of Physiology, University of Kentucky • [4]Pathology, Department of Neuropathology , University of Kentucky • [5]Department of Physiology , University of Kentucky

Graduate

Vascular cognitive impairment and dementia (VCID) is the second most common cause of dementia trailing Alzheimer's disease. Notably among the many vascular hallmarks of VCID, intracranial atherosclerosis (ICAS) is rapidly becoming a public health concern for both its role in stroke and subsequent cognitive dysfunction. The combination of lipoprotein retention, endothelial cell inflammation, monocyte/macrophage infiltration, intracellular cholesterol accumulation, impaired apoptotic cell clearance, and extracellular matrix degradation leads to formation of advanced, unstable atherosclerotic plaques that can limit or occlude blood flow to tissues causing acute or chronic tissue damage. The progression of atherosclerotic plaques within intracranial arteries contributes to the development of stroke and cognitive decline. Reducing low-density lipoprotein (LDL) concentration with statins is a primary therapeutic approach to stabilize atherosclerotic vascular disease (AVD), but treatment only reduces ischemic stroke risk by ~20% and does not appear to reduce VCID. This suggests that treatment of hypercholesterolemia alone is not an optimal approach for reducing VCID and additional therapies are likely needed. The obvious need for further therapies that regress or stabilize ICAS has been hampered by the paucity of suitable animal models. During an R01-funded study to determine the impact of microRNA-33 (miR-33) antagonism on cardiovascular AVD, we fortuitously discovered that our nonhuman primate (NHP) model had ICAS and other neurovascular hallmarks of VCID. Indeed, evaluation of intracranial arteries revealed that after 20 months on an atherogenic diet over 60% of NHPs (n=63) developed ≥1 atherosclerotic lesions within the circle of Willis (COW), the main arterial network that supplies the brain. Select atherosclerotic lesions from sections of COW suggested progressive characteristics of AVD such as: necrotic cores with CD68+ macrophages and a-SMA+ smooth muscle cell migration into the intima with increased staining of picro Sirius red (PSR)+ collagen initiating the formation of a fibrous cap. Furthermore, the initial preliminary data collected on a subset of animals (n=5) presented evidence of neurovascular hallmarks of VCID such as: gross ischemic lesions and infarcts, brain arteriolosclerosis (B-ASC), microinfarcts and microhemorrhages. Moreover as a surrogate for direct measures of reactive gliosis, the density of positive immunohistochemistry (IHC) staining for IBA1+ microglia and GFAP+ astrocytes was assessed on brain sections from 2 animals: one animal with ICAS and a control animal with no AVD. We are currently working on analyzing intracranial arteries and brains from our NHPs in hopes of making an impact on the field of VCID research where currently no treatments or preventative approaches have been developed for clinical trials.

7

Photothrombotic microinfarct technique for chronic, in vivo imaging of mouse vasculature and astrocyte networks using multiphoton microscopy

Nathan Farr [1] • *Pradoldej Sompol, PhD* [1] • *Chris Norris, PhD* [1]

[1]Sanders-Brown Center on Aging, University of Kentucky

Graduate

Vascular pathology contributes significantly to cognitive aging. Given the high incidence of cardiovascular disease in Kentucky and nationwide, vascular contributions to cognitive impairment and dementia (VCID) are a leading cause of dementia. The mechanisms by which vascular disease changes the brain are still largely unknown. Multiphoton microscopy helps by showing both the structure and the physiology of vessel-astrocyte interactions. Vascular pathology is modeled by delivering a precise infarct using LASER-activated thrombosis. Mice undergo cranial window surgery, whereby a four-millimeter fragment of skull is replaced with a circular glass coverslip. Stereotaxic coordinates allow for precise placement of the window above the area of interest, such as the hippocampus or barrel cortex. Following a period of post-operative recovery, mice are induced on isoflurane and placed under the objective lens of a multiphoton microscope. Retroorbital injection of rhodamine dextran allows for the visualization and mapping of the cortical vasculature using 5x and 20x Zeiss objectives. Images, Z-stacks, and movies are taken to demonstrate both the three-dimensional architecture of the cerebral vasculature as well as the cortical blood flow dynamics. Microinfarction is accomplished by first choosing a target vessel—capillary, arteriole, or venule—and, second, injecting the photocoagulant sodium salt Rose Bengal retroorbitally. LASER ablation of the vessel lumen creates a thrombus that occludes the target vessel, which can be confirmed by recording blood flow movies before and immediately after the insult. Blood flow dynamics can then be measured at various time intervals, such as 30 minutes, one hour, six hours, and 48 hours. Each mouse is secured under the objective using a custom stage and headpiece, ensuring that the mouse is positioned correctly across measurements. Ablation is directed and timed to minimize collateral damage to the surrounding tissue as well as potential vasogenic and cytotoxic edema formation. Adeno-associated virus injection of GCaMP6 allows for visualization of calcium signaling as astrocytes respond to infarction. Application of this technique in amyloidogenic APP/PS1 mice promises to illuminate the convergence of Alzheimer's disease (AD) and VCID related pathologies. Capillary flow stalls have also been observed with this technique in wild-type mice in the absence of infarction, sparking the question of the frequency and duration of stalls, as well as how that might be different in models of AD.

8

Targeting neuroinflammation in the context of Alzheimer's disease with comorbid vascular pathology

David Braun, PhD [1] • Donna Wilcock, PhD [1] • Linda Van Eldik, PhD [1]

[1]Sanders-Brown Center on Aging, University of Kentucky

Fellow

With each successive clinical failure of anti-amyloid treatment for Alzheimer's disease (AD), it is increasingly apparent that a singular focus on amyloid pathology is an untenable path to broadly effective treatments. Indeed, estimates of "pure" AD cases indicate that such patients make up less than 20% of the AD population, whereas the vast majority have one or more comorbidity. Furthermore, of the potential comorbidities, vascular pathology is the most prominent. Preclinical therapeutic development studies will therefore benefit from validation in systems where AD-type pathology is not the only factor contributing to cognitive dysfunction. To this end, we have been using a transient dietary hyperhomocysteinemia (HHcy) model to induce vascular dysfunction in a transgenic amyloid overexpression model of AD (APPswe/PS1dE9). AD mice (and wildtype littermates) were placed on vitamin B and folate-deficient HHcy diet for 8 weeks beginning at around 7.5 months of age, after the beginning of plaque deposition. Mice were then recovered on normal chow for 2 weeks, before beginning two weeks of treatment with our novel anti-inflammatory MW151 (5 mg/kg, I.P., daily). In the final week of treatment, mice underwent a battery of behavioral testing in the open field, novel spatial recognition task, novel object task, radial arm water maze, marble burying, and nesting. Characterization of the underlying interactive effects of the comorbid pathologies, and the efficacy of targeted anti-inflammatory treatment in this context, will help inform future therapeutic strategies in terms of relevant patient subpopulations, appropriate therapeutic window, and potential combinatorial treatments.

9

Common mechanisms in epilepsy and tauopathy

Ryan Cloyd [1] • Shon Koren [2] • Joe Abisambra, PhD [2] • Bret Smith, PhD [3]

[1]Physiology, University of Kentucky • [2]Neuroscience, University of Florida • [3]Neuroscience, University of Kentucky

Graduate

Objective:
Neurologic disorders are among the most significant health challenges facing society today. Although different neurologic disorders are often thought to be distinct from one another, evidence suggests similar processes may contribute to pathology in different diseases. Previous studies suggest that common disease mechanisms contribute to the development of epilepsy and tauopathy. The purpose of this study is to better characterize this relationship and explore potential therapeutic avenues to slow disease progress.
Methods/Results:
This study uses the pilocarpine-induced status epilepticus model of temporal lobe epilepsy to explore the effect of severe seizures on tau pathology. Brains were collected from mice at 6 or 24 hours after induced status epilepticus. Homogenates were analyzed via Western blot to look for changes in tau phosphorylation or activity of two major regulators of tau phosphorylation, GSK3β and PP2A. These data show that changes in tau phosphorylation dynamics occur at a much earlier time point after status epilepticus than has previously been described.
Conclusions:
The current project supports previous observations that seizures promote tau phosphorylation in vivo, but suggests that changes begin much earlier than previously thought. Further work is needed to understand how post-seizure changes in tau phosphorylation develop over longer periods of time. Additionally, future work will characterize the effect of tauopathy on electrical activity in vivo and in vivo.

Funding
NIH NIGMS 1T32GM118292-02, NIH NINDS 1R01NS902552-01, NIH NINDS 1R01 NS091329-01, Department of Defense AZ140097

10

Brain pathology and cognitive reserve are associated with executive functioning performance, not memory, in healthy older adults in an ADNI dataset

Chrisopher Bauer, PhD [1] • Tyler Hammond [2] • Chris Brown, PhD [2] • Brian Gold, PhD [1]

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

Fellow

Background: Alzheimer's Disease (AD) is a debilitating condition in which potential protective mechanisms are not well understood. Here, we sought to examine brain pathology and cognitive reserve and how they are associated with memory and executive function (EF) in both healthy aging and diseased states. More specifically, for AD pathology, we focused on structural connectivity of major brain networks, as well as biochemical analysis of the CSF, as an independent predictor. We hypothesized that AD pathology would be negatively correlated with memory and EF, particularly in impaired individuals, while the opposite effect would be observed with cognitive reserve. Method: Using the Alzheimer's Disease Neuroimaging Initiative (ADNI) database, we selected all 203 participants with available DTI scans at the time of this study. Participants were divided into healthy (no MCI or AD) and impaired (MCI or AD) groups. An AD pathology composite consisting of FA values of major white matter tracts, volumetric measures, and Tau over Abeta was calculated using backwards elimination of variables in SPSS to include only statistically relevant variables. Similarly, a cognitive reserve composite was calculated using this same approach, ultimately including both education and ANART scores. Dependent variables memory performance and EF were estimated from composites of neurocognitive outcome measures. The association between AD pathology/cognitive reserve and memory/EF performance was tested using the general linear model. Results: The AD pathology composite was negatively correlated with memory composite scores in impaired individuals ($p < 0.001$), but not in healthy older adults. AD pathology was negatively correlated with EF in both groups ($p < 0.001$, $p < 0.040$). Likewise, cognitive reserve was positively correlated with memory only in impaired individuals ($p < 0.032$), but not healthy older adults. Cognitive reserve was positively correlated with EF in both groups ($p< 0.013$, $p < 0.001$). Conclusion: We provide pilot data suggesting that both a brain pathology and cognitive reserve composite can separately predict memory and EF performance in impaired individuals, and EF performance in healthy older adults. These results imply that EF may be affected by clinically silent disease burden even before any diagnosis, and that cognitive reserve could be important during this phase to reduce disease-related decline. Further work is necessary to see if cognitive reserve truly moderates this association. The preservation of networks in prodromal AD through prophylactic interventions remains critical.

11

Prebiotic diet restores metabolism and reduces risk for Alzheimer's disease by modulating gut microbiome in an APOE4 Mouse Model

Lucille Yanckello [1] • Jared Hoffman, MS [1] • Ishita Parikh, PhD [2] • Jessie Hoffman, MS [1] • Stefan Green [3] • George Chilipala [3] • Ai-Ling Lin, PhD [4]

[1]Pharmacology and Nutritional Sciences, University of Kentucky • [2]Sanders-Brown Center on Aging • [3]DNA Services Facility, University of Illinois at Chicago • [4]Sanders Brown Center on Aging, University of Kentucky

Graduate

Objectives: Apolipoprotein ε4 (APOE4) allele is the strongest genetic risk factor for Alzheimer's disease. APOE4 carriers have brain metabolic dysfunctions decades before the clinical symptoms of dementia occur. Emerging evidence shows that the dysbiosis of gut microbiome in APOE4 carriers may play a critical role in determining brain metabolic integrity. The goal of the study was to use prebiotic diet (Inulin) to restore the gut microbiome of mice with human APOE4 genes (E4FAD mice). We hypothesized that modulating the gut microbiota with the prebiotic inulin will restore metabolic function and reduce risk for AD in asymptomatic E4FAD mice.

Methods: At 3 months of age the E4FAD mice were fed for 4 months with either control or inulin diet. We used 16S rRNA amplification and sequencing to determine gut microbiota diversity and species variations; magnetic resonance spectroscopy to measure metabolites in the brain; and, non-targeted UPLC-MS/MS and GC-MS analyses to determine metabolic profiles of blood and caecum.

Results: Our results indicate that prebiotic inulin significantly altered the gut microbiota of E4FAD mice, increasing beneficial gut microbiota including Erysipelotrichaceae spp. and Allobaculum spp., which produce the short chain fatty acid butyrate, and reducing those associated with inflammation including Clostridiaceae spp. and Ruminococcus spp. Inulin-fed mice also showed significant changes in alpha and beta diversity compared to the controls. Blood metabolomics of E4FAD mice showed alterations in pathways including the mitochondrial TCA cycle, pentose phosphate pathways and tryptophan metabolites, which all relate to improve metabolic integrity. We also found significantly increased scyllo-inositol in the brain and fecal culture. As scyllo-inositol has been used in clinic to reduce amyloid-beta (Aβ) aggregation, our finding indicate that Inulin may reduce Alzheimer's risk for the APOE4 carriers.

Conclusion: Through our nutrigenomic analysis of the inulin diet, we propose that prebiotic diet may positively modulate the gut microbiota and the metabolic profile to decrease AD-like pathology, inflammation and Aβ burden in E4FAD mice. Our approach in this study is translatable and potentially an actionable preventative measure for APOE4 carriers. We believe that preventative precision medicine involving nutritional interventions is the most applicable to systems wide disease such as AD.

Funding: NIH/NIDDK T323048107792, NIH/NIA R01AG054459, NIEHS/NIH P42ES007380

12

Therapeutic targeting of TREM2 in Alzheimer's disease

Brittani Price [1] • Tiffany Sudduth [1] • Erica Weekman, PhD [1] • Danielle Hawthorne [1] • Abigail Woolums [1] • Arnon Rosenthal, PhD [2] • Donna Wilcock, PhD [1]

[1]Sanders Brown Center on Aging, Department of Physiology , University of Kentucky • [2]Alector, LLC

Graduate

Triggering receptor expressed on myeloid cells-2 (TREM2) is a lipid and lipoprotein binding receptor expressed by activated innate immune cells. Homozygous TREM2 loss of function mutations cause early onset progressive presenile dementia while heterozygous, function-reducing point mutations triple the risk of Alzheimer's disease (AD). This is due in part to a reduction in microglia survival, proliferation, chemotaxis and phagocytic activity. Although human genetic findings support the notion that loss of TREM2 function exacerbates neurodegeneration, it is not clear whether activation of TREM2 in a disease state would result in therapeutic benefits. Here we show that chronic activation of TREM2, in the 5XFAD mouse model of AD, leads to reversal of AD gene expression signature, recruitment of microglia, decreased amyloid deposition, and improvement in spatial learning and novel object recognition memory. These findings indicate that TREM2 activators may be effective for the treatment of AD and possibly other neurodegenerative disorders.

13

Identifying predictive fluid biomarkers for vascular cognitive impairment and dementia (VCID)

Tiffany Sudduth [1] • Qiang Cheng [2] • Erin Abner, PhD [1] • Richard Kryscio, PhD [1] • Peter Nelson, MD, PhD [1] • Brian Gold, PhD [3] • Zachary Winder [1] • Sonya Anderson [1] • Omar Al-Janabi [1] • Ahmed Bahrani [1] • Gregory Jicha, MD [1] • Donna Wilcock, PhD [1]

[1]Sanders-Brown Center on Aging, University of Kentucky • [2]University of Kentucky • [3]Neuroscience, University of Kentucky

Staff

Vascular cognitive impairment and dementia (VCID) is the second leading cause of dementia. Currently, diagnosis for VCID is limited to clinical signs of cognitive impairment partnered with vascular injury seen most often as white matter hyperintensities (WMH) on MRI neuroimaging. There is a growing need in the research and clinical communities to develop earlier and more sensitive biomarkers for VCID. We leveraged an existing clinical cohort recruited to enrich for VCID that included extensive MRI imaging, CSF and plasma collection, along with comprehensive cognitive batteries. We examined the cross-sectional CSF (where available) and plasma from 66 individuals for SAA, CRP, VCAM-1, ICAM-1, FGF, Flt1, PIGF, Tie-2, VEGF-A, VEGF-C, VEGF-D, IFNγ, IL10, IL12p70, IL13, IL1β, IL2, IL4, IL6, IL8, TNFα, MMP1, MMP3, MMP9, MMP2, and MMP10 using Meso-Scale Discoveries multiplexed ELISAs.
We applied machine learning approaches to determine whether there were predictive biomarkers of either white matter hyperintensity volume or cognitive impairment. We identified nine fluid biomarkers that, collectively, had an 80% predictive accuracy for cognitive impairment (distinguishing MCI or normal cognition). These biomarkers were CSF PIGF, MMP2, SAA, CRP; plasma MMP9, ICAM-1, VEGF, VCAM-1, VEGF-D. We identified seven fluid biomarkers that, collectively, had a 70% predictive accuracy for white matter hyperintensity volume. These biomarkers were CSF IL8, IL6, VEGFD, PIGF; plasma TNFα, IL10, MMP10.
Collectively, our data strongly support the expansion of our studies to a larger cohort to evaluate these panels of biomarkers and explore their utility for diagnosis, and tracking response to future therapies in clinical trials.

14

Novel NFAT inhibitor Q134R ameliorates synaptic deficits in a mouse model of Alzheimer's disease

Pradoldej Sompol, PhD [1] • Jenna Gollihue, PhD [1] • Susan Kraner, PhD [1] • Irina Artiushin [1] • Ryan Cloyd [1] • Shon Koren [1] • Grant Nation [1] • Joe Abisambra, PhD [1] • Orsolya Huzian [2] • Laszlo Puskas, PhD [2] • Christopher Norris, PhD [1]

[1]Sanders-Brown Center on Aging, University of Kentucky • [2]Avidin Ltd.

Staff

The calcineurin (CN)/Nuclear Factor of Activated T cells (NFAT) transcriptional pathway is hyperactivated at early stages of Alzheimer's disease (AD). Using a common mouse model of AD, we previously reported high levels of activation and/or expression of CN and the NFAT4 isoform in activated astrocytes. Inhibition of CN/NFATs in AD mouse models, using genetic or pharmacologic approaches, typically yields many beneficial effects including reduced neuroinflammation and amyloid pathology, along with greater neuroprotection and improved synapse function. Here, we investigated the effects of Q134R, a novel small chemical compound, developed and tested for human use by Avidin Biotechnology, on NFAT signaling in neural tissue. Similar to the CN inhibitor-cyclosporine, Q134R suppressed IL-1β- or ionomycin-induced NFAT activation in primary rat astrocyte and neuron cultures, but, unlike cyclosporine, did not inhibit CN-dependent dephosphorylation of other non-NFAT substrates. When delivered to 15-month-old APP/PS1 mice (twice daily P.O. for two weeks), Q134R (4 mg/kg) reduced GFAP volume and inhibited the nuclear localization of NFAT4 in hippocampal astrocytes. To investigate long-term treatment effects, we administered Q134R (4mg/kg) or vehicle twice daily (P.O.) for three months to WT and APP/PS1 mice starting at six-months-of-age. Compared to vehicle control, Q134R significantly increased CA3-CA1 synaptic strength and long-term potentiation in brain slices from APP/PS1 mice. Moreover, synaptic indices in Q134R-treated APP/PS1 mice were qualitatively and quantitatively similar to WT mice. The results demonstrate that Q134R inhibits hyperactive NFAT signaling en route to protecting synaptic function during the progression of AD-like pathology. The findings offer important proof-of-concept support for the use of small chemical NFAT inhibitors, like Q134R, in the treatment of AD and related neurodegenerative disorders.

15

Changes in dorsal hippocampal calcium levels and behavior before, during, and after AD pathology in the 5XFAD mouse

Adam Ghoweri [1] • Lara Ouillette [2] • Hilaree Frazier [1] • Katie Anderson [1] • John Gant, PhD [1] • Rachel Parent [2] • Geoffrey Murphy, PhD [2] • Olivier Thibault, PhD [1]

[1]Pharmacology and Nutritional Sciences, University of Kentucky • [2]Molecular and Integrative Physiology, University of Michigan School of Public Health

Graduate

Background
As the projected rise of individuals affected by Alzheimer's disease is expected to triple by 2050, the need for the characterization of associated molecular mechanisms and the development of novel therapeutic treatments is indispensable. One potential mechanism highlighted in the calcium hypothesis of brain aging and dementia, describes a state of altered calcium handling in neurons that has an impact on several physiological parameters, including an increase in the Ca2+-dependent potassium potential, the afterhyperpolarization (AHP). Moreover, a hallmark marker of neuronal aging in field CA1 of the hippocampus is the increase in the AHP, accompanied with elevated levels of calcium. Though the strong association between calcium and the AHP has been illustrated in normal aging, how the two phenomena contribute to disease-state aging remains largely unknown and perhaps even less is known in models of AD. Recent work has reported reduced levels of L-type voltage sensitive calcium channels (L-VSCCs) in older APP and PS-1 transgenic mice, suggesting calcium dysregulation in AD mouse models may vary from that seen in aging.
Methods/Results
In this study, we are identifying the effects of aging on the calcium-dependent afterhyperpolarization and intracellular calcium levels in the 5XFAD model on a C57Bl6 congenic background. Using sharp electrode electrophysiology and calcium imaging (OGB-1), we are observing no differences between WT and 5xFAD mice at 1.5 months of age, but an attenuated AHP in the transgenic animals compared to the wildtype animals at 4 months of age. Analyses of behavior data (MWM) does not show deficit until later (6-7 months). This evidence suggests that reduced neuronal calcium signaling, as opposed to elevated calcium signaling could be a precipitating factor in the manifestation of behavioral deficits.
Conclusions
Overall these data corroborate the decreased L-VSCCs see in previous studies from our group and suggest alteration in behavior is not always tied to an increase in neuronal calcium. Indeed, calcium levels and kinetic analyses are suggesting reductions in calcium handling could well increase excitability and stimulate the onset of cognitive decline. Such measures provide new insights into dysregulated calcium and neuronal health, and directly address disease phenotype progression over time.

Supported by: RO1AG058171 and NIH T32 TRIAD Training Grant AG057461

16

Characterizing neuroinflammatory responses in apolipoprotein E genotypes

Courtney M Kloske [1] • Abigail Woolums [1] • Tiffany L Sudduth [1] • Soyna Anderson [1] • Ela Patel [1] • Erin Abner, PhD [1] • David Fardo, PhD [1] • Peter T Nelson, MD, PhD [1] • Donna M Wilcock, PhD [1]

[1]University of Kentucky

Graduate

Alzheimer's disease (AD) is characterized by two neuropathological hallmarks: amyloid plaques and neurofibrillary tangles. Additionally, AD is characterized by an inflammatory response to amyloid-beta, inducing the activation and proliferation of microglia. While the majority of AD cases have no clear genetic cause, a few genetic risk factors have been identified that increase the risk of developing AD. One of the most significant risk factors is Apolipoprotein E (ApoE), a plasma protein that transports cholesterol in the brain. A mutation in triggering receptor expressed on myeloid cells 2 (TREM2), a microglial surface receptor, also has a significant, albeit rare, genetic association with AD. Of the three identified ApoE alleles, E4 confers an increased risk of AD, with ApoE4/4 homozygotes showing the greatest risk of AD. ApoE3 is the most common allele in the human population, and ApoE3/3 is thought to be the control phenotype. The ApoE2 allele has been shown to be protective for AD, but this allele is relatively rare in the population. Despite an understanding of how each isoform of ApoE impacts disease risk, there is limited understanding of how ApoE drives disease progression. Interestingly, ApoE was shown to bind to TREM2 and alter microglial gene expression and function, suggesting a role for ApoE in the inflammatory response seen in AD. There are in vitro studies showing ApoE allele impacts the inflammatory response by microglia, however, complete, unbiased assessment of neuroinflammation in human AD tissue has not been performed. In the current study, we used the Human Neuroinflammation Nanostring panel to assess the neuroinflammatory profile in the superior medial temporal gyrus (SMTG) and cerebellar regions of Braak V/VI, Thal 5 ApoE3/3 (N= 9), Braak V/VI, Thal 5 ApoE4/4 (N=10), and Braak I/II Thal 0 ApoE3/3 (N=5). RNA was extracted from frozen frontal cortex (Brodman's area 9), and frozen cerebellum. We found significant inflammatory genes differentially expressed between the ApoE3/3-AD and ApoE4/4-AD brains. Of particular note, genes associated with microglia function, autophagy, and epigenetics pathways were significantly different. Most of these expression differences were decreased in ApoE4/4 compared to ApoE3/3 but some were increased. These results suggest differential inflammatory responses may contribute to disease risk, disease progression, and potentially responsiveness to therapeutic interventions.

17

CSF amylin – effect modifier of the Aβ-AD relationship

Deepak Kotiya, PhD [1] • Han Ly [1] • Lei Chen, PhD [2] • Florin Despa, PhD [1]

[1]Pharmacology and Nutritional Sciences, University of Kentucky • [2]Department of Physiology, University of Kentucky

Fellow

Background: Accumulating evidence from several laboratories indicates that patients with Alzheimer's disease (AD) have cerebral mixed deposits formed by β amyloid (Aβ) and amylin, a pancreatic hormone that crosses the blood-brain barrier and has amyloidogenic properties. In contrast to amylin from humans, rodents have non-amyloidgenic amylin, which does not accumulate in the brain or other tissues. Here, we speculated the difference in amyloidogenicity between human and rodent amylin species to test the hypothesis that chronically elevated levels of amylin in cerebrospinal fluid (CSF) promote mixed amylin-Aβ pathology worsening the behavior changes in a rat model of mixed amylin-Aβ pathology. Methods: For studying amylin-Aβ pathology, we crossed rats expressing human amylin in the pancreas (HIP) rats with AD rats to generate ADHIP rats. Littermate AD and wild-type (WT) rats expressing the non-amyloidogenic rat amylin served as negative controls for brain amylin deposition. Behavior was tested in ADHIP, AD and WT littermate rats at 8 months of age (when all rats have normal behavior), at 12 months of age (when HIP and ADHIP rats develop amylin pathology) and at 16 months of age, by the Novel Object Recognition (NOR) and Morris water maze (MWM) tasks. Amylin-Aβ interaction in CSF was assessed by immunoprecipitation of amylin (1 ml CSF/rat; n=4 rats/group) followed by ELISA for amylin. The formation of mixed amylin-Aβ pathology in the brain was tested by immunohistochemistry. Results: In ADHIP rats, behavior changes have developed at ~12 months of age, which was four months earlier than in AD littermate rats. Brain dysfunction in ADHIP rats correlated with elevated blood levels of aggregated amylin. The lower performance in ADHIP rats compared with age-matched rats in the other groups correlated with the development of mixed Aβ-amylin oligomers in CSF and mixed Aβ-amylin plaques in the brains of ADHIP rats. Conclusions: Finding mixed amylin-Aβ oligomers in ADHIP rats indicates that CSF amylin level is effect modifier of the Aβ-AD relationship. The formation of "mixed" amylin-Aβ oligomers in vivo is consistent with in vitro and in silico studies showing that amylin-Aβ interaction can promote robust growth of mixed amylin-Aβ amyloids.

18

Amylin dyshomeostasis – a non-Alzheimer's disease process contributing to an Alzheimer's disease phenotype

Han Ly [1] • Nirmal Verma, PhD [1] • Fengen Wu [1] • Jeffrey Walton, PhD [2] • Florin Despa, PhD [1]

[1]Pharmacology and Nutritional Sciences, University of Kentucky • [2]Biomedical Engineering, University of California at Davis

Graduate

Background: Recent studies from several laboratories showed that amylin, an amyloidogenic peptide that kills the pancreatic β-cells in patients with type-2 diabetes, forms neuritic deposits and mixed amylin-Aβ plaques in brains of individuals with Alzheimer's Disease (AD). Here, we tested the hypothesis that systemic amylin dyshomeostasis accelerates the development of AD.
Methods: TgF344-19 AD rats (which overexpress human APPSwe/PS1dE9 from a PrP promoter) were crossed with rats overexpressing human amylin in the pancreas (HIP rats). The newly generated ADHIP rats were compared with HIP, AD and wild-type (WT) littermates (males; n=10/group) by Morris Water Maze (MWM). MWM tests were performed at 12 months of age (when HIP rats develop amylin pathology) and 17 months of age (when AD rats have fully developed AD-like pathology). Brain structure in each rat was assessed by T2-weighted MRI (7T; TR: 3000ms; TE: 24ms). At 12 and 17 months of age, n=5 animals from each group were investigated for cellular markers of the blood-brain barrier (BBB) injury and interaction of amylin with Aβ.
Results: Amylin dyshomeostasis greatly accelerated brain structural abnormalities in ADHIP rats, as demonstrated by enlarged ventricles and brain atrophy. Compared to WT controls, ADHIP, AD, and HIP rats have impaired learning ($P<0.05$). The latency to the platform is significantly longer for ADHIP rats compared to AD rats ($P=0.03$) indicating a faster decline in brain function in ADHIP rats.
Staining of brain sections using cellular markers of astrocytes (GFAP), microglia/macrophages (CD11b), microglia (Iba1) and reactive microglia (CD68) demonstrates BBB breakdown in ADHIP and HIP rats. Cerebral vascular amylin deposits, microhemorrhages, loss of endothelial cell coverage in capillaries and white matter rarefaction were also seen in ADHIP and HIP, but not AD and WT rats. At 17 months of age, the latency to the platform was similar for ADHIP and AD rats. Histologic analysis revealed mixed Aβ-amylin plaques in brains of ADHIP rats.
Conclusion: Systemic amylin dyshomeostasis and subsequent accumulation of oligomerized amylin in the brain greatly accelerates AD-like pathology by provoking BBB breakdown and Aβ-amylin deposition in the brain parenchyma.

19

Mitochondria associated ER membrane is a novel subcellular location for microRNA in mammalian brain

Wang-Xia Wang, PhD [1] • Paresh Prajapati , PhD [2] • Peter Nelson, MD, PhD [1] • Joe Springer, PhD [2]

[1]Sanders Brown Center on Aging, University of Kentucky • [2]Spinal Cord and Brain Injury Research Center, University of Kentucky

Faculty

Mitochondria associated ER membrane (MAM) is an ER subdomain in direct contact with mitochondria. MAM serves as a subcellular platform in the communication between mitochondria and ER and plays key roles in lipid synthesis and exchange, calcium transport and homeostasis, mitochondria dynamics, as well as formation of autophagosome and inflammasome complexes. Disruption of mitochondria and ER contacts is associated with a variety of pathophysiological conditions and human diseases including impaired or overactive immune function (inflammation), metabolic diseases, cancers, and neurodegenerative diseases, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Here we present our recent findings from human and rat brains indicating that MAM is a novel subcellular localization for microRNAs (miRNAs). MiRNAs are evolutionally conserved, non-coding small RNAs that regulate gene expression post-transcriptionally. MiRNAs have been implicated in a broad range of normal biological and pathophysiological processes such as development, differentiation, metabolism, immune response, neurogenesis and neurodegeneration thus involved in an array of human diseases including AD. We performed subcellular fractionation from human and rodent cerebral cortex and analyzed protein and miRNA expression in purified MAM, mitochondria, ER, and cytosol fractions. The human brains were short-postmortem samples collected from research subjects of the U.K. AD Center autopsy cohort. RT-qPCR analysis revealed for the first time that MAM contains a substantial number of miRNAs. In addition, we found that mitochondria uncoupling or traumatic brain injury in rats resulted in a shift of mitochondria-associated miRNAs between the mitochondria, MAM, and ER, and may reflect the presence of a miRNA shuttling mechanism. Regardless, our findings indicate the existence of a novel interorganelle network in which miRNAs may be distributed and shuttled between organelles (including MAM) and the cytosol as a mechanism for making appropriate responses to cellular demands and stressors.

20

Time course of neuropathological changes in hyperhomocysteinemic amyloid depositing mice

Charles Seaks [1] • Erica Weekman, PhD [1] • Tiffany Sudduth [1] • Brittani Price [1] • Abigail Woolums [1] • Danielle Hawthorne [1] • Donna Wilcock, PhD [1]

[1]Physiology, Sanders-Brown Center on Aging

Graduate

Vascular contributions to cognitive impairment and dementia (VCID) are the second leading cause of dementia behind Alzheimer's disease (AD). Co-morbidity of VCID with AD is common, with over 60% of AD cases also presenting VCID pathology. VCID's contribution to clinical dementia is increasingly recognized; however, the mechanisms underlying VCID are not well understood, due, in part, to a lack of relevant animal models. We have previously shown that hyperhomocysteinemia (HHcy), induced by a diet deficient in folate, vitamins B6 and B12, and enriched in methionine, provides a mouse model of VCID, which can be applied to APP/PS1 mice to produce a co-morbidity model. While the pathological characteristics of HHcy have been characterized in our mouse models, the time course of these changes is unclear. In this study, neuroinflammation, microhemorrhages, amyloid deposition, and cognition were assessed at 2, 4, 6, 10, 14, and 18 weeks after initiation of the diet in our co-morbidity model. Immunohistochemistry for CD11b, a microglial marker, showed an increase starting at 6 weeks on diet. Proinflammatory markers, including IL-1b, TNFa, IL-6, and IL-12a, were also increased after 6 weeks on diet. Cognition was assessed using two-day radial arm water maze, and revealed a sharp decline in cognitive function at 10 weeks on diet. Microhemorrhage quantity was determined using Prussian blue staining and magnetic resonance imaging. There was an increase in microhemorrhages in the APP/PS1 mice after 14 weeks on the HHcy diet. Finally, at 14 weeks on the HHcy diet, APP/PS1 mice showed a redistribution of amyloid deposition from the parenchyma to the vasculature. These results indicate that neuroinflammation occurs first, followed by cognitive decline, and then progresses to microhemorrhages and amyloid redistribution to the vasculature. This suggests that neuroinflammation is an initiator in HHcy mediated VCID and presents a possible therapeutic target.

21

Use and combination of potentially inappropriate medications is associated with incident dementia

Ashley Martinez, PharmD [1] • Gregory Jicha, MD, PhD [2] • Daniela Moga, MD, PhD [3]

[1]Pharmaceutical Sciences, University of Kentucky • [2]Sanders-Brown Center on Aging and Neurology, University of Kentucky • [3]Sanders-Brown Center on Aging, Pharmacy Practice and Science, University of Kentucky

Graduate

Background/Objective: A growing body of literature supports the hypothesis that potentially inappropriate medication (PIM) use in older adults may be associated development of dementia, highlighting a critical concern among clinicians and researchers. However, the relationship between potentially inappropriate drug-drug interactions (PI-DDI) and cognitive function remains poorly characterized. Large administrative claims databases can provide an opportunity to study these issues. We used one such database to investigate the association of PIM use and PI-DDI (defined using 2015 Beers' Criteria) with incident dementia.

Methods/Results: Using data from Truven Health Marketscan Research Database® (2009-2016), we conducted a retrospective cohort study to investigate the association between PIM use and PI-DDI (defined using 2015 Beers' Criteria) and incident dementia. After a three year run-in period of continuous eligibility to identify dementia-free subjects aged ≥65 years, subjects were followed until disenrollment or incident dementia. Incident dementia was identified either by diagnosis or receipt of an anti-dementia prescription. We used Cox proportional hazard regressions (generating hazard ratios [HR] and 95% confidence intervals [CI]) to measure the association between PIM use and PI-DDI, and incident dementia, adjusted for comorbidities, total number of medications, and demographics.

2,568,479 subjects were included (54.4% female, mean age 74.0), of which 47.4% used any PIM. The most common PIMs were gastrointestinal medications (primarily proton pump inhibitors), followed by CNS-active medications (driven by benzodiazepine and non-benzodiazepine hypnotics). After a median follow-up of 2.1 years, those using CNS-active and strongly anticholinergic potentially inappropriate medications were more likely to develop dementia than those without these PIMs (HR [95% CI] 1.31 [1.29-1.32] and 1.22 [1.21-1.24] respectively). An interaction term between PI-DDI and PIM use was statistically significant (HR [95% CI] 1.54 [1.36-1.73]).

Conclusions: Our study suggests that individuals who use PIMs develop dementia at a higher rate than those without PIM use, and that PI-DDIs magnify that association. Further research should investigate the underlying biological plausibility of this association, especially for medications that do not act on the central nervous system. Understanding the complexities of PIM use may aid in designing interventions to lessen cognitive burden and reduce PIM use in the aging population at risk for dementias.

22

Sex differences in brain activity and memory performance correlates in healthy older adults

Emily Willis [1] • Lucas Broster, MD, PhD [2] • Haiqing Huang, PhD [3] • Erin Abner, PhD [4] • Richard Kryscio, PhD [5] • Frederick Schmitt, PhD [6] • Gregory Jicha, MD, PhD [7] • Linda Van Eldik, PhD [8] • Charles Smith, MD [7] • Mingzhou Ding, PhD [3] • Yang Jiang, PhD [1]

[1]Behavioral Sciences, University of Kentucky • [2]Behavioral Sciences, College of Medicine, University of Kentucky • [3]Biomedical Engineering, University of Florida • [4]Department of Epidemiology, Sanders-Brown Center on Aging • [5]Biostatistics, Sanders-Brown Center on Aging • [6]Neurology, Psychiatry, Psychology, Behavioral Sciences, Sanders-Brown Center on Aging • [7]Neurology, MRISC, Sanders-Brown Center on Aging • [8]Anatomy and Neurobiology, Sanders-Brown Center on Aging

Undergraduate

Patients with Alzheimer's disease and related dementia (ADRD) have diminished memory performance, i.e. less accurate, more false alarm, and increased reaction times during a memory task. For many healthy older adults, it is not clear whether poor performance is part of normal aging or a risk of mild cognitive impairment (MCI) induced by ADRD. In the US, there are more older males than females who suffer from MCIs. Here we test the hypothesis that sex differences have differential effects on functional brain responses associated with either memory accuracy or response times. 44 older adults (25 females and 19 males; aged 65-93), from the University of Kentucky Alzheimer's Disease Center (UKADC) cohort, participated in the Bluegrass Short-Term (BeST) memory task under magnetic resonance imaging (MRI). Liner regression analyses were performed on event-related functional MRI and individual performance results both in separate sexes and in total. We found in all 44 subjects that the bilateral hippocampi and the right frontal eye field (FEF) showed significance, and that the dorsolateral prefrontal cortex (DLPFC) showed no significance. However, only females showed significant negative correlation in the left hippocampus in with reaction time ($R2=0.1578$; $p < 0.05$), whereas males showed no significant correlation in the hippocampus with any behavioral measures. We also discovered that in the frontal eye field, only males showed significant correlation in both the left and right frontal eye fields for measures of accuracy (including false alarms). In the dorsolateral prefrontal cortex (a classical region for working memory), we discovered that males showed significant correlation with false alarm and accuracy for both the left and right regions, whereas females showed significance in reaction time of only the right region. These results indicate that our hypothesis that sex differences have an impact on brain activity in correlation with memory performance is supported. The present results will allow us to test the next step hypothesis whether the memory performance and brain activity measures are associated with cerebrospinal fluid (CSF) AD biomarkers ß-amyloid (Aß42) and tau-related neurodegeneration (p-Tau181), hallmark for AD pathology.

23

A subset of gene expression profiles in human post-mortem brain aging and Alzheimer's disease show exaggerated changes in female AD subjects

Kendra Hargis-Staggs, PhD [1] • Eleanor Johnson [1] • Nada Porter, PhD [1] • Olivier Thibault, PhD [1] • Eric Blalock, PhD [1]

[1]Pharmacology and Nutritional Sciences, University of Kentucky

Fellow

Sporadic Alzheimer's disease (AD) is increasing in parallel to the aging population, with the majority (~2/3) of AD cases affecting women. AD is a complex disease characterized by amyloid beta and neurofibrillary tangle pathologies, and risk factors include head injury, high blood pressure, high cholesterol, and inheritance of certain gene variants (e.g., ABCA1, APOEe4, APOEe2, etc.). Despite the vast amount of research, successful therapies that modify the disease have yet to be developed. One reason could be that some unmodifiable risk factors, such as aging and sex, contribute to vulnerability and progression in AD as well, but their molecular roles have not been robustly assessed. We hypothesize that a subset of age-related transcriptional changes precede, and are exaggerated by, AD, and further that female AD sufferers will have exaggerated expression compared to males for this restricted subset of robustly identified genes. To address this, we looked at transcriptional profiles of normal human aging (8 profiles) and AD (9 profiles) to test for a statistically significant agreement for Aging or AD changes across independent samples from different labs. We further tested for a statistically significant relationship between robust aging and AD-related changes. We found 85 genes that were significantly changed with age, worsened in AD, and exaggerated in female vs male subjects These genes also showed very strong directional and magnitude-of-change agreement between normal aging and AD. Taken together, this panel of genes likely contains key candidate molecules for intervention testing and rationale therapeutic development.

24

Acute vs Chronic NFAT inhibition using the novel drug Q134R in a mouse model of Alzheimer's disease

Jenna Gollihue, PhD [1] • Pradoldej Sompol, PhD [1] • Susan Kraner, PhD [1] • Irina Artuishin, MS [1] • Laszlo Puskas, PhD [2] • Orsolya Huzian, PhD [2] • Christopher Norris, PhD [1]

[1]Sanders Brown Center on Aging, University of Kentucky • [2]Avidin Ltd.

Fellow

Objectives: Astrocytic activation is a common occurrence in neurological disorders including Alzheimer's disease (AD). Calcineurin dysregulation in activated astrocytes can dephosphorylate nuclear factor of activated T cells (NFAT). This transcription factor is located in the cytosol, but upon dephosphorylation translocates to the nucleus where it influences transcription and gene expression that can alter inflammation, calcium signaling, and cell survival. One of the potential treatments for AD, therefore, is to block the activation or translocation of NFAT. Pharmacological therapeutics to inhibit NFAT via calcineurin (tacrolimus, cyclosporine) have been developed but show adverse effects; this is probably due to the number of other substrates calcineurin is responsible for regulating independently of NFAT. However, a novel blood brain barrier permeant drug, Q134R, is purported to directly inhibit NFAT activation downstream of calcineurin without such side effects. Our lab has previously shown in vitro that using the Q134R drug inhibits NFAT activity in primary astrocytes without affecting calcineurin activity. The purpose of this project was to determine changes in NFAT activation and resulting changes in hippocampal-dependent memory in an APP/PS1 mouse model of AD.
Methods/Results: 12 month old wild type and APP/PS1 mice were treated with 4mg/kg Q134R or vehicle for one week. Behavioral Y maze showed an increase in performance back to wild type levels when APP/PS1 mice were given the drug twice daily. Further, NFAT4 localization shifted from the nucleus to the cytosol (activated to inactivated state) in hippocampal astrocytes following two weeks of treatment. To maximize the effects of the drug, we tested a chronic dosing paradigm that consisted of oral administration of the drug at the optimal dosage (4mg/kg/2x daily) for 3 months. Interestingly, when the drug was administered chronically, differences in spatial memory as well as shifts in NFAT localization were not as apparent.
Conclusions: Our results lead us to believe that chronic use of the Q134R drug may incite a compensatory mechanism that still allows for NFAT activation and translocation to the nucleus. This study focused on NFAT4, a subtype of NFAT that is located in astrocytes. Future assessments will examine whether NFAT activation is altered by Q134R in other cell types such as neurons (NFAT3). Additionally, time course and dose response studies can be performed to determine the optimal drug dosage and duration for maintaining spatial memory integrity in APP/PS1 mice.

Grant support: NIA R01 AG027297 and ADDF (20160902)

25

Temporal and anatomical effects on tangle count in rTg4510 mice after TBI

Shelby Meier [1] • Emad Chisti [2] • Kathryn Saatman, PhD [1] • Jose Abisambra, PhD [3]

[1]Physiology, University of Kentucky • [2]Sanders Brown Center on Aging, University of Kentucky • [3]Neuroscience, University of Florida

Graduate

Background: Traumatic brain injury (TBI) increases the risk of developing tauopathies such as chronic traumatic encephalopathy and Alzheimer's disease. While our understanding of the effects of TBI on tau phosphorylation has advanced quickly, very little is known about the effects of TBI on mature tangle pathology. Here we used the TET-OFF tau transgenic mouse model (rTg4510) to investigate the effects of TBI on established tau tangles.

Methods: At 3 months of age, male and female rTg4510 mice were fed a doxycycline diet (625 ppm) to suppress tau expression and were kept on this diet until euthanized. At 4.5 months of age mice were injured using an electromagnetic controlled cortical impact (CCI) device set to 5.0 m/s with 100ms dwell time. Injury depth with the 3.0mm rounded metal tip was set to 1.0mm from the dura. Sham animals underwent all procedures except impact. Animals were euthanized at 24 hours or 7 days post injury and transcardially perfused with 0.9% saline and 4% PFA. Fixed whole brains were sectioned at 25um on a freezing microtome. Sections were mounted and immunofluorescently labeled with FSB solution to identify beta sheet enriched structures found in mature tangles. FSB labeled tissue was imaged using a Nikon TiE and C2+ confocal microscope.

Results: Tangles were counted in cortical regions medial and lateral from the surgery/impact site. Tangle counts were averaged from four sections of tissue per animal. We found that tangle count significantly increased over time in the medial cortex regardless of injury, but tangle count significantly decreased after injury in the lateral cortex regardless of time after injury. We found that the number of tangles were not significantly different between male and female mice after injury. We also found no significant difference between ipsilateral and contralateral tangle counts after injury.

Conclusions: Our data provide novel insight into temporal and anatomical effects of TBI on pre-formed tangles. These data suggest the possibility of regional differences in response to injury that could impact development of tau pathology and support further investigation to understand the mechanisms driving these observed differences.

26

Flow cytometry distinguishes synaptosome populations containing a progression of AD pathologic markers in human autopsy brain and a transgenic model

Harry LeVine, III, PhD [1] • Sergey Matveev, PhD [2] • Chris Norris, PhD [3] • H. Peter Spielmann, PhD [4]

[1]Sanders-Brown Center on Aging. Molecular and Cellular Biochemistry, University of Kentucky • [2]Sanders-Brown Center on Aging, University of Kentucky • [3]Sanders-Brown Center on Aging, Pharmacology and Nutritional Science, University of Kentucky • [4]Molecular and Cellular Biochemistry, University of Kentucky

Faculty

Objective: One of the impediments to developing disease-modifying therapeutics for Alzheimer's disease (AD) is that current animal models do not fully recapitulate the pathological details or the severity of the cognitive dysfunction of the human disease. Our objective is to elucidate the key differences between the disease process in humans and animal models of AD.
Two differences between AD in humans and animal models of AD are: 1) in humans, AD Aβ amyloid pathology binds the imaging ligand, Pittsburgh Compound B (PIB), and 2) in humans, tau pathology develops along with severe cognitive symptoms.
In animal models of AD the Aβ pathology binds a minimal amount of PIB, and neither tau pathology nor severe cognitive symptoms develop. Previously we found that isolated synaptic endings (synaptosomes) from human AD brain bind much more 3H-PIB than synaptosomes from non-cognitively impaired (NCI) human brain. This suggested that this synaptic, non-plaque PIB binding could be a marker for an early step in a progression of pathologic events that begins in the synapse. We hypothesize that in animal models either some component for the disease process to advance is missing or that the models somehow successfully reverse or block the pathological changes.
Methods/Results: In order to detect affected synapses at early stages of the disease, we applied flow cytometry to analyze individual synaptosomes isolated from human frontal cortex of a series of autopsy cases. Complement C1q marks damaged synaptic endings, but additional processes are required to trigger pruning of the synapse. We find that in PSD95+ particles (combined with size defined as synaptosomes) in a given NCI human brain, a proportion of synaptosomes contain C1q, as well as Aβ, and fluorescent CN-PIB in a pattern that suggests a sequential process. The proportion of affected frontal cortex synaptosomes in an NCI brain increases until in an AD brain with cognitive symptoms almost all synaptosomes are affected. To probe the mechanisms leading to differences between human and AD animal models in synaptic AD-related events, we analyzed synaptosomes isolated from cortical tissue of 6-9 month old strain-control and 5XFAD AD model mice. Using flow cytometry, in 5XFAD mice, despite robust human sequence Aβ plaque pathology, we observe only a small fraction of affected CN-PIB+ synaptosomes, which is absent from the control mice. Future studies using flow isolation of interesting populations of synaptosomes from both humans and the 5XFAD mouse model will allow us to explore altered biochemical and genetic pathways to better understand the AD disease process and inform improvement of animal models.

27

Anti-oxidative therapy prevents Aβ-induced oxidative damage of theblood-brain barrier in an Alzheimer's disease mouse model

Atcharaporn Ontawong [1] • *Björn Bauer, PhD* [2] • *Chutima Srimaroeng, PhD* [3] • *Anika Hartz, PhD* [1]

[1]Sanders-Brown Center on Aging, University of Kentucky • [2]Pharmaceutical Sciences, University of Kentucky • [3]Physiology, Chiang Mai University

Graduate

Alzheimer's disease (AD) is a progressive neurodegenerative disease. One hallmark of AD is the accumulation of amyloid β (Aβ) in the brain, a process that contributes to memory decline. Recent evidence shows that Aβ triggers oxidative stress, which leads to AD progression. Importantly, several studies indicate an inverse correlation between oxidative stress and cognition suggesting that in AD patients, oxidative damage and memory decline are mechanistically linked. Indeed, several pre-clinical and clinical studies have reported that antioxidant therapy (e.g., vitamin C and E) helps to delay AD progression. Based on these data, we hypothesized the reducing oxidative stress in AD can help to slow cognitive decline.
To test this hypothesis, we used a reactive oxygen species (ROS) scavenger to determine the effect of anti-oxidant therapy on Aβ-induced oxidative stress and blood-brain barrier function in a mouse AD model. We utilize 5XFAD model, a model of early and aggressive Aβ accumulation associated with inflammatory astrocyte activation and cognitive decline and are currently treating 5XFAD mice with anti-oxidants. We are evaluating memory deficits using the Y-maze test, oxidative stress with the TBARS assay and Aβ clearance with a transport assay. Our preliminary findings suggest that anti-oxidative therapy might be a potential strategy to improve blood-brain barrier function, which is anticipated to lower brain Aβ burden and slow cognitive decline.

28

RNA integrity is associated with weakened expression of genes in the synaptic pathway in human cadaver brain tissue samples

Eleanor Johnson [1] • *Kendra Hargis-Staggs, PhD* [1] • *Eric Blalock, PhD* [1]

[1]Pharmacology and Nutritional Sciences, University of Kentucky

Graduate

RNA degradation can be influenced by many factors, including post-mortem interval and tissue pH. The degree to which RNA is degraded prior to quantification affects downstream measurement (e.g., in situ hybridization, RT-PCR, transcriptional profiling). Agilent Technologies introduced the RNA Integrity Number (RIN) in 2006, and over the last decade RIN has become widely adopted as a de facto standard to quantify RNA degradation across samples and labs. Recent studies have shown that RIN (ranging from 1- worst, to 10- best) influences mRNA levels, though relatively little work has been done to determine whether that RNA damage is random or is targeted to certain biological pathways. We hypothesized that RIN's influence on gene expression would:
a) show a strong positive correlation in control tissue (degrading RNA signal as RIN declines)
b) show a robust effect across independent studies
c) target mRNA in specific pathways
To test this, we identified and downloaded four publically available human post-mortem transcriptional profiling datasets that included disambiguated RIN scores for each array, used Affymetrix transcriptional profiling technology, and examined post-mortem human frontal cortex samples. To isolate RIN-selective effects, only the profiles of control samples from within each study were examined. RIN was tested for correlation with each gene's relative expression level within each study. In general, RIN correlations to gene expression in individual studies showed False Discovery Rates in the 0.15-0.3 range, indicating a relatively strong 'RIN Effect'. To determine whether this RIN Effect was robust across studies, the three studies with the strongest RIN Effects were statistically analyzed for similarity. We report a consistent and selective correlation between worsened RIN and declining synaptic gene expression, suggesting that the synapses are more vulnerable to the RNA degradation in human brain tissue. Our results further indicate that this RIN-gene expression correlation becomes pronounced when RIN < 7. Finally, these results indicate that this RIN Effect may create transcriptional pathway-specific blind spots that are not amenable to downstream RIN-correcting strategies.

29

Brain metabolite changes with age in cohort of individuals with Down Syndrome

Kathryn O'Connor, PhD [1] • Katie McCarty [2] • Roberta Davis [2] • Josh Lykins [3] • Gregory Jicha, MD, PhD [4] • Allison Caban-Holt, PhD [5] • Donita Lightener, MD [6] • William Robertson, MD [6] • Stacey Brothers [2] • Ai-Ling Lin, PhD [7] • David Powell, PhD [8] • Frederick Schmitt, PhD [4] • Elizabeth Head, PhD [7]

[1]Spinal Cord and Brain Injury Research Center, Sanders-Brown Center on Aging • [2]Sanders-Brown Center on Aging • [3]Department of Molecular and Cellular Biochemistry, University of Kentucky • [4]Department of Neurology, Sanders-Brown Center on Aging • [5]Department of Behavioral Science, Sanders-Brown Center on Aging • [6]Department of Neurology, University of Kentucky • [7]Department of Pharmacology & Nutritional Sciences, Sanders-Brown Center on Aging • [8]Magnetic Resonance Imaging and Spectroscopy Center, University of Kentucky

Fellow

Introduction: Alzheimer's disease (AD) affects 5.7 million Americans and is characterized by specific neuropathology and resulting clinical symptoms. By age 40, nearly all individuals with Down Syndrome (DS) have significant AD neuropathology. Identifying factors associated with AD initiation and progression may identify potential interventions. Brain metabolites, which measure neuron health, may change before more substantial and permanent neuropathology emerges, thus serving as a potential prodromal marker. We hypothesized that certain brain metabolites would change with age and change more rapidly after age 40 when significant neuropathology is present in people with DS.
Methods: A DS cohort completed annual cognitive tests and Magnetic Resonance Spectroscopy (MRS). MRS measured the following brain metabolites: myo-inositol (MI), N-acetylaspartate (NAA), and glutamate-glutamine complex (Glx). The ratio of NAA:MI was calculated, as previous studies found that the NAA:MI ratio distinguished between individuals with dementia from those without dementia. Linear mixed models evaluated whether MRS measures changed with age. All models controlled for sex and MRS acquisition method. Follow up analysis tested whether rates of change differed by age of significant neuropathology, assumed to be represented by age 40. Jenks natural breaks indicated that 42.3 year cutoff minimizes the intra-class variance indicating the most natural cutoff.
Results: 61 participants (N = 25 Male) had 132 observations, with an average follow-up time of 1.9 years. Age was significantly associated with changes in NAA ($b = -0.009$, $p < 0.001$), Glx ($b = -0.012$, $p < 0.001$), and NAA:MI ($b = -0.016$, $p < 0.001$). Follow up analyses indicated that brain metabolites changed at different rates before age 42.3 versus after age 42.3 Specifically, MI increased significantly after age 42.3 ($b = 0.005$, $p = 0.008$), but no change in MI with age prior to age 42.3 ($p = 0.33$). Glx decreased significantly prior to age 42.3 ($b = -0.024$, $p = 0.001$), but there was no change in Glx after age 42.3.
Conclusions: The current study demonstrated that MRS metabolites change with age, indicating worse neuronal health with increasing age. Moreover, changes in certain brain metabolites are not linear. Increasing MI after age 42.3 indicates increased neuroinflammation, while decreasing Glx before age 42.3 indicates neuronal loss or injury. Thus, changes in Glx may serve as a potential measure of prodromal AD, while MI may represent the degree of neuroinflammatory response to neuropathology. Future studies should evaluate whether changes in Glx predicts conversion to dementia and whether increased MI is associated with greater cognitive impairment in people with DS.

Funding: R01 HD064993; T32 AG057461

30

Precision biochemical profile of Alzheimer's disease and APOE genotype

Tyler Hammond [1] • Ishita Parikh, PhD [2] • Peter Nelson, MD, PhD [3] • Scott McCulloch, PhD [4] • Ai-Ling Lin, PhD [5]

[1]Neuroscience, University of Kentucky • [2]Oklahoma Medical Research Foundation • [3]Pathology, University of Kentucky • [4]Metabolon, Inc • [5]Pharmacology and Nutritional Sciences, University of Kentucky

Graduate

Background: The cure for Alzheimer's disease (AD) has remained elusive for more than 20 years. The amyloid hypothesis has led researchers to target amyloid in drug discovery to no avail. It is possible that there are underlying metabolic processes that lead to the deposition of amyloid in brain tissue and that these processes differ based on APOE status. Here we measure the metabolites of AD brains and control brains with and without the APOE4 genotype to understand whether differences are implicated in the underlying disease pathology.
Purpose/Objectives: To understand the effect that cognitive status and APOE genotype have on an individual's biochemical profile to help guide personalized nutrition.
Methods: The global biochemical profiles of post-mortem human brain tissue was determined using mass spectroscopy. 24 subjects from 4 different cohorts were analyzed: APOE3 Control, APOE3 Alzheimer's disease, APOE4 control, and APOE4 Alzheimer's Disease. Metabolites were quantified using global untargeted metabolomics (HD4) and compared between cohorts using Welch's two-sample t-test.
Results: Many metabolites were significantly different between the 4 cohorts. Most notably, AD brain tissue regardless of APOE genotype had increases in products related to metabolic syndrome (betaine ratio 0.6:1, alanine ratio 1.2:1, phosphatidycholine ratio 0.8:1), mitochondrial dysfunction (cysteinylglycine ratio 1.6:1, succinate ratio 5:1), and kidney dysfunction (arginine ratio 1.2:1, myo-inositol ratio 1.3:1, GPC ratio 1.3:1) compared to control brain tissue. APOE4 AD brain tissue had elevated free fatty acids (eg: nonadecanoate ratio 1.5:1, erucate ratio 2.2:1) and altered endocannabinoid metabolism (anandamide ratio 0.7:1) compared to APOE3 AD brain tissue.
Conclusions: Many metabolites differed between the 4 cohorts. Metabolites associated with metabolic syndrome, mitochondrial dysfunction, and kidney dysfunction were all associated with AD. Free fatty acids were elevated in APOE4 genotypes. The different biochemical profiles of the different cohorts suggest that precision nutrition should be implemented depending on disease status and genotype in order to optimize management. Further investigation is needed to determine whether dietary interventions could be designed to alter harmful metabolites leading to pathology. Future studies with larger sample sizes are needed to confirm whether these metabolites are consistently abnormal in AD and APOE4 human brain tissue.

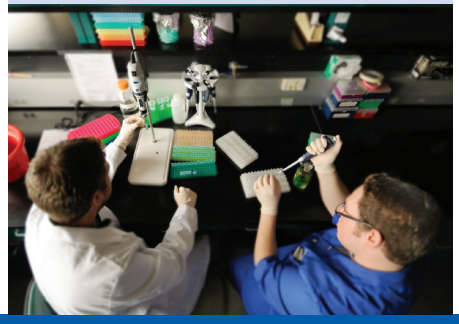
Sanders-Brown Center on Aging

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

- Someone in the US develops Alzheimer's disease every 65 seconds
- One in three seniors die with Alzheimer's or another dementia
- An estimated 5.7 million persons in the U.S. have Alzheimer's disease
- Older African-Americans are about twice as likely to have Alzheimer's or other dementias as older whites
- Almost two-thirds of Americans with Alzheimer's are women



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

- Basic and clinical research in Alzheimer's disease
- Neurodegenerative disorders
- Stroke
- Normal 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 plan to 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 to delay or prevent age-related brain diseases such as Alzheimer's disease, so that our volunteers, patients and caregivers become the beneficiaries of our advances in knowledge.

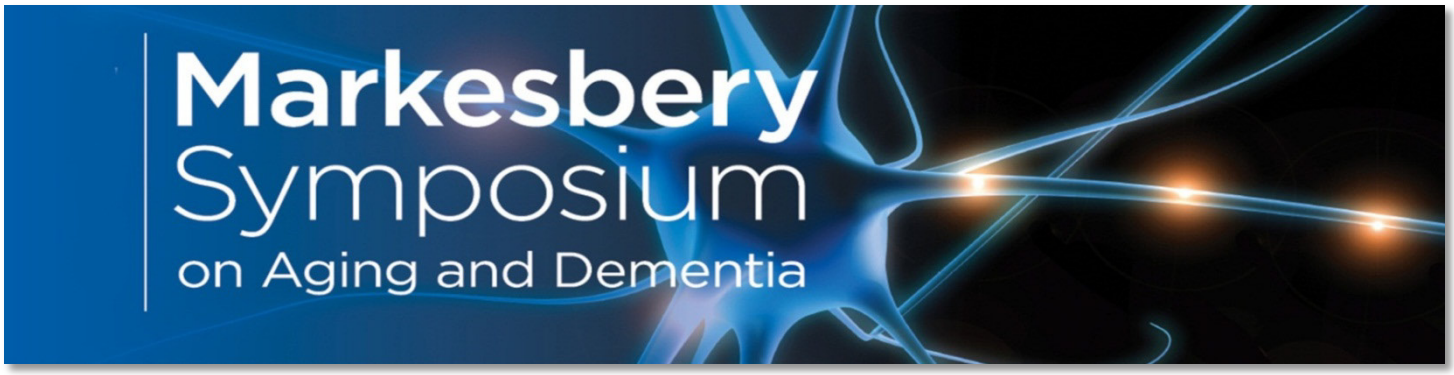
Unless science finds a way to slow the progression of this devastating disease, the United States will see a nearly 50 percent increase in the number of victims by 2030. In addition to the direct impact on the patient, Alzheimer's disease also affects the lives of family members and friends.

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

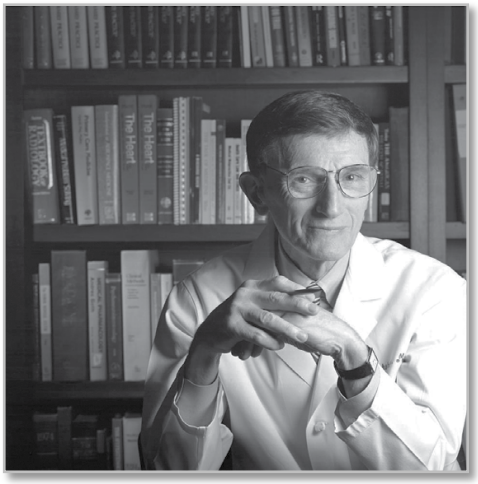
- Alzheimer's disease is the only top 10 cause of death in the United States that cannot be prevented, cured or even slowed
- Between 2000 and 2015 deaths from heart disease have decreased by 11% while deaths from Alzheimer's have increased 123%
- No cure or preventive measure currently exists for Alzheimer's disease, but a number of promising therapies are being developed and tested, including several at the University of Kentucky.
- By investing in the development of therapies now, we can save billions of dollars and heartache in the future. You can help through financial donations, or by participating in one of our research programs.

From the 2018 Alzheimer's Association Facts and Figures publication.

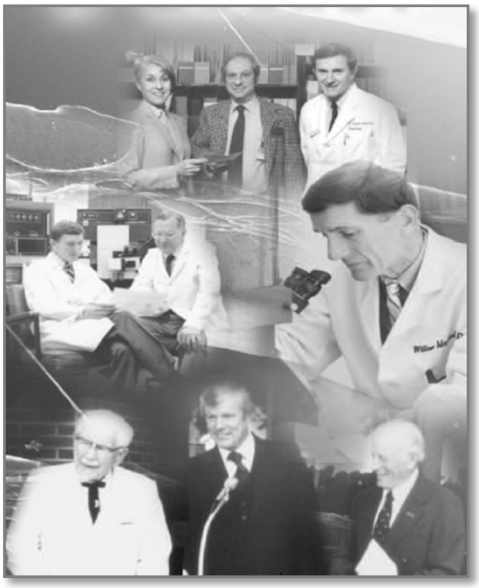
Please help us today in our fight against Alzheimer's disease. For more information on research, clinical trials and ways to get involved, contact us at 859-323-6040 or visit our website www.centeronaging.uky.edu



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 30 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.

Notes

Sanders-Brown
Center on Aging

101 Sanders-Brown Building | Lexington, Kentucky 40536-0230
(859) 323-6040 | www.centeronaging.uky.edu